Others have identified objectives in the form of questions. For example, can an agency that supports research also effectively regulate that research? Is the focus of attention on this issue accurate—the narrow view is concern about accidents—the longer range view deals with question of the potential impact of crossing species barriers? Is it necessary at this time to continue using *E. coli*? How much effort should be devoted to making available alternative and more acceptable test organisms? Shouldn't the potential risks of genetic engineering be postponed by delaying research in this field until the ethical and social problems of more immediately available biomedical application of other techniques in genetics are resolved. The list of questions is almost endless and includes critical legal issues associated with patent policies for discoveries in this field of research. This is an area under special study at this time by the Director of NIH.

Since the momentum of research on DNA recombinant molecules is increasing, it now appears that the basic science policy actions to be taken are those which will provide the most appropriate solution to these questions and similar objectives. The interface between legislation and research will determine which direction these actions will take.

APPENDIXES

APPENDIX I

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APPENDIX 2

1324
SCIENTIFIC AMERICAN OFFPRINTS

THE MANIPULATION OF GENES

by Stanley N. Cohen

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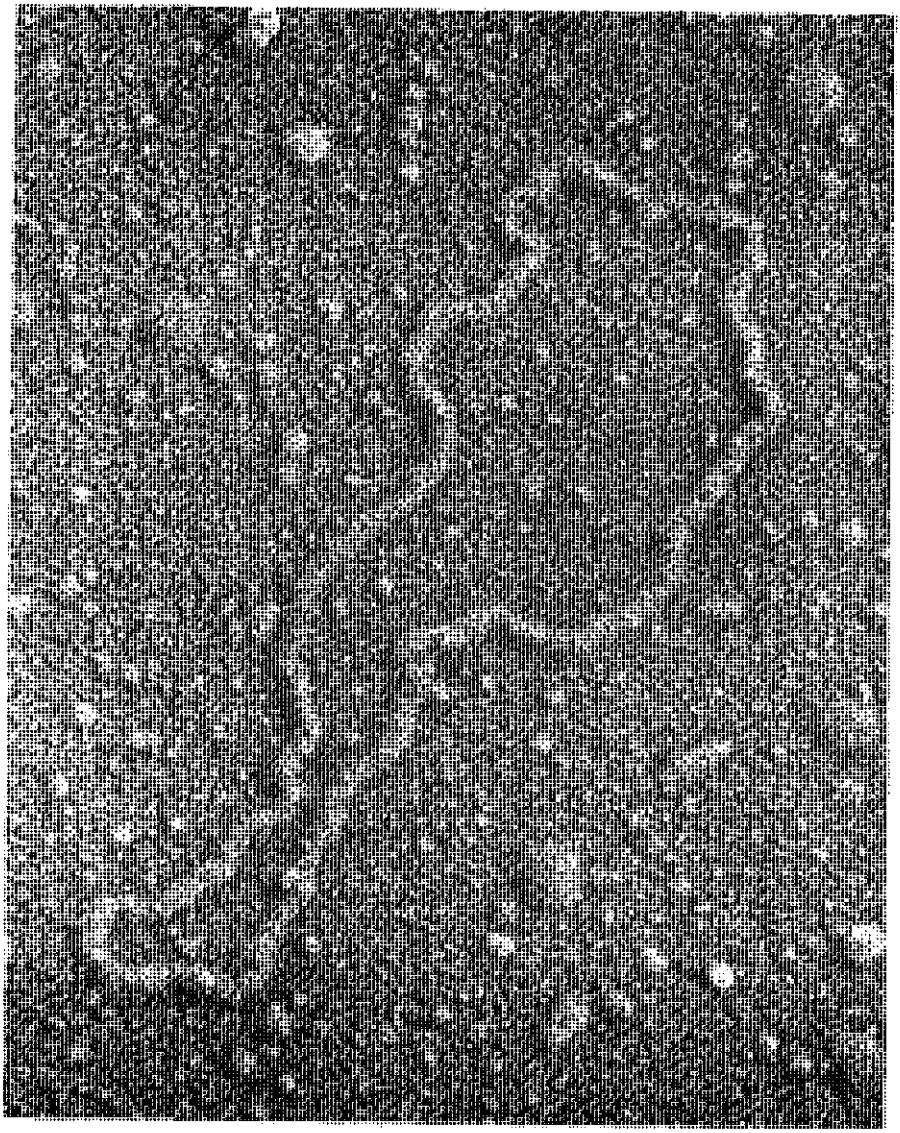


PLASMID *pSC101* is shadowed with platinum-palladium and enlarged *230,000 diameters in an electron micrograph made by the* author. A plasmid is a molecule of DNA that exists apart from the chromosome in a bacterium and replicates on its own, often carrying the genes for some supplementary activity such as resistance to antibiotics. This plasmid, a small one made by shearing a larger plasmid native to the bacterium *Escherichia coli*, is a circular, or closed-loop, molecule of DNA about three micrometers in circumference that carries the genetic information for replicating itself in *E. coli* and for conferring resistance to the antibiotic tetracycline. It was the "vehicle" for the first gene-manipulation experiments by the author and his colleagues. Foreign DNA was spliced to it and the plasmid was introduced into *E. coli*, where it replicated and expressed both its own and the foreign DNA's genetic information.

THE MANIPULATION OF GENES

Techniques for cleaving DNA and splicing it into a carrier molecule make it possible to transfer genetic information from one organism to an unrelated one. There the DNA replicates and expresses itself

by Stanley N. Cohen

Mythology is full of hybrid creatures such as the Sphinx, the Minotaur and the Chimera, but the real world is not; it is populated by organisms that have been shaped not by the union of characteristics derived from very dissimilar organisms but by evolution within species that retain their basic identity generation after generation. This is because there are natural barriers that normally prevent the exchange of genetic information between unrelated organisms. The barriers are still poorly understood, but they are of fundamental biological importance.

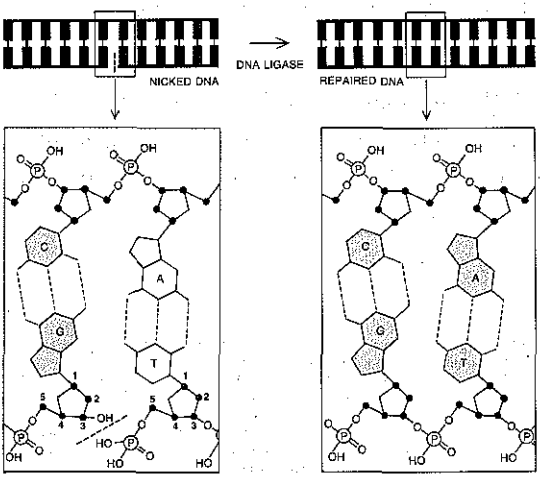
The basic unit of biological relatedness is the species, and in organisms that reproduce sexually species are defined by the ability of their members to breed with one another. Species are determined and defined by the genes they carry, so that in organisms that reproduce asexually the concept of species depends on nature's ability to prevent the biologically significant exchange of genetic material—the nucleic acid DNA—between unrelated groups.

The persistence of genetic uniqueness is perhaps most remarkable in simple organisms such as bacteria. Even when they occupy the same habitat most bacterial species do not exchange genetic information. Even rather similar species of bacteria do not ordinarily exchange the genes on their chromosomes, the structures that carry most of their genetic information. There are exceptions, however. There are bits of DNA, called plasmids, that exist apart from the chromosomes in some bacteria. Sometimes a plasmid can pick up a short segment of DNA from the chromosome of its own cell and transfer it to the cell of a related bacterial species, and sometimes the plasmid and the segment of chromosomal DNA can become integrated into the chromosome of the recipient cell. This transfer of genes between species by extrachromosomal elements has surely played some role in bacterial evolution, but apparently it has not been widespread in nature. Otherwise the characteristics of the common bacterial species would not have remained so largely intact over the huge number of bacterial generations that have existed during the era of modern bacteriology.

In 1973 Annie C. Y. Chang and I at the Stanford University School of Medicine and Herbert W. Boyer and Robert B. Helling at the University of California School of Medicine at San Francisco reported the construction in a test tube of biologically functional DNA molecules that combined genetic information from two different sources. We made the molecules by splicing together segments of two different plasmids found in the colon bacillus *Escherichia coli* and then inserting the composite DNA into *E. coli* cells, where it replicated itself and expressed the genetic information of both parent plasmids. Soon afterward we introduced plasmid genes from an unrelated bacterial species, *Staphylococcus aureus*, into *E. coli*, where they too expressed the biological properties they had displayed in their original host; then, applying the same procedures with John F. Morrow of Stanford and Howard M. Goodman in San Francisco, we were able to insert into *E. coli* some genes from an animal: the toad *Xenopus laevis*.

We called our composite molecules DNA chimeras because they were conceptually similar to the mythological Chimera (a creature with the head of a lion, the body of a goat and the tail of a serpent) and were the molecular counterparts of hybrid plant chimeras produced by agricultural grafting. The procedure we described has since been used and extended by workers in several laboratories. It has been called plasmid engineering, because it utilizes plasmids to introduce the foreign genes, and molecular cloning, because it provides a way to propagate a clone, or line of genetically alike organisms, all containing identical composite DNA molecules. Because of the method's potential for creating a wide variety of novel genetic combinations in microorganisms it is also known as genetic engineering and genetic manipulation. The procedure actually consists of several distinct biochemical and biological manipulations that were made possible by a series of independent discoveries made in rapid succession in the late 1960's and early 1970's. There are four essential elements: a method of breaking and joining DNA molecules derived from different sources; a suitable gene carrier that can replicate both itself and a foreign DNA segment linked to it; a means of introducing the composite DNA molecule, or chimera, into a functional bacterial cell, and a method of selecting from a large population of cells a clone of recipient cells that has acquired the molecular chimera.

In 1967 DNA ligases—enzymes that can repair breaks in DNA and under certain conditions can join together the loose ends of DNA strands—were discovered almost simultaneously in five laboratories. A DNA strand is a chain of nucleotides, each consisting of a deoxyribose sugar ring, a phosphate group and one of four organic bases: adenine, thymine, guanine and cytosine. The sugars and phosphates form the backbone of the strand, from which the bases project. The individual nucleotide building blocks are connected by phosphodiester bonds between the carbon atom at position No. 3 on one sugar and the carbon atom at position No. 5 on the adjacent sugar. Double-strand DNA, the form found in most organisms, consists of two



DNA LIGASE is an enzyme that repairs "nicks," or breaks in one strand of a double-strand molecule of DNA (*top*). A strand of DNA is a chain of nucleotides (*bottom*), each consisting of a deoxyribose sugar and a phosphate group and one of four organic bases: adenine (*A*), thymine (*T*), guanine (*G*) and cytosine (*C*). The sugars and phosphates constitute the backbone of the strand, and paired bases, linked by hydrogen bonds (*broken black lines*), connect two strands. The ligase catalyzes synthesis of a bond at the site of the break (*broken colored line*) between the phosphate of one nucleotide and the sugar of the next nucleotide.

chains of nucleotides linked by hydrogen bonds between their projecting bases. The bases are complementary: adenine (*A*) is always opposite thymine (*T*), and guanine (*G*) is always opposite cytosine (*C*). The function of the ligase is to repair "nicks," or breaks in single DNA strands, by synthesizing a phosphodiester bond between adjoining nucleotides [*see illustration above*].

In 1970 a group working in the laboratory of H. Gobind Khorana, who was then at the University of Wisconsin, found that the ligase produced by the bacterial virus T4 could sometimes catalyze the end-to-end linkage of completely separated double-strand DNA segments. The reaction required that the ends of two segments be able to find each other; such positioning of two DNA molecules was a matter of chance, and so the reaction was inefficient. It was clear that efficient joining of DNA molecules required a mechanism for holding the two DNA ends together so that the ligase could act.

An ingenious way of accomplishing this was developed and tested independently in two laboratories at Stanford: by Peter Lobban and A. Dale Kaiser and by David Jackson, Robert Symons and Paul Berg. Earlier work by others had shown that the ends of the DNA molecules of certain bacterial viruses can be joined by base-pairing between complementary sequences of nucleotides that are naturally present on single-strand segments projecting from the ends of those molecules: *A*'s pair with *T*'s, *G*'s pair with *C*'s and the molecules are held together by hydrogen bonds that form between the pairs. The principle of linking DNA molecules by means of the single-strand projections had been exploited in Khorana's laboratory for joining short synthetic sequences of nucleotides into longer segments of DNA.

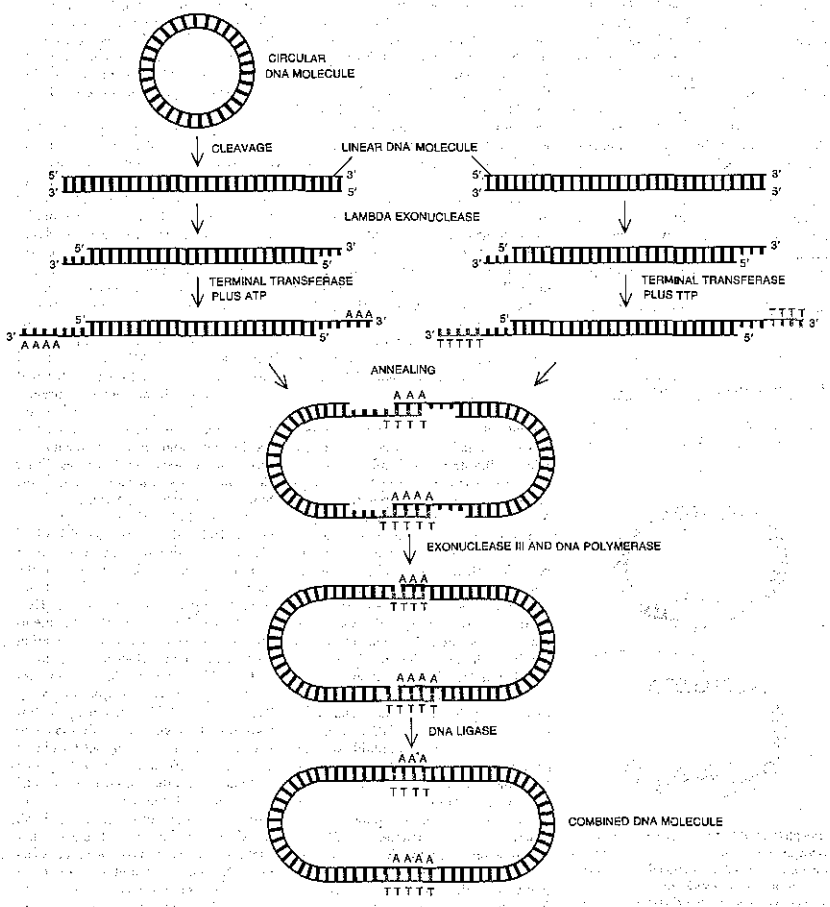
The Stanford groups knew too that an enzyme, terminal transferase, would catalyze the stepwise addition, specifically at what are called the 3′ ends of single strands of DNA, of a series of identical nucleotides. If the enzyme worked also with double-strand DNA, then a block of identical nucleotides could be added to one population of DNA molecules and a block of the complementary nucleotides could be added to another population from another source. Molecules of the two populations could then be annealed by hydrogen bonding and sealed together by DNA ligase. The method was potentially capable of joining any two species of DNA. While Lobban and Kaiser tested the terminal-transferase procedure with the DNA of the bacterial virus P22, Jackson, Symons and Berg applied the procedure to link the DNA of the animal virus SV40 to bacterial-virus DNA.

The SV40 and bacterial-virus DNA molecules Berg's group worked with are closed loops, and the loops had first to be cleaved to provide linear molecules with free ends for further processing and linkage [*see illustration on opposite page*]. (As it happened, the particular enzyme chosen to cleave the loops was the *Eco* RI endonuclease, which was later to be used in a different procedure for making the first biologically functional gene combinations. At the time, however, the enzyme's special property of producing complementary single-strand ends all by itself had not yet been discovered.)

The cleaved linear molecules were treated with an enzyme, produced by the bacterial virus lambda, called an exonuclease because it operates by cutting off nucleotides at the end of a DNA molecule. The lambda exonuclease chewed back the 5′ ends of DNA molecules and thus left projecting single-strand ends that had 3′ termini to which the blocks of complementary nucleotides could be added. The next step was to add, with the help of terminal transferase, a block of *A*'s at the 3′ end of one of the two DNA species to be linked and a block of *T*'s at the 3′ ends of the other species. The species were mixed together. Fragments having complementary blocks at their ends could find each other, line up and become annealed by hydrogen bonding, thus forming combined molecules. To fill the gaps at the 5′ ends of the original segments the investigators supplied nucleotides and two more enzymes: exonuclease III and DNA polymerase. Finally the nicks in the molecules were sealed with DNA ligase.

The method of making cohesive termini for joining DNA molecules in the first successful genetic-manipulation experiments was conceptually and operationally different from the terminal-transferase procedure. It was also much simpler. It depended on the ability of one of a group of enzymes called restriction endonucleases to make complementary-ended fragments during the cleavage of DNA at a site within the molecule, instead of requiring the addition of new blocks of complementary nucleotides to DNA termini.

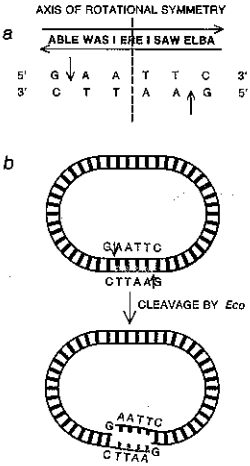
Viruses grown on certain strains of *E. coli* were known to be restricted in their ability to grow subsequently on other strains. Investigations had shown that this restriction was due to bacterial enzymes that recognize specific sites on a "foreign" viral DNA and cleave that DNA. (To protect its own DNA the bacterial cell makes a modification enzyme that adds methyl groups to nucleotides constituting the recognition sites for the restriction endonuclease, making them resistant to cleavage.) Restriction endonucleases (and modification methylases) are widespread in microorganisms; genes for making them were found on viral chromosomes and extrachromosomal plasmid DNA as well as on many bacterial chromosomes. During the early 1970's the nucleotide sequences at the cleavage sites recognized by several re-



TERMINAL-TRANSFERASE procedure for joining DNA molecules involves a number of steps, each dependent on a different enzyme. If one of the molecules to be joined is a closed loop, it must first be cleaved. The linear molecules are treated with lambda exonuclease, an enzyme that cuts nucleotides off the 5′ end of DNA strands (the end with a phosphate group on the No. 5 carbon). Then specific nucleotides are added to the 3′ end (the end with an OH group on the No. 3 carbon) by the action of the enzyme terminal transferase. One DNA species is supplied with adenosine triphosphate (ATP), the other with thymidine triphosphate (TTP), so that *A* nucleotides are added to one species and complementary *T* nucleotides to the other. When the two species are mixed, the complementary bases pair up, annealing the molecules. Nucleotides and the enzymes DNA polymerase and exonuclease III are added to fill gaps and DNA ligase is added to seal the DNA backbones. The result is a double molecule composed of two separate DNA segments.

striction endonucleases were identified. In every instance, it developed, the cleavage was at or near an axis of rotational symmetry: a palindrome where the nucleotide base sequences read the same on both strands in the 5′-to-3′ direction [*see illustration below*].

In some instances the breaks in the DNA strands made by restriction enzymes were opposite each other. One particular endonuclease, however, the *Eco RI* enzyme isolated by Robert N. Yoshimori in Boyer's laboratory in San Francisco, had a property that was of special interest. Unlike the other nucleases known at the time, this enzyme introduced breaks in the two DNA strands that were separated by several nucleotides. Because of the symmetrical, palindromic arrangement of the nucleotides in the region of cleavage this separation of the cleavage points on the two strands yielded DNA termini with projecting complementary nucleotide sequences: "sticky" mortise-and-tenon ter-



RESTRICTION ENDONUCLEASES cleave DNA at sites where complementary nucleotides are arranged in rotational symmetry: a palindrome, comparable to a word palindrome (*a*). The endonuclease *Eco RI* has the additional property of cleaving complementary strands of DNA at sites (*colored arrows*) four nucleotides apart. Such cleavage (*b*) yields DNA fragments with complementary, overlapping single-strand ends. As a result the end of any DNA fragment produced by *Eco RI* cleavage can anneal with any other fragment produced by the enzyme.

mini. The *Eco RI* enzyme thus produced in one step DNA molecules that were functionally equivalent to the cohesive-end molecules produced by the complicated terminal-transferase procedure.

The experiments that led to the discovery of the capabilities of *Eco RI* were reported independently and simultaneously in November, 1972, by Janet Mertz and Ronald W. Davis of Stanford and by another Stanford investigator, Vittorio Sgaramella. Sgaramella found that molecules of the bacterial virus P22 could be cleaved with *Eco RI* and would then link up end to end to form DNA segments equal in length to two or more viral-DNA molecules. Mertz and Davis observed that closed-loop SV40-DNA molecules cleaved by *Eco RI* would reform themselves into circular molecules by hydrogen bonding and could be sealed with DNA ligase; the reconstituted molecules were infectious in animal cells growing in tissue culture. Boyer and his colleagues analyzed the nucleotide sequences at the DNA termini produced by *Eco RI*, and their evidence confirmed the complementary nature of the termini, which accounted for their cohesive activity.

In late 1972, then, several methods were available by which one could join double-strand molecules of DNA. That was a major step in the development of a system for manipulating genes. More was necessary, however. Most segments of DNA do not have an inherent capacity for self-replication; in order to reproduce themselves in a biological system they need to be integrated into DNA molecules that can replicate in the particular system. Even a DNA segment that can replicate in its original host was not likely to have the specific genetic signals required for replication in a different environment. If foreign DNA was to be propagated in bacteria, as had long been proposed in speculative scenarios of genetic engineering, a suitable vehicle, or carrier, was required. A composite DNA molecule consisting of the vehicle and the desired foreign DNA would have to be introduced into a population of functional host bacteria. Finally, it would be necessary to select, or identify, those cells in the bacterial population that took up the DNA chimeras. In 1972 it still seemed possible that the genetic information on totally foreign DNA molecules might produce an aberrant situation that would prevent the propagation of hybrid molecules in a new host.

Molecular biologists had focused for many years on viruses and their relations with bacteria, and so it was natural that bacterial viruses were thought of as the most likely vehicles for genetic manipulation. For some time there had been speculation and discussion about using viruses, such as lambda, that occasionally acquire bits of the *E. coli* chromosome by natural recombination mechanisms for cloning DNA from foreign sources. It was not a virus, however, but a plasmid that first served as a vehicle for introducing foreign genes into a bacterium and that provided a mechanism for the replication and selection of the foreign DNA.

A ubiquitous group of plasmids that confer on their host bacteria the ability to resist a number of antibiotics had been studied intensively for more than a decade. Antibiotic-resistant *E. coli* isolated in many parts of the world, for example, were found to contain plasmids, designated *R* factors (for "resistance"), carrying the genetic information for products that in one way or another could interfere with the action of specific antibiotics [see "Infectious Drug Resistance," by Tsutomu Watanabe; SCIENTIFIC AMERICAN, December, 1967]. Double-strand circular molecules of *R*-factor DNA had been separated from bacterial chromosomal DNA by centrifugation in density gradients and had been characterized by biochemical and physical techniques [see "The Molecule of Infectious Drug Resistance," by Royston C. Clowes; SCIENTIFIC AMERICAN, April, 1973].

In 1970 Morton Mandel and A. Higa of the University of Hawaii School of Medicine had discovered that treatment of *E. coli* with calcium salts enabled the bacteria to take up viral DNA. At Stanford, Chang and I, with Leslie Hsu, found that if we made the cell membranes of *E. coli* permeable by treating them with calcium chloride, purified *R*-factor DNA could be introduced into them [*see illustration on opposite page*]. The *R*-factor DNA is taken up in this transformation process by only about one bacterial cell in a million, but those few cells can be selected because they live and multiply in the presence of the antibiotics to which the *R* factor confers resistance, whereas other cells die. Each transformed cell gives rise to a clone that contains exact replicas of the parent plasmid DNA molecules, and so we reasoned that plasmids might serve as vehicles for propagating new genetic information in a line of *E. coli* cells.

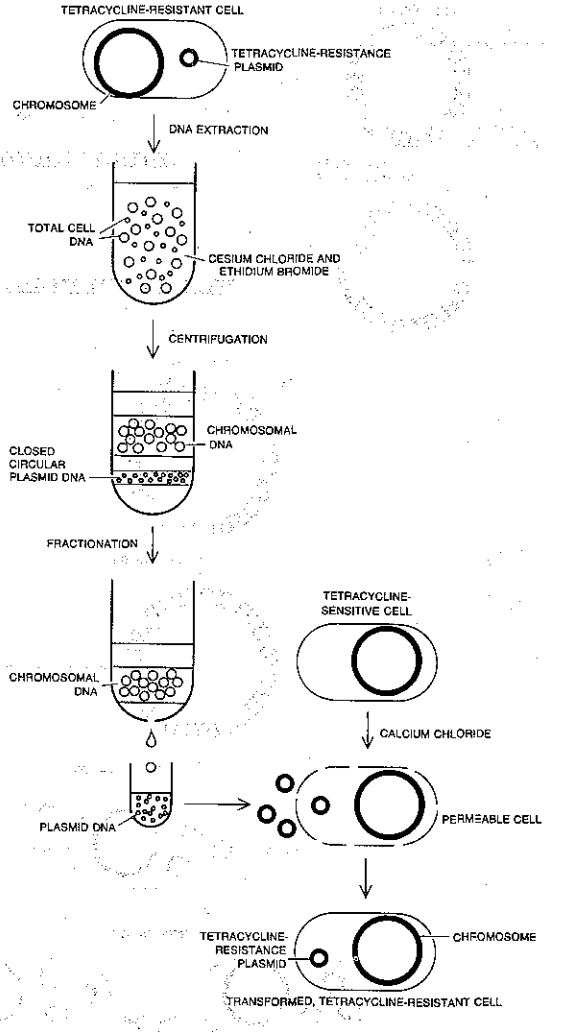
In an effort to explore the genetic and molecular properties of various regions of the *R*-factor DNA we had begun to take plasmids apart by shearing their DNA mechanically and then transforming *E. coli* with the resulting

fragments. Soon afterward we began to cleave the plasmids with the *Eco RI* enzyme, which had been shown to produce multiple site-specific breaks in several viruses. It might therefore be counted on to cleave all molecules of a bacterial plasmid in the same way, so that any particular species of DNA would yield a specific set of cleavage fragments, and do so reproducibly. The fragments could then be separated and identified according to the different rates at which they would migrate through a gel under the influence of an electric current.

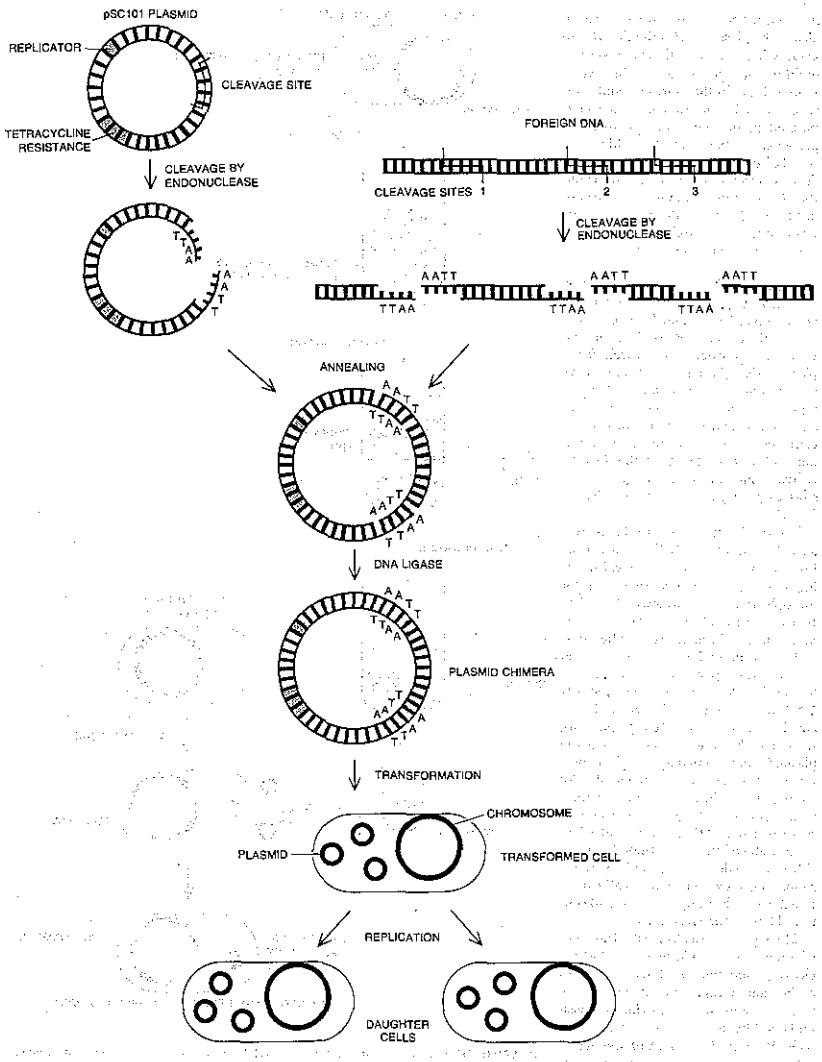
When the DNA termini produced by *Eco RI* endonuclease were found to be cohesive, Chang and I, in collaboration with Boyer and Helling in San Francisco, proceeded to search for a plasmid that the enzyme would cleave without affecting the plasmid's ability to replicate or to confer antibiotic resistance. We hoped that if such a plasmid could be found, we could insert a segment of foreign DNA at the *Eco RI* cleavage site, and that it might be possible to propagate the foreign DNA in *E. coli*.

In our collection at Stanford there was a small plasmid, *pSC101*, that had been isolated following the mechanical shearing of a large plasmid bearing genes for multiple antibiotic resistance. It was less than a twelfth as long as the parent plasmid, but it did retain the genetic information for its replication in *E. coli* and for conferring resistance to one antibiotic, tetracycline. When we subjected *pSC101* DNA to cleavage by *Eco RI* and analyzed the products by gel electrophoresis, we found that the enzyme had cut the plasmid molecule in only one place, producing a single linear fragment. We were able to join the ends of that fragment again by hydrogen bonding and reseal them with DNA ligase, and when we introduced the reconstituted circular DNA molecules into *E. coli* by transformation, they were biologically functional plasmids: they replicated and conferred tetracycline resistance.

The next step was to see if a fragment of foreign DNA could be inserted at the cleavage site without interfering with replication or expression of tetracycline resistance and thus destroying the plasmid's ability to serve as a cloning vehicle. We mixed the DNA of another *E. coli* plasmid, which carried resistance to the antibiotic kanamycin, with the *pSC101* DNA. We subjected the mixed DNA to cleavage by *Eco RI* and then to ligation, transformed *E. coli* with the resulting DNA and found that some of the transformed bacteria were indeed resist-



PLASMID DNA can be introduced into a bacterial cell by the procedure called transformation. Plasmids carrying genes for resistance to the antibiotic tetracycline (*top left*) are separated from bacterial chromosomal DNA. Because differential binding of ethidium bromide by the two DNA species makes the circular plasmid DNA denser than the chromosomal DNA, the plasmids form a distinct band on centrifugation in a cesium chloride gradient and can be separated (*bottom left*). The plasmid DNA is mixed with bacterial cells that are not resistant to tetracycline and that have been made permeable by treatment with a calcium salt. The DNA enters the cells, replicates there and makes the cells resistant to tetracycline.



FOREIGN DNA is spliced into the pSC101 plasmid and introduced with the plasmid into the bacterium *Escherichia coli*. The plasmid is cleaved by the endonuclease *Eco RI* at a single site that does not interfere with the plasmid's genes for replication or for resistance to tetracycline (*top left*). The nucleotide sequence recognized by *Eco RI* is present also in other DNA, so that a foreign DNA exposed to the endonuclease is cleaved about once in every 4,000 to 16,000 nucleotide pairs on a random basis (*top right*). Fragments of cleaved foreign DNA are annealed to the plasmid DNA by hydrogen bonding of the complementary base pairs, and the new composite molecules are sealed by DNA ligase. The DNA chimeras, each consisting of the entire plasmid and a foreign DNA fragment, are introduced into *E. coli* by transformation, and the foreign DNA is replicated by virtue of the replication functions of the plasmid.

ant to both tetracycline and kanamycin. The plasmids isolated from such transformants contained the entire *pSC101* DNA segment and also a second DNA fragment that carried the information for kanamycin resistance, although it lacked replication functions of its own. The results meant that the *pSC101* could serve as a cloning vehicle for introducing at least a nonreplicating segment of a related DNA into *E. coli*. And the procedure was extraordinarily simple.

Could genes from other species be introduced into *E. coli* plasmids, however? There might be genetic signals on foreign DNA that would prevent its propagation or expression in *E. coli*. We decided to try to combine DNA from a plasmid of another bacterium, the *pI258* plasmid of *Staphylococcus aureus*, with our original *E. coli* plasmid. The staphylococcal plasmid had already been studied in several laboratories; we had found that it was cleaved into four DNA fragments by *Eco RI*. Since *pI258* was not native to *E. coli* or to related bacteria, it could not on its own propagate in an *E. coli* host. And it was known to carry a gene for resistance to still another antibiotic, penicillin, that would serve as a marker for selecting any transformed clones. (Penicillin resistance, like combined resistance to tetracycline and kanamycin, was already widespread among *E. coli* strains in nature. That was important; if genes from a bacterial species that cannot normally exchange genetic information with the colon bacillus were to be introduced into it, it was essential that they carry only antibiotic-resistance traits that were already prevalent in *E. coli*. Otherwise we would be extending the species' antibiotic-resistance capabilities.)

Chang and I repeated the experiment that had been successful with two kinds of *E. coli* plasmids, but this time we did it with a mixture of the *E. coli*'s *pSC-101* and the staphylococcal *pI258*: we cleaved the mixed plasmids with *Eco RI* endonuclease, treated them with ligase and then transformed *E. coli*. Next we isolated transformed bacteria that expressed the penicillin resistance coded for by the *S. aureus* plasmid as well as the tetracycline resistance of the *E. coli* plasmid. These doubly resistant cells were found to contain a new DNA species that had the molecular characteristics of the staphylococcal plasmid DNA as well as the characteristics of *pSC101*.

The replication and expression in *E. coli* of genes derived from an organism ordinarily quite unable to exchange genes with *E. coli* represented a breach in the barriers that normally separate biological species. The bulk of the genetic information expressed in the transformed bacteria defined it as *E. coli*, but the transformed cells also carried replicating DNA molecules that had molecular and biological characteristics derived from an unrelated species, *S. aureus*. The fact that the foreign genes were on a plasmid meant that they would be easy to isolate and purify in large quantities for further study. Moreover, there was a possibility that one might introduce genes into the easy-to-grow *E. coli* that specify a wide variety of metabolic or synthesizing functions (such as photosynthesis or antibiotic production) and that are indigenous to other biological classes. Potentially the *pSC101* plasmid and the molecular-cloning procedure could serve to introduce DNA molecules from complex higher organisms into bacterial hosts, making it possible to apply relatively simple bacterial genetic and biochemical techniques to the study of animal-cell genes.

Could animal-cell genes in fact be introduced into bacteria, and would they replicate there? Boyer, Chang, Helling and I, together with Morrow and Goodman, immediately undertook to find out. We picked certain genes that had been well studied and characterized and were available, purified, in quantity: the genes that code for a precursor of the ribosomes (the structure on which proteins are synthesized) in the toad *Xenopus laevis*. The genes had properties that would enable us to identify them if we succeeded in getting them to propagate in bacteria. The toad DNA was suitable for another reason: although we would be constructing a novel biological combination containing genes from both animal cells and bacteria, we and others expected that no hazard would result from transplanting the highly purified ribosomal genes of a toad.

Unlike the foreign DNA's of our earlier experiments, the toad genes did not express traits (such as antibiotic resistance) that could help us to select bacteria carrying plasmid chimeras. The tetracycline resistance conferred by *pSC101* would make it possible to select transformed clones, however, and we could then proceed to examine the DNA isolated from such clones to see if any clones contained a foreign DNA having the molecular properties of toad ribosomal DNA. The endonuclease-generated fragments of toad ribosomal DNA have characteristic sizes and base compositions; DNA from the transformed cells could be tested for those characteristics. The genes propagated in bacteria could also be tested for nucleotide-sequence homology with DNA isolated directly from the toad.

When we did the experiment and analyzed the resulting transformed cells, we found that the animal-cell genes were indeed reproducing themselves in generation after generation of bacteria by means of the plasmid's replication functions. In addition, the nucleotide sequences of the toad DNA were being transcribed into an RNA product in the bacterial cells.

Within a very few months after the first DNA-cloning experiments the procedure was being used in a number of laboratories to clone bacterial and animal-cell DNA from a variety of sources. Soon two plasmids other than *pSC101* were discovered that have a single *Eco RI* cleavage site at a location that does not interfere with essential genes. One of these plasmids is present in many copies in the bacterial cell, making it possible to "amplify," or multiply many times, any DNA fragments linked to it. Investigators at the University of Edinburgh and at Stanford went on to develop mutants of the virus lambda (which ordinarily infects *E. coli*) that made the virus too an effective cloning vehicle. Other restriction endonucleases were discovered that also make cohesive termini but that cleave DNA at different sites from the *Eco RI* enzymes, so that chromosomes can now be taken apart and put together in various ways.

The investigative possibilities of DNA cloning are already being explored intensively. Some workers have isolated from complex chromosomes certain regions that are implicated in particular functions such as replication. Others are making plasmids to order with specific properties that should clarify aspects of extrachromosomal-DNA biology that have been hard to study. The organization of complex chromosomes, such as those of the fruit fly *Drosophila*, is being studied by cloning the animal genes in bacteria. Within the past few months methods have been developed for selectively cloning specific genes of higher organisms through the use of radioactively labeled RNA probes. Instead of purifying the genes to be studied before introducing them into bacteria, one can transform bacteria with a heterogeneous population of animal-cell DNA and then isolate those genes that produce a particular species of RNA. It is also possible to isolate groups of genes that are expressed concurrently at a particular stage in the animal's development.

The potential seems to be even broader. Gene manipulation opens the pros-

pect of constructing bacterial cells, which can be grown easily and inexpensively, that will synthesize a variety of biologically produced substances such as antibiotics and hormones, or enzymes that can convert sunlight directly into food substances or usable energy. Perhaps it even provides an experimental basis for introducing new genetic information into plant or animal cells.

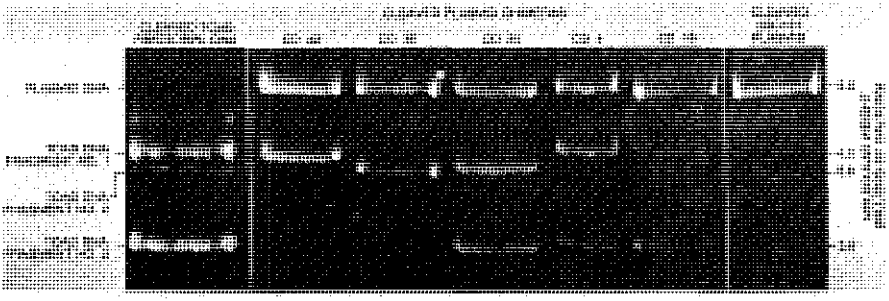
It has been clear from the beginning of experimentation in molecular cloning that the construction of some kinds of novel gene combinations may have a potential for biological hazard, and the scientific community has moved quickly to make certain that research in genetic manipulation would not endanger the public. For a time after our initial experiments the *pSC101* plasmid was the only vehicle known to be suitable for cloning foreign DNA in *E. coli*, and our colleagues asked for supplies with which to pursue studies we knew were of major scientific and medical importance. Investigators normally facilitate the free exchange of bacteria and other experimental strains they have isolated or developed, but Chang and I were concerned that manipulation of certain genes could give rise to novel organisms whose infectious properties and ecological effects could not be predicted. In agreeing to provide the plasmid we therefore asked for assurance that our colleagues would neither introduce tumor viruses into bacteria nor create antibiotic-resistance combinations that were not already present in nature; we also asked the recipients not to send the plasmid on to other laboratories, so that we could keep track of its distribution.

When still other cloning vehicles were discovered, it became apparent that a more general mechanism for ensuring experimental safety in gene-manipulation research was advisable. The groundwork for such control had been established earlier: the National Academy of Sciences had been urged to consider the "possibility that potentially biohazardous consequences might result from widespread or injudicious use" of these techniques and had asked Paul Berg to form an advisory committee that would consider the issue. Berg too had been concerned about the potential hazards of certain kinds of experimentation for some years, and had himself decided to abandon plans to try to introduce genes from the tumor virus SV40 into bacteria because of the possible danger if the experiment were successful.

Berg brought together a number of investigators, including some who were then directly involved in molecular cloning, in the spring of 1974. In a report released in July and in a letter to leading professional journals the members of the committee expressed their "concern about the possible unfortunate consequences of indiscriminate application" of the techniques and formally asked all investigators to join them in voluntarily deferring two types of experiments (which had, as a matter of fact, been avoided by informal consensus up until that time). Experiments of Type I involved the construction of novel organisms containing combinations of toxin-producing capabilities or of antibiotic-resistance genes not found in nature. Type 2 experiments involved the introduction of DNA from tumor viruses or other animal viruses into bacteria; the committee noted that "such recombinant molecules might be more easily disseminated to bacterial populations in humans and other species, and might thus increase the incidence of cancer or other diseases."

The Academy committee was concerned largely because of our inability to assess the hazards of certain experiments accurately before the experiments were undertaken. Guidelines for safety had long been available in other areas of potentially hazardous research, such as studies involving known disease-causing bacteria and viruses, radioactive isotopes or toxic chemicals. Because of the newness of the microbial gene-manipulation methods, no such guidelines had yet been developed for work in this area, however; there was the possibility that potentially hazardous experiments might proceed before appropriate guidelines could be considered and implemented. We recognized that most work with the new methods did not and would not involve experiments of a hazardous nature but we recommended the deferral of Type I and Type II experiments until the hazards were more carefully assessed, until it was determined whether or not the work could be undertaken safely and until adequate safety precautions were available. The committee also proposed that an international meeting be held early in 1975 to consider the matter more fully.

Such a meeting was held in February at the Asilomar Conference Center near Pacific Grove, Calif. It brought together 86 American biologists and 53 investigators from 16 other countries, who spent three and a half days reviewing progress in the field of molecular cloning and formulating guidelines that would allow most types of new hereditary characteristics to be introduced into bacteria and

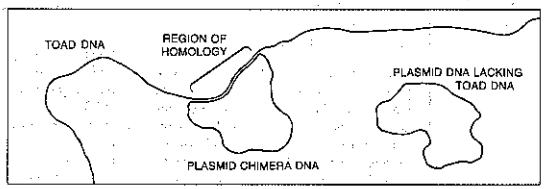


GEL ELECTROPHORESIS demonstrates the presence of toad DNA in chimeric plasmids. Fragments of DNA migrate through a gel at different rates under the influence of an electric current, depending on their size. Linear molecules of plasmid DNA (*right*) and the cleavage products of toad ribosomal DNA (*left*) therefore have characteristic sizes and migrate characteristic distances in a given time. The bands of DNA, visualized by a fluorescent dye, are photographed in ultraviolet. All five chimeric plasmids (*center*) contain a plasmid DNA molecule; in addition each chimera includes one or more fragments characteristic of original toad DNA.

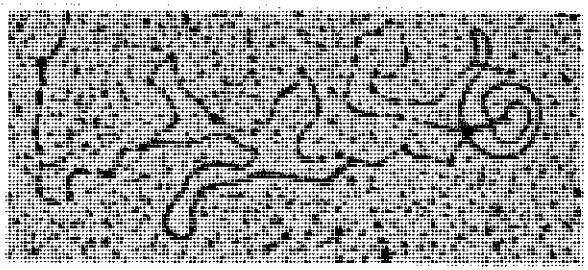
viruses safely. Invited nonscientists from the fields of law and ethics participated in the discussions and decisions at Asilomar, along with representatives of agencies that provide Federal funds for scientific research; the meetings were open to the press and were fully reported. The issues were complex and there were wide differences of opinion on many of them, but there was consensus on three major points. First, the newly developed cloning methods offer the prospect of dealing with a wide variety of important scientific and medical problems as well as other problems that trouble society, such as environmental pollution and food and energy shortages. Second, the accidental dissemination of certain novel biological combinations may present varying degrees of potential risk. The construction of such combinations should proceed only under a graded series of precautions, principally biological and physical barriers, adequate to prevent the escape of any hazardous organisms; the extent of the actual risk should be explored by experiments conducted under strict containment conditions. Third, some experiments are potentially too hazardous to be carried out for the present, even with the most careful containment. Future research and experience may show that many of the potential hazards considered at the meeting are less serious and less probable than we now suspect. Nevertheless, it was agreed that standards of protection should be high at the beginning and that they can be modified later if the assessment of risk changes.

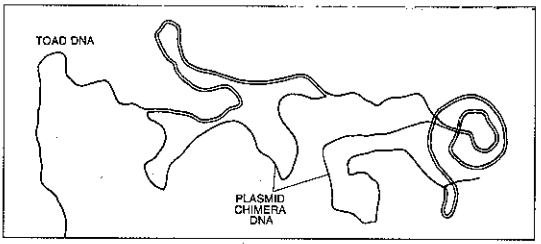
Physical containment barriers have long been used in the U.S. space-exploration program to minimize the possibility of contamination of the earth by extraterrestrial microbes. Containment procedures are also employed routinely to protect laboratory workers and the public from hazards associated with radioactive isotopes and toxic chemicals and in work with disease-causing bacteria and viruses. The Asilomar meeting formulated the additional concept of biological barriers, which involve fastidious cloning vehicles that are able to propagate only in specialized hosts and equally fastidious bacterial strains that are unable to live except under stringent laboratory conditions.

In the past the scientific community has commonly policed its own actions informally, responding to ethical concerns with self-imposed restraint. Usually, but not always, society at large has also considered the public well-being in determining how knowledge obtained by basic scientific research should be applied. Extensive public scrutiny and open discussion by scientists and nonscientists of the possible risks and benefits of a particular line of basic research has been rare, however, when (as in this case) the hazards in question are only potential and, for some experiments, even hypothetical. As this article is being written it is still too early to know what the long-range outcome of the public discussions initiated by scientists working in genetic manipulation will be. One can hope that the forthright approach and the rigorous standards that have been adopted for research in the cloning of recombinant DNA molecules will promote a sharper focus on other issues relevant to public and environmental safety.



HETERODUPLEX ANALYSIS identifies regions of a toad DNA (*black*) that have been incorporated in a chimeric plasmid DNA molecule. DNA isolated from toad eggs and the DNA of the chimera are denatured, that is, each natural double-strand molecule is split into two single strands of DNA, by alkali treatment. The toad and the chimeric DNA's are mixed together, and any complementary sequences are allowed to find each other. The toad DNA incorporated in the chimeras has nucleotide sequences that are complementary to sequences in the DNA taken directly from the animal source. Those homologous sequences anneal to form heteroduplex double-strand DNA that can be identified in electron micrographs.





PRESENCE OF TOAD DNA in two separate chimeric plasmid molecules is demonstrated by an electron micrograph made by John F. Morrow at the Stanford University School of Medicine. As is indicated in the drawing (*bottom*), there are DNA strands from two plasmids and a strand of toad DNA. The micrograph shows thickened regions of DNA where nucleotide sequences are homologous and two single strands have been annealed. The toad DNA in the chimeras codes for ribosomes, and the space between the two heteroduplex regions is compatible with the spacing of multiple ribosomal genes in toad DNA.

The Author

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APPENDIX 3

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APPENDIX 4

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Summary Statement of the Asilomar Conference on Recombinant DNA Molecules*

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I. INTRODUCTION AND GENERAL CONCLUSIONS

This meeting was organized to review scientific progress in research on recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. Impressive scientific achievements have already been made in this field and these techniques have a remarkable potential for furthering our understanding of fundamental biochemical processes in pro- and eukaryotic cells. The use of recombinant DNA methodology promises to revolutionize the practice of molecular biology. Although there has as yet been no practical application of the new techniques, there is every reason to believe that they will have significant practical utility in the future.

Of particular concern to the participants at the meeting was the issue of whether the pause in certain aspects of research in this area, called for by the Committee on Recombinant DNA Molecules of the National Academy of Sciences, U.S.A. in the letter published in July, 1974** should end; and, if so, how the scientific work could be undertaken with minimal risks to workers in laboratories, to the public at large, and to the animal and plant species sharing our ecosystems.

The new techniques, which permit combination of genetic information from very different organisms, place us in an area of biology with many unknowns. Even in the present, more limited conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. It is this ignorance that has compelled us to conclude that it would be wise to exercise considerable caution in performing this research. Nevertheless, the participants at the Conference agreed that most of the work on construction of recombinant DNA molecules should proceed provided that appropriate safeguards, principally biological and physical barriers adequate to contain the newly created organisms, are employed. Moreover, the standards of protection should be greater at the beginning and modified as improvements in the methodology occur and assessments of the risks change. Furthermore, it was agreed that there are certain experiments in which the potential risks are of such a serious nature that they ought not to be done with presently available containment facilities. In the longer term, serious problems may arise in the large scale application of this methodology in industry, medicine, and agriculture. But it was also recognized that future research and experience may show that many of the potential biohazards are less serious and/or less probable than we now suspect.

II. PRINCIPLES GUIDING THE RECOMMENDATIONS AND CONCLUSIONS

Although our assessments of the risks involved with each of the various lines of research on recombinant DNA molecules may differ, few, if any, believe that this methodology is free from any risk. Reasonable principles for dealing with these potential risks are: (*i*) that containment be made an essential consideration in the experimental design and, (*ii*) that the effectiveness of the containment should match, as closely as possible, the estimated risk. Consequently, whatever scale of risks is agreed upon, there should be a commensurate scale of containment. Estimating the risks will be difficult and intuitive at first but this will improve as we acquire additional knowledge; at each stage we shall have to match the potential risk with an appropriate level of containment. Experiments requiring large scale operations would seem to be riskier than equivalent experiments done on a small scale and, therefore, require more stringent containment procedures. The use of cloning vehicles or vectors (plasmids, phages) and bacterial hosts with a restricted capacity to multiply outside of the laboratory would reduce the potential biohazard of a particular experiment. Thus, the ways in which potential biohazards and different levels of containment are matched may vary from time to time, particularly as the containment technology is improved. The means for assessing and balancing risks with appropriate levels of containment will need to be reexamined from time to time. Hopefully, through both formal and informal channels of information within and between the nations of the world, the way in which potential biohazards and levels of containment are matched would be consistent.

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* Summary statement of the report submitted to the Assembly of Life Sciences of the National Academy of Sciences and approved by its Executive Committee on 20 May 1975.

Requests for reprints should be addressed to: Division of Medical Sciences, Assembly of Life Sciences, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

** Report of Committee on Recombinant DNA Molecules: "Potential Biohazards of Recombinant DNA Molecules," *Proc. Nat. Acad. Sci. USA* 71, 2593–2594, 1974.

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Containment of potentially biohazardous agents can be achieved in several ways. The most significant contribution to limiting the spread of the recombinant DNAs is the use of biological barriers. These barriers are of two types: (i) fastidious bacterial hosts unable to survive in natural environments, and (ii) nontransmissible and equally fastidious vectors (plasmids, bacteriophages, or other viruses) able to grow only in specified hosts. Physical containment, exemplified by the use of suitable hoods, or where applicable, limited access or negative pressure laboratories, provides an additional factor of safety. Particularly important is strict adherence to good microbiological practices which, to a large measure can limit the escape of organisms from the experimental situation, and thereby increase the safety of the operation. Consequently, education and training of all personnel involved in the experiments is essential to the effectiveness of all containment measures. In practice, these different means of containment will complement one another and documented substantial improvements in the ability to restrict the growth of bacterial hosts and vectors could permit modifications of the complementary physical containment requirements.

Stringent physical containment and rigorous laboratory procedures can reduce but not eliminate the possibility of spreading potentially hazardous agents. Therefore, investigators relying upon "disarmed" hosts and vectors for additional safety must rigorously test the effectiveness of these agents before accepting their validity as biological barriers.

III. RECOMMENDATIONS FOR MATCHING TYPES OF CONTAINMENT WITH TYPES OF EXPERIMENTS

No classification of experiments as to risk and no set of containment procedures can anticipate all situations. Given our present uncertainties about the hazards, the parameters proposed here are broadly conceived and meant to provide provisional guidelines for investigators and agencies concerned with research on recombinant DNAs. However, each investigator bears a responsibility for determining whether, in his particular case, special circumstances warrant a higher level of containment than is suggested here.

A. Types of containment

1. Minimal Risk. This type of containment is intended for experiments in which the biohazards may be accurately assessed and are expected to be minimal. Such containment can be achieved by following the operating procedures recommended for clinical microbiological laboratories. Essential features of such facilities are no drinking, eating, or smoking in the laboratory, wearing laboratory coats in the work area, the use of cotton-plugged pipettes or preferably mechanical pipetting devices, and prompt disinfection of contaminated materials.

2. Low Risk. This level of containment is appropriate for experiments which generate novel biotypes but where the available information indicates that the recombinant DNA cannot alter appreciably the ecological behavior of the recipient species, increase significantly its pathogenicity, or prevent effective treatment of any resulting infections. The key features of this containment (in addition to the minimal procedures mentioned above) are a prohibition on mouth pipetting, access limited to laboratory personnel, and the use of biological safety cabinets for procedures likely to produce aerosols (e.g., blending and sonication). Though existing vectors may be used in conjunction with low risk procedures, safer vectors and hosts should be adopted as they become available.

3. Moderate Risk. Such containment facilities are intended for experiments in which there is a probability of generating an agent with a significant potential for pathogenicity or ecological disruption. The principle features of this level of containment, in addition to those of the two preceding classes, are that transfer operations should be carried out in biological safety cabinets (e.g., laminar flow hoods), gloves should be worn during the handling of infectious materials, vacuum lines must be protected by filters, and negative pressure should be maintained in the limited access laboratories. Moreover, experiments posing a moderate risk must be done only with vectors and hosts that have an appreciably impaired capacity to multiply outside of the laboratory.

4. High Risk. This level of containment is intended for experiments in which the potential for ecological disruption or pathogenicity of the modified organism could be severe and thereby pose a serious biohazard to laboratory personnel or the public. The main features of this type of facility, which was designed to contain highly infectious microbiological agents, are its isolation from other areas by air locks, a negative pressure environment, a requirement for clothing changes and showers for entering personnel, and laboratories fitted with treatment systems to inactivate or remove biological agents that may be contaminants in exhaust air and liquid and solid wastes. All persons occupying these areas should wear protective laboratory clothing and shower at each exit from the containment facility. The handling of agents should be confined to biological safety cabinets in which the exhaust air is incinerated or passed through Hepa filters. High risk containment includes, in addition to the physical and procedural features described above, the use of rigorously tested vectors and hosts whose growth can be confined to the laboratory.

B. Types of experiments

Accurate estimates of the risks associated with different types of experiments are difficult to obtain because of our ignorance of the probability that the anticipated dangers will manifest themselves. Nevertheless, experiments involving the construction and propagation of recombinant DNA molecules using DNAs from (i) prokaryotes, bacteriophages, and other plasmids, (ii) animal viruses, and (iii) eukaryotes have been characterized as minimal, low, moderate and high risks to guide investigators in their choice of the appropriate containment. These designations should be viewed as interim assignments which will need to be revised upward or downward in the light of future experience.

The recombinant DNA molecules themselves, as distinct from cells carrying them, may be infectious to bacteria or higher organisms. DNA preparations from these experiments, particularly in large quantities, should be chemically inactivated before disposal.

1. Prokaryotes, Bacteriophages, and Bacterial Plasmids. Where the construction of recombinant DNA molecules and their propagation involves prokaryotic agents that are known to exchange genetic information naturally, the experiments

can be performed in minimal risk containment facilities. Where such experiments pose a potential hazard, more stringent containment may be warranted.

Experiments involving the creation and propagation of recombinant DNA molecules from DNAs of species that ordinarily do not exchange genetic information, generate novel biotypes. Because such experiments may pose biohazards greater than those associated with the original organisms, they should be performed, at least, in low risk containment facilities. If the experiments involve either pathogenic organisms or genetic determinants that may increase the pathogenicity of the recipient species, or if the transferred DNA can confer upon the recipient organisms new metabolic activities not native to these species and thereby modify its relationship with the environment, then moderate or high risk containment should be used.

Experiments extending the range of resistance of established human pathogens to therapeutically useful antibiotics or disinfectants should be undertaken only under moderate or high risk containment, depending upon the virulence of the organism involved.

2. Animal Viruses. Experiments involving linkage of viral genomes or genome segments to prokaryotic vectors and their propagation in prokaryotic cells should be performed only with vector–host systems having demonstrably restricted growth capabilities outside the laboratory and with moderate risk containment facilities. Rigorously purified and characterized segments of non-oncogenic viral genomes or of the demonstrably non-transforming regions of oncogenic viral DNAs can be attached to presently existing vectors and propagated in moderate risk containment facilities; as safer vector–host systems become available such experiments may be performed in low risk facilities.

Experiments designed to introduce or propagate DNA from non-viral or other low risk agents in animal cells should use only low risk animal DNAs as vectors (e.g., viral, mitochondrial) and manipulations should be confined to moderate risk containment facilities.

3. Eukaryotes. The risks associated with joining random fragments of eukaryote DNA to prokaryotic DNA vectors and the propagation of these recombinant DNAs in prokaryotic hosts are the most difficult to assess.

A priori, the DNA from warm-blooded vertebrates is more likely to contain cryptic viral genomes potentially pathogenic for man than is the DNA from other eukaryotes. Consequently, attempts to clone segments of DNA from such animal and particularly primate genomes should be performed only with vector–host systems having demonstrably restricted growth capabilities outside the laboratory and in a moderate risk containment facility. Until cloned segments of warm-blooded vertebrate DNA are completely characterized, they should continue to be maintained in the most restricted vector–host system in moderate risk containment laboratories; when such cloned segments are characterized, they may be propagated as suggested above for purified segments of virus genomes.

Unless the organism makes a product known to be dangerous (e.g., toxin, virus), recombinant DNAs from cold-blooded vertebrates and all other lower eukaryotes can be constructed and propagated with the safest vector–host system available in low risk containment facilities.

Purified DNA from any source that performs known functions and can be judged to be non-toxic, may be cloned with currently available vectors in low risk containment facilities. (Toxic here includes potentially oncogenic products or substances that might perturb normal metabolism if produced in an animal or plant by a resident microorganism.)

4. Experiments to be Deferred. There are feasible experiments which present such serious dangers that their performance should not be undertaken at this time with the currently available vector–host systems and the presently available containment capability. These include the cloning of recombinant DNAs derived from highly pathogenic organisms (i.e., Class III, IV, and V etiologic agents as classified by the United States Department of Health, Education and Welfare), DNA containing toxin genes, and large scale experiments (more than 10 liters of culture) using recombinant DNAs that are able to make products potentially harmful to man, animals, or plants.

IV. IMPLEMENTATION

In many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard.††,‡‡ Until these are established, we urge individual scientists to use the proposals in this document as a guide. In addition, there are some recommendations which could be immediately and directly implemented by the scientific community.

A. Development of safer vectors and hosts

An important and encouraging accomplishment of the meeting was the realization that special bacteria and vectors which have a restricted capacity to multiply outside the laboratory can be constructed genetically, and that the use of these organisms could enhance the safety of recombinant DNA experiments by many orders of magnitude. Experiments along these lines are presently in progress and in the near future, variants of λ bacteriophage, non-transmissible plasmids, and special strains of *Escherichia coli* will become available. All of these vectors could reduce the potential biohazards by very large factors and improve the methodology as well. Other vector–host systems, particularly modified strains of *Bacillus subtilis* and their relevant bacteriophages and plasmids, may also be useful for particular purposes. Quite possibly safe and suitable vectors may be found for eukaryotic hosts such as yeast and readily cultured plant and animal cells. There is likely to be a continuous development in this area and the participants at the meeting agreed that improved vector–host systems which reduce the biohazards of recombinant DNA research will be made freely available to all interested investigators.

B. Laboratory procedures

It is the clear responsibility of the principal investigator to inform the staff of the laboratory of the potential hazards of

†† Advisory Board for the Research Councils, "Report of the Working Party on the Experimental Manipulation of the Genetic Composition of Micro-Organisms. Presented to Parliament by the Secretary of State for Education and Science by Command of Her Majesty, January 1975." London: Her Majesty's Stationery Office, 1975, 23pp.

‡‡ National Institutes of Health Recombinant DNA Molecule Program Advisory Committee.

such experiments before they are initiated. Free and open discussion is necessary so that each individual participating in the experiment fully understands the nature of the experiment and any risk that might be involved. All workers must be properly trained in the containment procedures that are designed to control the hazard, including emergency actions in the event of a hazard. It is also recommended that appropriate health surveillance of all personnel, including serological monitoring, be conducted periodically.

C. Education and reassessment

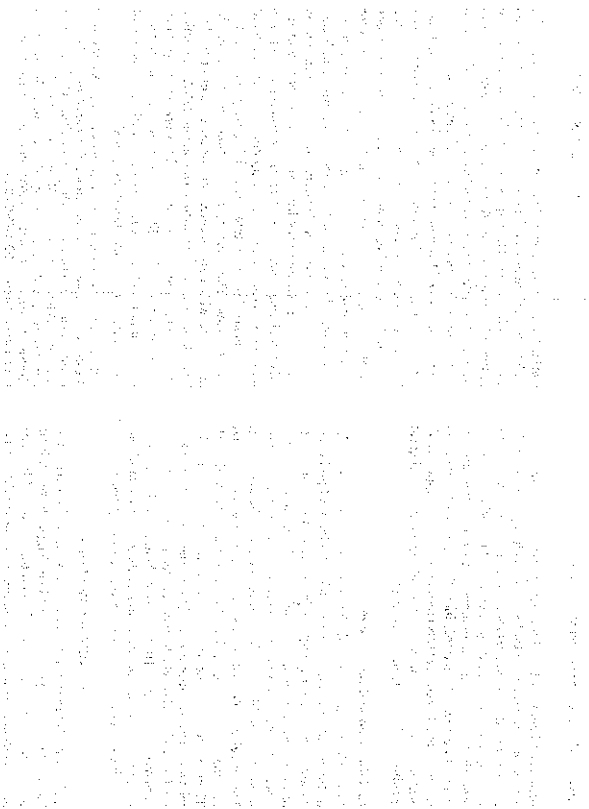
Research in this area will develop very quickly and the methods will be applied to many different biological problems. At any given time it is impossible to foresee the entire range of all potential experiments and make judgments on them. Therefore, it is essential to undertake a continuing reassessment of the problems in the light of new scientific knowledge. This could be achieved by a series of annual workshops and meetings, some of which should be at the international level. There should also be courses to train individuals in the relevant methods since it is likely that the work will be taken up by laboratories which may not have had extensive experience in this area. High priority should also be given to research that could improve and evaluate the containment effectiveness of new and existing vector–host systems.

V. NEW KNOWLEDGE

This document represents our first assessment of the potential biohazards in the light of current knowledge. However, little is known about the survival of laboratory strains of bacteria and bacteriophages in different ecological niches in the outside world. Even less is known about whether recombinant DNA molecules will enhance or depress the survival of their vectors and hosts in nature. These questions are fundamental to the testing of any new organism that may be constructed. Research in this area needs to be undertaken and should be given high priority. In general, however, molecular biologists who may construct DNA recombinant molecules do not undertake these experiments and it will be necessary to facilitate collaborative research between them and groups skilled in the study of bacterial infection or ecological microbiology. Work should also be undertaken which would enable us to monitor the escape or dissemination of cloning vehicles and their hosts.

Nothing is known about the potential infectivity in higher organisms of phages or bacteria containing segments of eukaryotic DNA and very little about the infectivity of the DNA molecules themselves. Genetic transformation of bacteria does occur in animals, suggesting that recombinant DNA molecules can retain their biological potency in this environment. There are many questions in this area, the answers to which are essential for our assessment of the biohazards of experiments with recombinant DNA molecules. It will be necessary to ensure that this work will be planned and carried out; and it will be particularly important to have this information before large scale applications of the use of recombinant DNA molecules is attempted.

The work of the committee was assisted by the National Academy of Sciences–National Research Council Staff: Artemis P. Simopoulos (Executive Secretary) and Elena O. Nightingale (Resident Fellow), Division of Medical Sciences, Assembly of Life Sciences, and supported by the National Institutes of Health (Contract NO1-OD-5-2103) and the National Science Foundation (Grant GBMS75-05293).



APPENDIX 5

THE SECRETARY OF HEALTH, EDUCATION, AND WELFARE,
Washington, D.C.

CHARTER

RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

Purpose

In accordance with Section 301 of the Public Health Service Act (42 U.S.C. 241), the Secretary of Health, Education, and Welfare is directed to conduct research, investigations, experiments, demonstrations, and studies relating to the causes, diagnosis, treatment, control and prevention of physical diseases and impairments of man. In carrying out this mandate, exploration of the genetics of microbial agents and of animal cells hy use of the technology of study of DNA (deoxyribonucleic acid) recombinants offers tremendous promise of uncovering basic aspects of health and disease, and is appropriate for support by the National Institutes of Health. However, the use of this technology has various possible hazards because new types of organisms, some potentially pathogenic, can be introduced into the environment if there are no effective controls. The technology is also capahle of producing microbial organisms which can be useful or harmful to agriculture or industry, and thus secondarily affect human health. The goal of the Committee is to investigate the current state of knowledge and technology regarding DNA recombinants, their survival in nature, and transferability to other organisms; to recommend programs of research to assess the possibility of spread of specific DNA recombinants and the possible hazards to public health and to the environment; and to recommend guidelines on the basis of the research results. This Committee is a technical committee, established to look at a specific problem.

Authority

42 U.S.C. 217a. This Committee is established in accordance with, and is governed by, the provisions of Public Law 92–463, which sets forth standards for the formation and use of advisory committees.

Function

The Recombinant DNA Molecule Program Advisory Committee shall advise the Secretary, Health, Education, and Welfare, the Assistant Secretary for Health, Department of Health, Education, and Welfare, and the Director, National Institutes of Health, concerning a program for the evaluation of potential biological and ecological hazards of DNA recombinants of various types, for developing procedures which will minimize the spread of such molecules within human and other populations, and for devising guidelines to be followed by investigators working with potentially hazardous recombinants. The Committee may recommend special workshops for exploration of particualr problems.

Structure

The Committee shall consist of twelve members, including the Chairman. Members shall be selected by the Secretary, or his designee, from authorities knowledgeable in the fields of molecular biology, virology, genetics and microbiology.

Members shall be invited to serve for overlapping 4-year terms; terms of more than two years are contingent upon the renewal of the Committee by appropriate action prior to its termination.

Management and staff services shall he provided by the Division of Research Grauts, Office of the Associate Director for Scientific Review, who shall designate an Executive Secretary.

Meetings

Meetings shall be held approximately four times a year at the call of the Chairman, with the advance approval of a government official who also approves the agenda. A government official is present at all meetings.

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Meetings shall be open to the public except as determined otherwise by the Secretary; notice of all meetings shall be given to the public.

Meetings shall be conducted, and records of the proceedings kept, as required by applicable laws and Departmental regulations.

Recombinant DNA Molecule Program Advisory Committee

CHAIRMAN

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VICE CHAIRMAN

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APPENDIX 6

ADVISORY COMMITTEE TO THE DIRECTOR, NIH

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CONTENTS

	Page
Participants	2
Public Interest Groups Notified	5
Opening Remarks, Dr. Fredrickson, Chairman	6
Safeguards for Recombinant DNA Research, Dr. Berg	11
Development of NIH Policy, Dr. Stetten	27
Proposed Guidelines: Summary & Review, Dr. Singer	34
Viewpoints of the Drafting Committee, Drs. Hogness & Curtiss	60
Committee Discussion	70
Physical Containment, Dr. Barkley	77
Committee Discussion	87
Questions from Public	104
Public Statements to Committee	107
Adjournment, First Day	152
Further Public Comments, Second Day	152
Summary of Major Issues, Dr. Stetten	176
Committee Discussion	180
Adjournment, Second Day	208
APPENDIXES	209

A: Communication from Boston Area Recombinant DNA Group

B: "The Detrick Experience as a Guide to the Probable Efficacy of P4 Microbiological Containment Facilities for Studies on Microbial Recombinant DNA Molecules," A.G. Wedum, Jan. 20, 1976

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APPENDIX 7

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APPENDIX 8

federal register

WEDNESDAY, JULY 7, 1976

PART II:

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

■

RECOMBINANT DNA RESEARCH

Guidelines

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

RECOMBINANT DNA RESEARCH

Guidelines

On Wednesday, June 23, 1976, the Director, National Institutes of Health, with the concurrence of the Secretary of Health, Education, and Welfare, and the Assistant Secretary for Health, issued guidelines that will govern the conduct of NIH supported research on recombinant DNA molecules. The NIH is also undertaking an environmental impact assessment of these guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969.

The NIH Guidelines establish carefully controlled conditions for the conduct of experiments involving the production of such molecules and their insertion into organisms such as bacteria. These Guidelines replace the recommendations contained in the 1975 *Summary Statement of the Asilomar Conference on Recombinant DNA Molecules*. The latter would have permited research under less strict conditions than the NIH Guidelines.

The chronology leading to the present Guidelines is described in detail in the NIH Director's decision document that follows. In summary, scientists engaged in this research called, in 1974, for a moratorium on certain kinds of experiments until an international meeting could be convened to consider the potential hazards of recombinant DNA molecules. They also called upon the NIH to establish a committee to provide advice on recombinant DNA technology.

The international meeting was held at the Asilomar Conference Center, Pacific Grove, California, in February 1975. The consensus of this meeting was that certain experiments should not be done at the present time, but that most of the work on construction of recombinant DNA molecules should proceed with appropriate physical and biological barriers. The Asilomar Conference report also made interim assignments of the potential risks associated with different types of experiments. The NIH then assumed responsibility for translating the broadly based Asilomar recommendations into detailed guidelines for research.

The decision by the NIH Director on these Guidelines was reached after extensive scientific and public airing of the issues during the sixteen months which have elapsed since the Asilomar Conference. The issues were discussed at public meetings of the Recombinant DNA Molecule Program Advisory Committee (Recombinant Advisory Committee) and the Advisory Committee to the NIH Director. The Recombinant Advisory Committee extensively debated three different versions of the Guidelines during this period.

The Advisory Committee to the NIH Director, augmented with consultants representing law, ethics, consumer affairs and the environment, was asked to advise as to whether the proposed Guidelines balanced responsibility to protect the public with the potential benefits through the pursuit of new knowledge. The many different points of view expressed at this meeting were taken into consideration in the decision.

The NIH recognizes a special obligation to disseminate information on these guidelines as widely as possible. Accordingly, the Guidelines will be sent to all of the approximately 25,000 NIH grantees and contractors. Major professional societies which represent scientists working in this area will also be asked to endorse the Guidelines. The Guidelines will be sent to medical and scientific journals and editors of these journals will be asked to request that investigators include a description of the physical and biological containment procedures used in any recombinant research they report on. International health and scientific organizations will also receive copies of the guidelines for their review.

Filing of an environmental impact statement will provide opportunity for the scientific community, Federal, State and local agencies and the general public to address the potential benefits and hazards of this research area. In order for there to be further opportunity for public comment and consideration, these guidelines are being offered for general comment in the FEDERAL REGISTER. It must be clearly understood by the reader that the material that follows is *not* proposed rulemaking in the technical sense, but is a document on which early public comment and participation is invited.

Please address any comments on these draft policies and procedures to the Director, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014. All comments should be received by November 1, 1976.

Additional copies of this notice are available from the Acting Director, Office of Recombinant DNA Activities, National Institute of General Medical Sciences, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014.

DONALD S. FREDRICKSON,
Director,
NIH National Institutes of Health.

JUNE 25, 1976.

DECISION OF THE DIRECTOR, NATIONAL INSTITUTES OF HEALTH TO RELEASE GUIDELINES FOR RESEARCH ON RECOMBINANT DNA MOLECULES

JUNE 23, 1976.

INTRODUCTION

I. General Policy Considerations.
A. Science Policy.
B. Implementation Within the NIH.
C. Implementation Beyond the Purview of NIH.
D. Environmental Policy.
II. Methods of Containment (See *Guidelines* II).
III. Prohibited Experiments (See *Guidelines* III, A).
IV. Permissible Experiments: *E. Coli* K-12 Host-Vector Systems (See *Guidelines* III, B, 1).
V. Classification of Experiments Using the *E. Coli* K-12 Containment Systems (See *Guidelines* III, B, 2).
VI. Classification of Experiments Using Containment Systems Other than *E. Coli* K-12 (See *Guidelines* III, B, 4).
VII. Roles and Responsibilities (See *Guidelines* IV).

INTRODUCTION

Today, with the concurrence of the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health, I am releasing guidelines that will govern the conduct of NIH-supported research on recombinant DNA molecules (molecules resulting from the recombination in cell-free systems of segments of deoxyribonucleic acid, the material that determines the hereditary characteristics of all known cells). These guidelines establish carefully controlled conditions for the conduct of experiments involving the insertion of such recombinant genes into organisms, such as bacteria. The chronology leading to the present guidelines and the decision to release them are outlined in this introduction.

In addition to developing these guidelines, NIH has undertaken an environmental impact assessment of these guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969 (NEPA). The guidelines are being released prior to completion of this assessment. They will replace the current Asilomar guidelines, discussed below, which in many instances allow research to proceed under less strict conditions. Because the NIH guidelines will afford a greater degree of scrutiny and protection, they are being released today, and will be effective while the environmental impact assessment is under way.

Recombinant DNA research brings to the fore certain problems in assessing the potential impact of basic science on society as a whole, including the manner of providing public participation in those assessments. The field of research involved is a rapidly moving one, at the leading edge of biological science. The experiments are extremely technical and complex. Molecular biologists active in this research have means of keeping informed, but even they may fail to keep abreast of the newest developments. It is not surprising that scientists in other fields and the general public have difficulty in understanding advances in recombinant DNA research. Yet public awareness and understanding of this line of investigation is vital.

It was the scientists engaged in recombinant DNA research who called for a moratorium on certain kinds of experiments in order to assess the risks and devise appropriate guidelines. The capability to perform DNA recombinations, and the potential hazards, had become apparent at the Gordon Research Conference on Nucleic Acids in July 1973. Those in attendance voted to send an open letter to Dr. Philip Handler, President of the National Academy of Sciences, and to Dr. John R. Hogness, President of the Institute of Medicine, NAS. The letter, appearing in *Science 181*, 1114, (1973), suggested "that the Academies

[sic] establish a study committee to consider this problem and to recommend specific actions or guidelines, should that seem appropriate."

In response, NAS formed a committee, and its members published another letter in *Science 185*, 303, (1974). Entitled "Potential Biohazards of Recombinant DNA Molecules," the letter proposed:

> First, and most important, that until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring * * * [certain] experiments * * *.
>
> Second, plans to link fragments of animal DNAs to bacterial plasmid DNA or bacteriophage DNA should be carefully weighted * * *.
>
> Third, the Director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (i) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (ii) developing procedures which will minimize the spread of such molecules within human and other populations; and (iii) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.
>
> Fourth, an international meeting of involved scientists from all over the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

On October 7, 1974, the NIH Recombinant DNA Molecule Program Advisory Committee (hereafter "Recombinant Advisory Committee") was established to advise the Secretary, HSW, the Assistant Secretary for Health, and the Director, NIH, "concerning a program for developing procedures which will minimize the spread of such molecules within human and other populations, and for devising guidelines to be followed by investigators working with potentially hazardous recombinants."

The international meeting proposed in the *Science* article (*185*, 303, 1974) was held in February 1975 at the Asilomar Conference Center, Pacific Grove, California. It was sponsored by the National Academy of Sciences and supported by the National Institutes of Health and the National Science Foundation. One hundred and fifty people attended, including 52 foreign scientists from 15 countries, 16 representatives of the press, and 4 attorneys.

The conference reviewed progress in research on recombinant DNA molecules and discussed ways to deal with the potential biohazards of the work. Participants felt that experiments on construction of recombinant DNA molecules should proceed, provided that appropriate biological and physical containment is utilized. The conference made recommendations for matching levels of containment with levels of possible hazard for various types of experiments. Certain experiments were judged to pose such serious potential dangers that the conference recommended against their being conducted at the present time.

A report on the conference was submitted to the Assembly of Life Sciences, National Research Council, NAS, and approved by its Executive Committee on May 20, 1975. A summary statement of the report was published in *Science 188*, 991 (1975), *Nature 225*, 442, (1975), and the *Proceedings of the National Academy of Sciences 72*, 1981, (1975). The report noted that "in many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard. Until these are established, we urge individual scientists to use the proposals in this document as a guide."

The NIH Recombinant Advisory Committee held its first meeting in San Francisco immediately after the Asilomar conference. It proposed that NIH use the recommendations of the Asilomar conference as guidelines for research until the committee had an opportunity to elaborate more specific guidelines, and that NIH establish a newsletter for informal distribution of information. NIH accepted these recommendations.

At the second meeting, held on May 12–13, 1975, in Bethesda, Maryland, the committee received a report on biohazard-containment facilities in the United States and reviewed a proposed NIH contract program for the construction and testing of microorganisms that would have very limited ability to survive in natural environments and would thereby limit the potential hazards. A subcommittee chaired by Dr. David Hogness was appointed to draft guidelines for research involving recombinant DNA molecules, to be discussed at the next meeting.

The NIH committee, beginning with the draft guidelines prepared by the Hogness subcommittee, prepared proposed guidelines for research with recombinant DNA molecules at its third meeting, held on July 18–19, 1975, in Woods Hole, Massachusetts.

Following this meeting, many letters were received which were critical of the guidelines. The majority of critics felt that they were too lax, others that they were too strict. All letters were reviewed by the committee, and a new subcommittee, chaired by Dr. Elizabeth Kutter, was appointed to revise the guidelines.

A fourth committee meeting was held on December 4–5, 1975, in La Jolla, California. For this meeting a "variorum edition" had been prepared, comparing line-for-line the Hogness, Woods Hole, and Kutter guidelines. The committee reviewed these, voting item-by-item for their preference among the three variations and, in many cases, adding new material. The result was the "Proposed Guidelines for Research Involving Recombinant DNA Molecules, "which were referred to the Director, NIH, for a final decision in December 1975.

As Director of the National Institutes of Health, I called a special meeting of the Advisory Committee to the Director to review these proposed guidelines. The meeting was held at NIH, Bethesda, on February 9–10, 1976. The Advisory Committee is charged to advise the Director, NIH, on matters relating to the broad setting—scientific, technological, and socioeconomic—in which the continuing development of the biomedical sciences, education for the health professions, and biomedical communications must take place, and to advise on their implications for NIH policy, program development, resource allocation, and administration. The members of the committee are knowledgeable in the fields of basic and clinical biomedical sciences, the social sciences, physical sciences, research, education, and communications. In addition to current members of the committee, I invited a number of former committee members as well as other scientific and public representatives to participate in the special February session.

The purpose of the meeting was to seek the committee's advice on the guidelines proposed by the Recombinant Advisory Committee. The Advisory Committee to the Director was asked to determine whether, in their judgment, the guidelines balanced scientific responsibility to the public with scientific freedom to pursue new knowledge.

Public responsibility weighs heavily in this genetic research area. The scientific community must have the public's confidence that the goals of this profoundly important research accord respect to important ethical, legal, and social values of our society. A key element in achieving and maintaining this public trust is for the scentific community to ensure an openness and candor in its proceedings. The meetings of the Director's Advisory Committee, the Asilomar group, and the Recombinant Advisory Committee have reflected the intent of science to be an open community in considering the conduct of recombinant DNA experiments. At the Director's Advisory Committee meeting, there was ample opportunity for comment and an airing of the issues, not only by the committee members but by public witnesses as well. All major points of view were broadly represented.

I have been reviewing the guidelines in light of the comments and suggestions made by participants at that meeting, as well as the written comments received afterward. As part of that review I asked the Recombinant Advisory Committee to consider at their meeting of April 1–2, 1976, a number of selected issues raised by the commentators. I have taken those issues and the response of the Recombinant Advisory Committee into account in arriving at my decision on the guidelines. An analysis of the issues and the basis for my decision follow.

I. GENERAL POLICY CONSIDERATIONS

A word of explanation might be interjected at this point as to the nature of the studies in question. Within the past decade, enzymes capable of breaking DNA strands at specific sites and of coupling the broken fragments in new combinations were discovered, thus making possible the insertion of foreign genes into viruses or certain cell particles (plas-

mids). These, in turn, can be used as vectors to introduce the foreign genes into bacteria or into cells of plants or animals in test tubes. Thus transplanted, the genes may impart their hereditary properties to new hosts. These cells can be isolated and cloned—that is, bred into a genetically homogeneous culture. In general, there are two potential uses for the clones so produced: as a tool for studying the transferred genes, and as a new useful agent, say for the production of a scarce hormone.

Recombinant DNA research offers great promise, particularly for improving the understanding and possibly the treatment of various diseases. There is also a potential risk—that microorganisms with transplanted genes may prove hazardous to man or other forms of life. Thus special provisions are necessary for their containment.

All commentators acknowledged the exemplary responsibility of the scientific community in dealing publicly with the potential risks in DNA recombinant research and in calling for a self-imposed moratorium on certain experiments in order to assess potential hazards and devise appropriate guidelines. Most commentators agreed that the process leading to the formulation of the proposed guidelines was a most responsible and responsive one. Suggestions by the commentators on broad policy considerations are presented below. They relate to the science policy aspects of the guidelines, the implementation of the guidelines for NIH grantees and contractors, and the scope and impact of the guidelines nationally and internationally.

A. Science policy considerations

Commentators were divided on how best to steer a course between stifling research through excessive regulation and allowing it to continue with sufficient controls. Several emphasized that the public must have assurance that the controls afford adequate protection against potential hazards. In the views of these commentators, the burden is on the scientific community to show that the danger is minimal and that the benefits are substantial and far outweigh the risks.

Opinion differed on whether the proposed guidelines were an appropriate response to the potential benefits and hazards. Several found the guidelines to so exaggerate safety procedures that inquiry would be unnecessarily retarded, while others found the guidelines weighted toward promoting research. The issue was how to strike a reasonable balance—in fact, a proper policy "bias"—between concerns to "go slow" and those to progress rapidly.

There was strong disagreement about the nature and level of the possible hazards of recombinant DNA research. Several commentators believed that the hazards posed were unique. In their view, the occurrence of an accident or the escape of a vector could initiate an irreversible process, with a potential for creating problems many times greater than those arising from the multitude of genetic recombinations that occur spontaneously in nature. These commentators stress the moral obligation on the part of the scientific community to do no harm.

Other commentators, however, found the guidelines to be adequate to the hazards posed. In their view, the guidelines struck an appropriate balance so that research could proceed cautiously. Still other commentators found the guidelines too onerous and restrictive in light of the potential benefits of this research for medicine, agriculture, and industry. Some felt that the guidelines are perhaps more stringent than necessary given the available evidence on the likelihood of hazards, but supported them as a compromise that would best serve the scientific community and the public at large. Many commentators urged that the guidelines be adopted as soon as possible to afford more specific direction to this research area.

I understand and appreciate the concerns of those who urge that this research proceed because of the benefits and of those who urge caution because of potential hazards. The guidelines issued today allow the research to go forward in a manner responsive and appropriate to hazards that may be realized in the future.

The object of these guidelines is to ensure that experimental DNA recombination will have no ill effects on those engaged in the work, on the general public, or on the environment. The essence of their construction is subdivision of potential experiments by class, decision as to which experiments should be permitted at present, and assignment to these of certain procedures for containment of recombinant organisms.

Containment is defined as physical and biological. Physical containment involves the isolation of the research by procedures which have evolved over many years of experience in laboratories studying infectious microorganisms. P1 containment—the first *physical* containment level—is that used in most routine bacteriology laboratories. P2 and P3 afford increasing isolation of the research from the environment. P4 represents the most extreme measures used for containing virulent pathogens, and permits no escape of contaminated air, wastes, or untreated materials. *Biological* containment is the use of vectors or hosts that are crippled by mutation so that the recombinant DNA is incapable of surviving under natural conditions.

The experiments now permitted under the guidelines involve no known additional hazard to the workers or the environment beyond the relatively low risk known to be associated with the source materials. The additional hazards are speculative and therefore not quantifiable. In a real sense they are considerably less certain than are the benefits now clearly derivable from the projected research.

For example, the ability to produce, through "molecular cloning," relatively large amounts of pure DNA from the chromosomes of any living organism will have a profound effect in many areas of biology. No other procedure, not even chemical synthesis, can provide pure material corresponding to particular genes. DNA "probes," prepared from the clones will yield precise evidence on the presence or absence, the organization, and the expression of genes in health and disease.

Potential medical advances were outlined by scientists active in this research area who were present at the meeting of the Director's Advisory Committee. Of enormous importance, for example, is the opportunity to explore the malfunctioning of cells in complicated diseases. Our ability to understand a variety of hereditary defects may be significantly enhanced, with amelioration of their expression a real possibility. There is the potential to elucidate mechanisms in certain cancers, particularly those that might be caused by viruses.

Instead of mere propagation of foreign DNA, the expression of the genes of one organism by the cell machinery of another may alter the new host and open opportunities for manipulating the biological properties of cells. In certain prokaryotes (organisms with a poorly developed nucleus, like bacteria), this exchange of genetic information occurs in nature. Such exchange explains, for instance, an important mechanism for the changing and spreading of resistance to antibiotics in bacteria. Beneficial effects of this mechanism might be the production of medically important compounds for the treatment and control of disease. Examples frequently cited are the production of insulin, growth hormone, specific antibodies, and clotting factors absent in victims of hemophilia.

Aside from the potential medical benefits, a whole host of other applications in science and technology have been envisioned. Examples are the large-scale production of enzymes for industrial use and the development of bacteria that could ingest and destroy oil spills in the sea. Potential benefits in agriculture include the enhancement of nitrogen fixation in certain plants, permitting increased food production.

While the projected research offers the possibility of many benefits, it must proceed only with assurance that potential hazards can be controlled or prevented. Some commentators are concerned that nature may maintain a barrier to the exchange of DNA between prokaryotes and eukaryotes (higher organisms, with a well-formed nucleus)—a barrier that can now be crossed by experimentalists. They further argue that expression of the foreign DNA may alter the host in unpredictable and undesirable ways. Conceivable harm could result if the altered host has a competitive advantage that would foster its survival in some niche within the ecosystem. Other commentators believe that the endless experiments in recombination of DNA which nature has conducted since the beginning of life on the earth, and which have accounted in part for the evolution of species, have most likely involved exchange of DNA between widely disparate species. They

argue that prokaryotes such as bacteria in the intestines of man do exchange DNA with this eukaryotic host and that the failure of the altered prokaryotes to be detected attests to a sharply limited capacity of such recombinants to survive. Thus nature, this argument runs, has already tested the probabilities of harmful recombination and any survivors of such are already in the ecosystem. The fact is that we do not know which of the above-stated propositions is corrected.

The international scientific community, as exemplified by the Asilomar conference and the deliberations attendant upon preparation of the present guidelines, has indicated a desire to proceed with research in a conservative manner. And most of the considerable public commentary on the subject, while urging caution, has also favored proceeding. Three European groups have independently arrived at the opinion that recombinant DNA research should proceed with caution. These are the Working Party on Experimental Manipulation of the Genetic Composition of Micro-Organisms, whose "Ashby Report" was presented to Parliament in the United Kingdom by the Secretary of State for Education and Science in January 1975; the Advisory Committee on Medical Research of the World Health Organization, which issued a press release in July 1975; and the European Molecular Biology Organization Standing Committee on Recombinant DNA, meeting in February 1976.

There is no means for a flat proscription of such research throughout the world community of science. There is also no need to attempt it. It is likely that the evaluation engendered in the preparation and application of these guidelines will lead to beneficial review of some of the containment practices in other work that is not technically defined as recombinant DNA research.

Recombinant DNA research with which these guidelines are concerned involves microorganisms such as bacteria or viruses or cells of higher organisms growing in tissue culture. It is extremely important for the public to be aware that this research is not directed to altering of genes in humans although some of the techniques developed in this research may have relevance if this is attempted in the future.

NIH recognizes its responsibility to conduct and support research designed to determine the extent to which certain potentially harmful effects from recombinant DNA molecules may occur. Among these are experiments, to be conducted under maximum containment, that explore the capability of foreign genes to alter the character of host or vector, rendering it harmful, as through the production of toxic products.

Given the general desire that no rare and unexpected event arising from this research shall cause irreversible damage, it is obvious that merely to establish conservative rules of conduct for one group of scientists is not enough. The precautions must be uniformly and unanimously observed. Second, there must be full and timely exchange of experiences so that guidelines can be altered on the basis of new knowledge. The guidelines must also be implemented in a manner that protects all concerned—the scientific workers most likely to encounter unexpected hazards and all forms of life within our biosphere. The responsibility of the scientists involved is as inescapable and extreme as is their opportunity to beneficially enrich our understanding.

B. Implementation considerations within the NIH

All the commentators had suggestions concerning the structure and function of decision making as it relates to the principal investigator, the local biohazards committee, the peer review group, and the NIH Recombinant Advisory Committee. These comments and my response on the section of the guidelines relating to roles and responsibilities of investigators, their institutions, and the National Institutes of Health are presented below.

Of considerable concern to all commentators was the process by which NIH would proceed to implement the guidelines. The scientific community generally urged that there be no Federal regulations, while some of the public commentators recommended the regulatory process.

Many who opposed changing the proposed guidelines into Federal regulations expressed concern for flexibility and administrative efficiency, which could best be achieved, in their view, through voluntary compliance. Other commentators, however, believed it imperative to proceed toward regulation. In their view, the guidelines could be implemented for purposes of NIH funding and would govern the conduct of experiments until regulations were in effect. Another commentator who thought regulation would be harmful rather than helpful suggested that if there were to be regulations, they should be along lines similar to those that govern the sale, distribution, use, and disposal of radioisotopes.

The question of how best to proceed now that the guidelines have been released deserves careful attention. I share the concern of those who feel that the guidelines must remain flexible. It is especially important that there be opportunity to change them quickly, based on new information relating to scientific evidence, potential risks, or safety aspects of the research program.

The suggestions for regulation need further attention at this time. The process for regulation not only involves the Director of NIH, but also the Assistant Secretary for Health and the Secretary of Health, Education, and Welfare. These guidelines are being promulgated now in order to afford additional protection to all concerned. Consideration of their conversion to regulations can proceed with continuing review of their content and present and future implications. Meanwhile, the NIH shall continue to provide the opportunity for public comment and participation at least equivalent to that provided if steps towards regulations were to proceed immediately. The guidelines will be published in the FEDERAL REGISTER forthwith to allow for further public comment.

C. Implementation considerations beyond the purview of NIH

Special concern has been expressed by many commentators regarding the application of the guidelines to research outside NIH by investigators other than its grantees or contractors. It has been urged that the guidelines be made applicable to recombinant DNA research conducted or supported by other agencies in HEW and by NSF, ERDA, DoD, and other governmental departments. Most commentators believe that these or similar guidelines should also govern research in the private sector, including industry, voluntary organizations, and foundations. Many feel that experiments conducted in colleges, universities, and even in high schools require some form of monitoring. And finally, all agree that in view of the potential hazards of recombinant DNA research to the biosphere, some form of international understanding on guidelines for the research is essential.

The committee, in the proposed guidelines, has suggested as one means of control that a description of the physical and biological containment procedures practiced in a research project be included in the publication of research results. In the scientific community this can be a powerful force for conformity, and we will undertake to present the recommendation to all appropriate journals. We are also prepared to take steps to disseminate the guidelines widely, and to arrange for a continual flow of information outward concerning the activities of the Recombinant Advisory Committee and the Advisory Committee to the Director, NIH, in the evolution of the guidelines and their implementation.

In response to these suggestions, I have already held a meeting with relevant HEW agencies and with representatives from other departments of the Federal Government. The purpose of the meeting was to exchange information on recombinant DNA research and to discuss the NIH guidelines. It served as an important beginning to address a common concern of these public institutions. A number of the representatives indicated that various departments might very well adopt the guidelines for research conducted both in-house and supported outside. Following up, I have begun preliminary discussions with the Assistant Secretary for Health and the Secretary of HEW, to determine possible methods to ensure adoption of the guidelines by all Federal agencies. Encouraged by these efforts, we held a meeting on June 2 with representatives of industry to provide them with full information about the guidelines and to help determine the present and future interests of industrial laboratories in this type of research. The meeting provided one of the first opportunities for industry representatives to convene for a discussion of this research area, and an industry com-

mittee under the auspices of the Pharmaceutical Manufacturers Association will be formed to review the guidelines for potential application to the drug industry. Further meetings will be scheduled with other groups that have an active interest in recombinant DNA research.

It is my hope that the guidelines will be voluntarily adopted and honored by all who support or conduct such research throughout the United States, and that at least very similar guidelines will obtain throughout the rest of the world. NIH places the highest priority on efforts to inform and to work with international organizations, such as the World Health Organization and the International Council of Scientific Unions, with a view to achieving a consensus on safety standards in this most important research area.

There has been considerable international cooperation and activity in the past, and I expect it to continue in the future. The aforementioned Ashby Report, presented to Parliament in January 1975, describes the advances in knowledge and possible benefits to society of the experiments involving recombinant DNA molecules, and attempts to assess the hazards in these techniques. The Asilomar meeting also had a number of international representatives, as mentioned previously. The European Molecular Biology Organization (EMBO) has been involved in considering guidelines for recombinant DNA research. They have closely followed the activities of NIH, and will thus be encouraged, I believe, to monitor their research with augmented cooperation and coordination. For example, EMBO recently announced plans for a voluntary registry of recombinant DNA research in Europe. Following this EMBO initiative, NIH shall similarly maintain a voluntary registry of investigators and institutions engaged in such research in the United States. Plans for establishing this registry are under way.

D. Environmental policy considerations

A number of commentators urged NIH to consider preparing an environmental impact statement on recombinant DNA research activity. They evoked the possibility that organisms containing recombinant DNA molecules might escape and affect the environment in potentially harmful ways.

I am in full agreement that the potentially harmful effects of this research on the environment should be assessed. As discussed throughout this paper, the guidelines are premised on physical and biological containment to prevent the release or propagation of DNA recombinants outside the laboratory. Deliberate release of organisms into the environment is prohibited. In my view, the stipulated physical and biological containment ensures that this research will proceed with a high degree of safety and precaution. But I recognize the legitimate concern of those urging that an environmental impact assessment be done. In view of this concern and ensuing public debate, I have reviewed the appropriateness of such an assessment and have directed that one be undertaken.

The purpose of this assessment will be to review the environmental effects, if any, of research that may be conducted under the guidelines. The assessment will provide further opportunity for all concerned to address the potential benefits and hazards of this most important research activity. I expect a draft of the environmental impact statement should be completed by September 1 for comment by the scientific community, Federal and State agencies, and the general public.

It should be noted that the development of the guidelines was in large part tantamount to conducting an environmental impact assessment. For example, the objectives of recombinant DNA research, and alternate approaches to reach those objectives, have been considered. The potential hazards and risks have been analyzed. Alternative approaches have been thoroughly considered, to maximize safety and minimize potential risk. And an elaborate review structure has been created to achieve these safety objectives. From a public policy viewpoint, however, the environmental impact assessment will be yet another review that will provide further opportunity for the public to participate and comment on the conduct of this research.

II. METHODS OF CONTAINMENT

Comments on the containment provisions of the proposed guidelines were directed to the definition of both physical and biological containment and to the safety and effectiveness of the prescribed levels. Several commentators found the concept of physical containment imprecise and too subject to the possibility for human error. Others questioned the concept of biological containment in terms of its safety and purported effectiveness in averting potential hazards. The commentators were divided on which method of containment would provide the most effective and safe system to avoid hazards. Several suggested that each of the physical containment levels be more fully explained.

W. Emmett Barkley, Ph.D., Director of the Office of Research Safety, National Cancer Institute, was asked to review the section on physical containment in light of these comments. Dr. Barkley convened a special committee of safety and health experts, who met to consider not only this section of the guidelines but also the section on the roles and responsibilities of researchers and their institutions. The committee thoroughly reviewed the section on physical containment and recommended a number of changes. The Recombinant Advisory Committee, meeting on April 1-2, 1976, reviewed the recommendations of the Barkley group. These are incorporated, with editorial revisions, in the final version of the guidelines.

The present section on physical containment is directly responsive to those commentators who asked for greater detail and explanation. Although different in detail, the four levels of containment approximate those given by the Center for Disease Control for human etiologic agents and by the National Cancer Institute for oncogenic viruses. For each of the proposed levels, optional items have been excluded, and only those items deemed absolutely necessary for safety are presented. Necessary facilities, practices, and equipment are specified. To give further guidance to investigators and their institutions, a supplement to the guidelines explains more fully safety practices appropriate to recombinant DNA research. And a new section has been added to ensure that shipment of recombinant DNA materials conforms, where appropriate, to the standards, prescribed by the U.S. Public Health Service, the Department of Transportation, and the Civil Aeronautics Board.

The section on physical containment is carefully designed to offer a constructive approach to meeting potential hazards for recombinant experiments at all levels of presumed risk. Certain commentators had suggested that the first level of physical containment (P1) be merged with the second level (P2). This suggestion, however, would tend to apply overly stringent standards for some experiments and might result in a lowering of standards necessary at the second level. I believe the level of control must be consistent with a reasonable estimate of the hazard; and the section on physical containment does provide this consistency. Accordingly, the first and second levels of physical containment remain as separate sections in the guidelines.

Because of the nature and operation of facilities required for experiments to be done at the fourth level of containment (P4), a provision has been included that the NIH shall review such facilities prior to funding them for recombinant DNA studies. The situation merits the special attention of experts who have maximum familiarity with the structure, operation, and potential problems of P4 installations. Several commentators advocated that NIH arrange for sharing of P4 facilities, both in the NIH intramural program and in institutions supported through NIH awards. In response to these suggestions, we are currently reviewing our facilities, including those at the Frederick Cancer Research Center (Fort Detrick), to determine how such a program can best be devised. It is most important that P4 facilities be made available to investigators. It should be noted that incidents of infection by even the most highly infectious and dangerous organisms are extremely infrequent at P4 facilities, and therefore the potential for hazard in certain complex experiments in recombinant DNA research is considerably reduced.

III. PROHIBITED EXPERIMENTS

1. Practically all commentators supported the present prohibition of certain experiments. There were suggestions for a clearer definition of the prohibition of certain experiments where increased antibiotic resistance may result. And it

was urged by some that the prohibition be broadened to include experiments that result in resistance to any antibiotic, irrespective of its use in medicine or agriculture. Consideration of such a suggestion must take into account that antibiotic resistance occurs naturally among bacteria, and that resistance is a valuable marker in the study of microbial genetics in general, and recombinants in particular.

In view of these concerns, however, the Recombinant Advisory Committee was asked to reconsider carefully the prohibition and related sections concerning antibiotic resistance. The committee noted that the prohibition relating to drug resistance was intended to ban those experiments that could compromise drug use in controlling disease agents in veterinary as well as human medicine and this is now clearly stated.

In the draft guidelines there were two statements concerning resistance to drugs which related to experiments with *E. coli*. The statements appeared to allow experiments that would extend the range of resistance of this bacterium to therapeutically useful drugs and disinfectants, and thus seemed to be in conflict with the general prohibition on such research. There are numerous reports in the scientific literature indicating that *E. coli* can acquire resistance to all antibiotics known to act against it. Since *E. coli* acquires resistance naturally, the prohibition directed against increasing resistance does not apply. The ambiguous statements have been deleted from the present guidelines. On the other hand, new language has been inserted in the section dealing with other prokaryote species to set containment levels for permitted experiments.[1]

2. The Recombinant Advisory Committee was also asked to clarify whether the prohibition of use of DNA derived from pathogenic organisms (those classified as 3, 4, and 5 by the Center for Disease Control, USPHS) also included the DNA from any host infected with these organisms. The committee explained that this prohibition did extend to experiments with cells known to be so infected. To avoid misunderstanding, the prohibition as now worded includes such cells. In addition, the prohibitions have been extended to include moderate-risk oncogenic viruses, as defined by the National Cancer Institute, and cells known to be infected with them.

3. Two other issues relating to the section on prohibited experiments were raised by Roy Curtiss III, Ph.D., Professor, Department of Microbiology, University of Alabama School of Medicine, Birmingham, who is a member of the Recombinant Advisory Committee. Dr. Curtiss noted that for the class of experiments prohibited on the basis of production of highly toxic substances, only substances from micro-organisms were cited as examples. He suggested that other examples be included, such as venoms from insects and snakes. The committee approved the suggestion and I concur.

In the proposed guidelines, release of organisms containing recombinant DNA molecules into the environment was prohibited unless a series of controlled tests had been done to leave no reasonable doubt of safety. Dr. Curtiss felt that the guidelines should provide greater specificity for testing and should include some form of review prior to release of the organism. I have decided that the guidelines should, for the present, prohibit any deliberate release of organisms containing recombinant DNA into the environment. With the present limited state of knowledge, it seems highly unlikely that there will be in the near future, any recombinant organism that is universally accepted as being beneficial to introduce into the environment. When the scientific evidence becomes available that the potential benefits of recombinant organisms, particularly for agriculture, are about to be realized, then the guidelines can be altered to meet the needs for release. It is most important that the potential environmental impact of the release be considered.

IV. PERMISSIBLE EXPERIMENTS: E. COLIK-12 HOST-VECTOR SYSTEMS

The continued use of *E. coli* as a host has drawn considerable comment, including some suggestions that its use be prohibited presently or within a specified time limit. It should be stressed that the use of *E. coli* as detailed in the guidelines is limited to *E. coli* K-12, a strain that has been carried in the laboratory for decades and does not involve the use of any strain of *E. coli* that is freshly isolated from a natural source. *E. coli* K-12 does not usually colonize the normal bowel, even when given in large doses, and exhibits little if any multiplication while passing through the alimentary canal. For years it has been the subject of more intense investigation than any other single organism, and knowledge of its genetic makeup and recombinant behavior exceeds greatly that pertaining to any other organism. I believe that because of this experience, *E. coli* K-12 will provide a host-vector system that is safer than other candidate microorganisms.

NIH recognizes the importance of supporting the development of alternative host-vector systems (such as *B. subtilis*, which has no ecological niche in man) and will encourage such development. It should be noted, however, that for each new host-vector system, the same questions of risk from altered properties attendant upon the presence of recombinant genes will apply as apply to *E. coli*. NIH does not believe it wise to set a time limit on replacement of *E. coli* systems by other organisms.

There were specific suggestions concerning the three levels of biological containment prescribed for use of *E. coli* K-12 host-vectors. Some commentators requested a more detailed explanation of the adequacy of protection for laboratory personnel with the first level of containment (EK1).[2] Sections of the guidelines dealing with physical containment and roles and responsibilities now specify the need for safety practices and accident plans.

For the second level of containment (EK2), it is required that a cloned DNA fragment be contained in a host-vector system that has no greater than a 10^{-8} probability of survival in a nonpermissive or natural environment. It was suggested that the selection of this level of biological containment and the appropriate tests for verification be more fully explained in the guidelines. The committee, in responding to a request for further examination of this point, reviewed at considerable length the testing for an EK2 system and recommended certain modifications. We have accepted the committee's new language that better explains testing of survival of a genetic marker carried on the vector, preferably on an inserted NDA fragment.

Possible tests to determine the level of biological containment afforded by these altered host-vector systems are outlined in this section. Because this is such a new area of scientific research and development, however, it is inappropriate to standardize such testing at the present time. Standards will gradually be set as more experience with EK2 host-vector systems is acquired. The committee, for example, during its April 1976 meetings gave its first approval to an EK2 host-vector system. What is necessary is that new and more effective tests be devised by investigators, and this effort is very likely to occur under the present guidelines. For example, one task recognized by the committee is to clarify how survival of the organism and the cloned DNA should be defined in terms of temperature, medium, and other variables.

It is also very important to note here that the stringent requirements set by the committee for EK2 biological containment jeopardize considerably the capacity of such crippled organisms to survive and replicate even under permissive laboratory conditions. More experience will be required to determine whether EK2 containment will permit some lines of important research to be followed.

Several commentators suggested that methods and procedures to confirm an

[1] Specifically, experiments that would extend resistance to therapeutically useful drugs must use P2 physical containment plus a host-vector comparable to EK1, or P2 containment plus a host-vector comparable to EK2.

[2] The EK1 system presently consists of a battery of different vectors and of *E. coli* K-12 mutants, all of which afford a considerable degree of biological containment. The diversity of vectors and of host mutants in this battery has permitted a wide range of important scientific questions to be attacked. For example, the availability of different vectors with cleavage sites for different restriction endonucleases have increased the kind of DNA segments that can be cloned. By contrast, the first EK2 host-vector systems are only now being considered by the Recombinant Advisory Committee. While NIH is supporting the development of more EK2 host-vector systems, it is not expected that a battery equivalent to that available for the EK1 system will be certified by the Recombinant Advisory Committee in the near future.

EK system at the third level of containment (EK3) be more fully explained. The Recombinant Advisory Committee was asked to consider this suggestion. After considerable discussion the committee declined to define the procedures more fully at this time, because development of an EK3 system is still far enough in the future not to warrant specific testing procedures. Further, it is not clear what tests are best suited. The language, therefore, remains general. The committee, however, is aware of the concerns for a more completely defined system of testing, and has considered the possibility of organizing a symposium for purposes of designating tests. In my view, more fully developed protocols for testing EK3 systems are warranted, and it is necessary that guidelines here be more fully developed before the committee proceeds to certify such a system. In this regard the NIH is prepared through the National Institute of Allergy and Infectious Diseases to support contracts to accomplish this task. We will seek the advice and assistance of the committee to define the scope of necessary work.

These guidelines also include a statement that for the time being no EK2 or EK3 host-vector system will be considered *bona fide* until the Recombinant Advisory Committee has certified it. I share the concern of the commentators that new host-vector systems require the highest quality of scientific review and scrutiny. At this early stage of development, it is most important that the committee provide that scrutiny. Further, I believe that until more experience has been gained, the committee should encourage and the NIH support research that will independently confirm and augment the data on which certification of EK2 host-vector systems are based.

V. CLASSIFICATION OF EXPERIMENTS USING THE E. COLI K-12 CONTAINMENT SYSTEMS

The guidelines assign different levels of containment for experiments in which DNA from different sources is to be introduced into an *E. coli* K-12 host-vector system. The variation is based on both facts and assumptions. There are some prokaryotes (bacteria) which constantly exchange DNA with *E. coli*. Here it is assumed that experimental conditions beyond those obtained in careful, routine microbiology laboratories are superfluous, because any exchange experiments have undoubtedly been performed already in nature.

In every instance of artificial recombination, consideration must be given to the possibility that foreign DNA may be translated into protein (expressed), and also to the possibility that normally repressed genes of the host may be expressed and thus change, undesirably, the characteristics of the cell. It is assumed that the more similar the DNAs of donor and host, the greater the probability of expression of foreign DNA, or of possible derepression of host genes. In those cases where the donor exchanges DNA with *E. coli* in nature, it is unlikely that recombination experiments will create new genetic combinations. When prokaryote donors not known to exchange DNA with *E. coli* in nature are used, however, there is a greater potential for new genetic combinations to be formed and be expressed. Therefore, it is required that experiments involving prokaryotic DNA from a donor that is not known to exchange DNA with *E. coli* in nature be carried out at a higher level of containment. Recombination using prokaryotic DNA from an organism known to be highly pathogenic is prohibited.

There are only limited data available concerning the expression of DNA from higher forms of life (eukaryotes) in *E. coli* (or any other prokaryote). Therefore, the containment prescriptions for experiments inserting eukaryotic DNA into prokaryotes are based on risks having quite uncertain probabilities.

On the assumption that a prokaryote host might translate eukaryotic DNA, it is further presumed that the product of that foreign gene would be most harmful to man if it were an enzyme, hormone, or other protein that was similar (homologous) to proteins already produced by or active in man. An example is a bacterium that could produce insulin. Such a "rogue" bacterium could be of benefit if contained, a nuisance or possibly dangerous if capable of surviving in nature. This is one reason that the higher the phylogenetic order of the eukaryote, the higher the recommended containment, at least until the efficiency of expression of DNA from higher eukaryotes in prokaryotes can be determined.

There is a second, more concrete reason for scaling containment upward as the eukaryote host becomes similiar to man. This is the concern that viruses capable of propagating in human tissue, and possibly causing diseases, can contaminate DNA, replicate in prokaryote hosts and infect the experimentalist. Such risks are greatest when total DNA from donor tissue is used in "shotgun" recombinant experiments; it diminishes to much lower levels when pure cloned DNA is used.

The commentators were clearly divided on the classification of containment criteria for different kinds of recombinant DNAs. Many commentators considered the guidelines too stringent and rigid. Others viewed the guidelines in certain instances as too permissive. And still others endorsed the guidelines as sensible and reasonable, affording the public an enormous degree of protection from the speculative risks. Several suggestions were made for the specific classes of experiments, and they follow:

1. Comment on the use of DNA from animals and plants in recombinant experiments varied widely. Some commentators suggested banning the use of DNA from primates, other mammals, and birds. Others suggested that higher levels of containment be used for all such experiments. Still others believed that the guidelines were too strict for experiments of this class. I have carefully reviewed the issues raised by the commentators and the responses of the committee to certain queries concerning use of animal and plant DNA in these experiments.

In my view, the classification for the use of DNA from primates, other mammals, and birds is appropriate to the potential hazards that might be posed. The physical and biological containment levels are very strict. For example, biological containment levels are at EK2 or EK3, and will effectively preclude experimentation until useful EK2 and EK3 systems are available. EK2 systems are still in the initial stages of development, and the first system was only certified at the most recent meeting of the Recombinant Advisory Committee. An EK3 host-vector system has yet to be tested, and its certification is far enough in the future to place a moratorium on those experiments requiring biological containment at an EK3 level. The physical containment levels of P3 or P4 themselves afford a very high degree of protection. I am satisfied that the guidelines demonstrate the caution and prudence that must govern the conduct of experiments in this category.

The guidelines allow reduced containment levels for primate DNA when it is derived from embryonic tissue or germline cells. This is based on evidence that embryonic material is less likely to contain viruses than is tissue from the adult. Obviously, the embryonic tissue must be free of adult tissue, and the present guidelines so indicate.

I have also carefully considered the special concerns arising from the use of DNA from cold-blooded vertebrates and other cold-blooded animals, because several commentators questioned the basis of lower physical and biological containment levels for DNA from these species. The Recombinant Advisory Committee has debated this extensively, and they were asked to do so once again in April.[2] The committee has now recommended high containment levels (P3+EK2) when the DNA is from a cold-blooded vertebrate known to produce a potent toxin. That recommendation is included in the present guidelines. Where no toxin is involved the committee supported lower

[2] A committee member, David S. Hogness, Ph. D., Professor, Department of Biochemistry, Stanford University, California, submitted a statement in support of lower containment levels based on current scientific evidence. That evidence is based on certain differences between cold- and warm-blooded vertebrates. One of the criteria used for the evaluation of the relative risk that might be encountered with different levels of shotgun experiment is the degree of sequence homology between the DNA of the given species and that of humans. This criterion is used to estimate the likelihood that segments of DNA from the given species might be integrated into the human genome by recombination; the greater the homology, the greater the likelihood of integration. Studies of sequence homologies indicate that there is a considerable degree of homology between human DNA and DNA from other primates, much less homology between primates and other mammals, and even lower but detectable homology between birds and primates. By contrast, no significant homologies between cold-blooded vertebrates and primates have been detected.

containment levels. The guidelines specify P3+EK2 levels for such work. There was considerable discussion concerning the advisability of recommending lower containment (P2+EK1) when the DNA is isolated from embryonic tissue or germ-line cells from cold-blooded vertebrates. Those supporting lower containment levels argued that the justification for P2+EK2 was the possibility that cold-blooded vertebrates may carry viruses and that the distinction between adult and germ-cell tissue is real. Others argued that, contrary to the situation with primate DNA, viruses are not a central problem with cold-blooded vertebrates and therefore no distinction should be made on the basis of tissue origin. Finally, the committee recommended, on a divided vote (8 to 4), to adopt P2+EK1 when the cold-blooded vertebrate DNA is isolated from embryonic tissue or germ-line cells. Upon reviewing these considerations, I have decided to retain the containment levels for embryonic or germ-line DNA from cold-blooded vertebrates as recommended by the committee.

In April the committee also reviewed, at our request, the classification of experiments where DNA is derived from other cold-blooded animals or lower eukaryotes. Several commentators, for example, had been concerned about the fact that insects are known to carry agents pathogenic to man. In the committee review, it was noted that viruses carried by insects and known to transmit disease to man are RNA rather than DNA viruses and do not reproduce via DNA copied from RNA. In order, however, to make the intent clearer, the guidelines have been rewritten for experiments of this class. New language is inserted to ensure that strict containment levels are employed when the DNA comes from known pathogens or species known to carry them. Further, to reduce the potential hazards, we have also included in the guidelines the requirement that any insect must be grown under laboratory conditions for at least 10 generations prior to its use as a DNA source.

2. As alluded to above, certain commentators expressed concern that when *E. coli* becomes the host of recombinant DNA from prokaryotes with which DNA is not usually exchanged, there is hazard of altered host characteristics resulting from translation of the DNA into functioning proteins. The committee was asked to review the guidelines and take into account this potential hazard. They agreed that the containment levels should be increased for this category of experiment, from P2+EK1 to either P2+EK2 or P3+EK1. That recommendation is included in the present guidelines.

Comments were made concerning that class of experiments in which the recombinant DNA, regardless of source, has been cloned. A clone is a population of cells derived from a single cell and therefore all the cells are presumed to be genetically identical. As outlined in the proposed guidelines, clones could be used at lower containment levels if they had been rigorously characterized and shown to be free of harmful genes. Several commentators inquired how the characterization was to be performed and the freedom from harmful genes demonstrated. Although the committee acknowledges that these terms are unavoidably vague, they do cite appropriate scientific methods to make relevant' determinations. Again, this is a rapidly changing area and more clarity and precision can be expected with experience. Reduced containment requirements for this class of experiment are warranted because of the purified nature of clones. Further, the granting agency must approve the clone before containment conditions can be reduced, thus providing an additional element of review.

4. Another comment was related to the use of DNA from organelles (intracellular elements that contain special groups of genes for particular cell functions). Concern was expressed about the potential contamination of purified organelle DNA with DNA from viruses because of the similarity of their structures. The committee agrees, and the guidelines now specify a requirement, that the organelles be isolated prior to extracting DNA, as a further means of reducing the hazard of viral contamination.

5. Some commentators were troubled about the lowering of containment for that class of experiments involving recombinations with cell DNA segments purified by chemical or physical methods. They asked that procedures for determining the state of purification be more fully detailed and that the Recombinant Advisory Committee certify the purity. There are, however, appropriate techniques, such as gel electrophoresis, with which a purity of 99 percent by mass can be achieved and ascertained. There is no way for the committee to certify these results beyond repeating the experiments themselves. These techniques are well documented and described in the literature. I do not believe it is necessary or feasible for the committee to review each procedure for purification of DNA.

6. Comments were made concerning the use of DNA derived from animal viruses. It was urged that containment levels for this class of experiment be increased. On the basis of my review, I find the containment conditions appropriate to the potential hazard posed. As defined in the guidelines, experiments are to be done at very strict levels of containment and these can be lowered only when the cloned DNA recombinants have been shown to be free of possibly harmful genes by suitable biochemical and biological tests. This also pertains to DNA that is copied from RNA viruses. In no instance are the guidelines more lenient, and in most instances they are more stringent than conditions obtaining in many laboratories where such viruses are studied in non-DNA-recombinant experiments.

VI. CLASSIFICATION OF EXPERIMENTS USING CONTAINMENT SYSTEMS OTHER THAN *E. COLI* K–12

1. No issue with regard to these guidelines raised more comment than the use of animal viruses as vectors. Of special concern to many commentators was the use of the simian (monkey) virus 40 (hereafter "SV40"). Some suggested a complete ban on the use of this virus; others urged its retention as a vector. SV40 is not known to produce any disease in man, although it can be grown in human cells and on very rare occasions has been isolated from humans. Many humans have received SV40 virus inadvertently in vaccines prepared from virus grown in monkey kidney-cell cultures. An intensive search has been made and is continuing for evidence that SV40 might cause cancer or be otherwise pathogenic for man. At present, it is my view that the extensive knowledge we have of SV40 virus provides us with sufficient sophistication to ensure its safe handling under the conditions developed for its use in the guidelines.

I believe work with SV40 should continue under the cost careful conditions, but I do recognize and appreciate the concerns expressed over its possible harmful effects in humans. In light of these concerns, I asked the Recombinant Advisory Committee to review this section of the guidelines. The committee reconsidered the containment conditions for this class of experiments and judged them appropriate to meet the potential hazards.[1]

This class of experiments will proceed under the most careful and stringent conditions. Work with SV40 virus will be done at the maximum level of physical containment (P4). The extraordinary precautions required in a P4 facility lessen the likelihood of a potential hazard from this work. Only defective SV40 virus will be used as vector; that is, the SV40 virus particles that carry the foreign DNA cannot multiply by themselves. When a number of strict conditions are met, this work will be permitted to go on at the third level of containment (P3), which in itself requires care and precision. It should be noted that SV4C virus and its DNA can be efficiently disinfected by Clorox and autoclaving. These are customary procedures for disinfecting glassware and other items used in SV40 animal-cell work.

Some commentators suggested that the containment criteria for experiments using polyoma virus as the vector be strengthened. There is no evidence that polyoma infects humans or replicates to any significant extent in human cells. It holds promise as a vector, as is more fully documented in an appendix to these guidelines.

2. Several commentators found the guidelines inadequate regarding experiments with plant host-vector systems. Because NIH shared these concerns, a group with extensive experience with plants was appointed to review this section. The group met concurrently with

[1] One member dissented from this position. During the discussion, additional language was recommended (and adopted) to ensure that the defective SV40-virus/helper-virus system, with its inserted non-SV40 DNA segment, does not replicate in human cells with significantly more efficiency than does SV40.

the Recombinant Advisory Committee in April 1976 and made several modifications. The suggested revisions were acceptable to the full committee, and we have included them in the guidelines.

The modifications are responsive to the stated concerns of the commentators. A description of greenhouse facilities is given, and physical containment conditions have been modified to take into account operations with whole plants. On the whole, the respective portions of the guidelines relating to plants are more fully explained and the intent is clarified.

I have also accepted the recommendation of the subcommittee to lower the biological containment level from EK2 to EK1 for experiments in which the DNA from plants is used in conjunction with the *E. coli* K–12 host-vector system, thereby setting containment in this instance at the same level required for experiments with lower-eukaryote DNA.

VII. ROLES AND RESPONSIBILITIES

1. Most commentators had suggestions for the section on the roles and responsibilities of investigators, their local institutions, and NIH. Commentators generally urged openness, candor, and public participation in the process, emphasizing shared responsibility and accountability from the local to the national level. We reviewed that section of the guidelines in light of these comments and have asked the Recombinant Advisory Committee to review certain issues.

It is clear that much of the success of the guidelines will lie in the wisdom with which they are implemented. Because of the importance of this section, especially in terms of safety programs and plans, we have carefully weighed the comments and suggestions made in this regard. NIH has a special responsibility to take a leading role in ensuring that safety programs are part of all recombinant DNA research. Dr. Barkley and a specially convened committee were asked to provide greater detail for safety, accident, and training plans for this section of the guidelines. Based on their recommendations, the section has been extensively rewritten to clarify the respective responsibilities of the principal investigator, the institution (including the institutional biohazards committee), the NIH initial review group (study section), the NIH Recombinant DNA Molecule Program Advisory Committee, and NIH staff.

This section has a definitive administrative framework for assuring that safety is an essential and integrated component of research involving recombinant DNA molecules. The guidelines require investigators to institute, monitor, and evaluate containment and safety practices and procedures. Before research is done, the investigator must have safety and accident plans in place and training exercises for the staff well under way.

Some commentators suggested that the investigator be required to obtain informed consent of laboratory personnel prior to their participation. Rather than rely explicitly on an informed consent document, the guidelines now make the investigator responsible for advising his program and support staff as to the nature and assessment of the real and potential biohazards. He must explain and provide for any advised or requested precautionary medical policies, vaccinations, or serum collections. Further, an appendix to the guidelines includes detailed explanations for dealing with accidents, as well as instructions for the training of staff in safety and accident procedures.

In response to suggestions for epidemiological monitoring, the guidelines now require the principal investigator to report certain categories of accidents, in writing, to appropriate officials. NIH is investigating procedures for long-term surveillance of workers engaged in recombinant DNA research.

2. A number of comments on the role and responsibilities of the institutional biohazards committee were received. Comments were directed to the structure of the committee, the scope of its responsibility, and the methods for operation. Comments on structure included suggestions that the committee have a broadly based representation, especially in terms of health and safety expertise. Some others suggested NIH require certain classes of representation. In response to these suggestions, the guidelines now recommend membership from a diversity of disciplines relevant to recombinant DNA molecule technology, biological safety, and engineering.

For broader representation beyond the immediate scientific expertise, the guidelines now recommend that local committees should possess, or have available, the competence necessary to determine the acceptability of their findings in terms of applicable laws, regulations, standards of practice, community attitudes, and health and environmental considerations. The names of and relevant background information on the committee members will be reported to NIH.

In response to suggestions that decisions of the committee be made publicly available, the guidelines now recommend that minutes of the meetings should be kept and made available for public inspection.

Commentators generally approved of the responsibility given to the institutional biohazards committee to serve as a source of advice and reference to the investigator on scientific and safety questions. It was further suggested that the committee's responsibility be broadened in the development, monitoring, and evaluation of safety standards and procedures. In response to these suggestions, the guidelines now indicate that the institutional biohazards committee has the responsibility to certify, and recertify annually, to NIH that the facilities, procedures, practices, training, and expertise of involved personnel have been reviewed and approved. The Recombinant Advisory Committee suggested that examination might be unnecessary for P1 facilities, but we believe that all facilities should be reviewed to emphasize the importance of safety programs.

Some commentators suggested that the guidelines should stipulate that the local committees be required to determine the containment conditions to be imposed for a given project (which the draft guidelines specifically noted was not their responsibility). The Recombinant Advisory Committee took exception to this suggestion. They urged NIH not to include these conditions as local requirements, arguing among other things that review by the NIH study sections would provide the necessary scrutiny at the national level and assure uniformity of standards in application of the guidelines. I do not believe that NIH should require the local institution to have its biohazards committee assess what containment conditions are required for a given project. On the other hand, the guidelines should not prohibit the local institution from having its biohazards committee perform this function. Accordingly, I have deleted the prohibition that appeared in the proposed guidelines.

Another suggestion was that the local committee ensure that research is carried out in accordance with standards and procedures under the Occupational Safety and Health Act (OSHA). This is an area of importance to the local institutions under Federal and State law, but need not be included as a requirement in the guidelines. NIH will maintain liaison with the Occupational Safety and Health Administration (Department of Labor) to ensure maximum Federal cooperation in this venture.

I would also encourage all institutions, as suggested by several commentators, to review their insurance compensation programs to determine whether their laboratory personnel, in the research area, are covered for injuries.

3. The commentators approved of having the NIH study sections responsible for making an independent evaluation of the classification of the proposed research under the guidelines, along with the customary judgment of the scientific merit of each grant application. This additional element of review will ensure careful attention to potential hazards in the research activity. The study sections will also scrutinize the proposed safeguards. Biological safety expertise shall be available to the study section for consultation and guidance in this regard.

4. Several commentators made suggestions concerning the structure, function, and scope of responsibility of the NIH Recombinant DNA Molecule Program Advisory Committee.

Comments on possible structural mechanisms for decision making included suggestions that there be a scientific and technical committee and a general advisory public policy committee. It was also suggested that the scientific committee include scientists who are not actively engaged in recombinant research, and that the public policy committee have a broad scientific and public representation.

I have carefully reviewed these comments and suggestions. In response, the following structure has been devised. The Recombinant Advisory Committee shall serve as the scientific and technical committee. Its membership shall continue to

include scientists who represent disciplines actively engaged in recombinant DNA research. In my view, it is most important that this committee have the necessary expertise to assure that the guidelines are of the highest scientific quality. The committee has provided this expertise in the past, and it must continue to do so. The committee shall also include members from other scientific disciplines.

It should be noted that the present committee recommended on its own initiative that a nonscientist be appointed. Emmette S. Redford, Ph.D., LL.D., Ashbel Smith Professor of Government and Public Affairs at the Lyndon B. Johnson School of Public Affairs, University of Texas at Austin, serves in that capacity. An ethicist has also been nominated for appointment.

The Advisory Committee to the Director, NIH, shall serve to provide the broader public policy perspectives. This committee, at its meeting on February 9-10, 1976, reviewed the proposed guidelines with the participation of public witnesses, and shall continue to provide such review for future activities of the Recombinant Advisory Committee.

In response to suggestions, the responsibilities of the Recombinant Advisory Committee have been expanded. In addition to reviewing the guidelines for possible modification as scientific evidence warrants, the committee will certify EK2 and EK3 systems. In response to requests by the investigator, local committee, or study section, the committee will also provide evaluation and review in order to advise on levels of required containment, on lowering of requirements when cloned recombinants are to be used, and on questions concerning potential biohazard and adequacy of containment provisions.

Commentators also asked that the committee review ongoing research initiated prior to the implementation of the guidelines. Now that the guidelines are being released, NIH-funded investigators in this field will be asked to give assurance, within a given period, that they will comply. Any investigators who constructed clones under the Asilomar guidelines will be asked to petition NIH for special consideration of their case, if the new guidelines require higher containment than did the Asilomar guidelines. Here the advice of the Recombinant Advisory Committee will be sought.

There were also suggestions that the committee certify chemical purification of recombinant DNA, but as I indicated earlier, these procedures are too well known to require NIH monitoring.

5. In light of comments received, NIH will provide review, through appropriate NIH offices, of data from institutional biohazards committees (including accident reports) and will ensure dissemination of these findings as appropriate. Dr. William Gartland will head the newly created NIH Office of Recombinant DNA Activities for these purposes. In addition, NIH will provide for rapid dissemination of information through its Nucleic Acid Recombinant Scientific Memoranda (NARSM), distributed by the National Institute for Allergy and Infectious Diseases. NIH will also provide an appropriate mechanism for approving and certifying clones before containment conditions can be lowered.

With these extended modifications, the section of the guidelines dealing with roles and responsibilities now sets forth a more fully developed review structure involving the principal investigator, local biohazards committees, and the Recombinant Advisory Committee, as well as peer review committees. Guidelines now provide extensive opportunity for advice, from the local to the national level. Several levels of review and scrutiny are provided, ensuring the highest standards for scientific merit and conditions for safety.

The Recombinant Advisory Committee in conjunction with the Director's Advisory Committee shall continue to serve as an ongoing forum for examining progress in the technology and safety of recombinant DNA research. Their responsibility, and that of the NIH Director, is to ensure that the guidelines, through modification when called for, reflect the soundest scientific and safety evidence as it accrues in this area. Their task, in a sense, is just beginning.

DONALD S. FREDRICKSON,
Director,
National Institute of Health.

GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

JUNE 1976

I. Introduction.
II. Containment:
A. Standard practices and training.
B. Physical containment levels: P1 level (Minimal); P2 Level (Low); P3 Level (Moderate); P4 Level (High).
C. Shipment.
D. Biological containment levels.
III. Experimental Guidelines:
A. Experiments that are not to be performed.
B. Containment guidelines for permissible experiments:
1. Biological containment criteria using *E. Coli* K-12 host-vectors; EK1 host-vectors; EK2 host-vectors; EK3 host-vectors.
2. Classification of experiments using the *E. Coli* K-12 containment systems:
(a) Shotgun experiments:
(i) Eukaryotic DNA recombinants;
(ii) Prokaryotic DNA recombinants;
(iii) Characterized clones of DNA recombinants derived from shotgun experiments.
(b) Purified cellular DNAs other than plasmids, bacteriophages, and other viruses.
(c) Plasmids, bacteriophages, and other viruses:
(i) Animal Viruses;
(ii) Plant Viruses;
(iii) Eukaryotic organelle DNAs;
(iv) Prokaryotic plasmid and phage DNAs.
3. Experiments with other prokaryotic host-vectors.
4. Experiments with eukaryotic host-vectors:
(a) Animal host-vector systems;
(b) Plant host-vector systems;
(c) Fungal or similar lower eukaryotic host-vector systems.
IV. Roles and Responsibilities:
A. Principal Investigator;
B. Institution;
C. NIH Initial Review Group (Study Sections);
D. NIH Recombinant DNA Molecule Program Advisory Committee;
E. NIH Staff.
V. Footnotes.
VI. References.
VII. Members of the Recombinant DNA Molecule Program Advisory Committee.

APPENDICES

A. Statement on the use of *Bacillus subtilis* in recombinant molecule technology.
B. Polyoma and SV40 Virus.
C. Summary of Workshop on the Design and Testing of Safer Prokaryotic Vehicles and Bacterial Hosts for Research on Recombinant DNA Molecules.
D. Supplementary Information on Physical Containment (Including Detailed Contents).

I. INTRODUCTION

The purpose of these guidelines is to recommend safeguards for research on recombinant DNA molecules to the National Institutes of Health and to other institutions that support such research. In this context we define recombinant DNAs as molecules that consist of different segments of DNA which have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of the host's genome.

This is the first attempt to provide a detailed set of guidelines for use by study sections as well as practicing scientists for evaluating research on recombinant DNA molecules. We cannot hope to anticipate all possible lines of imaginative research that are possible with this powerful new methodology. Nevertheless, a considerable volume of written and verbal contributions from scientists in a variety of disciplines has been received. In many instances the views presented to us were contradictory. At present, the hazards may be guessed at, speculated about, or voted upon, but they cannot be known absolutely in the absence of firm experimental data—and, unfortunately, the needed data were, more often than not, unavailable. Our problem then has been to construct guidelines that allow the promise of the methodology to be realized while advocating the considerable caution that is demanded by what we and others view as potential hazards.

In designing these guidelines we have adopted the following principles, which are consistent with the general conclusions that were formulated at the International Conference on Recombinant DNA Molecules held at Asilomar Conference Center, Pacific Grove, California, in February 1975 (3): (i) There are certain experiments for which the assessed potential hazard is so serious that they are not to be attempted at the present time. (ii) The remainder can be undertaken at the present time provided that the experiment is justifiable on the basis that new knowledge or benefits to humankind will accrue that cannot readily be obtained by use of conventional methodology and that appropriate safeguards are incorporated into the design and execution of the experiment. In addition to an insistence on the practice of good microbiological techniques, these safeguards consist of providing both physical

and biological barriers to the dissemination of the potentially hazardous agents. (iii) The level of containment provided by these barriers is to match the estimated potential hazard for each of the different classes of recombinants. For projects in a given class, this level is to be highest at initiation and modified subsequently only if there is a substantiated change in the assessed risk or in the applied methodology. (iv) The guidelines will be subjected to periodic review (at least annually) and modified to reflect improvements in our knowledge of the potential biohazards and of the available safeguards.

In constructing these guidelines it has been necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account existing and anticipated special procedures and information that will allow particular experiments to be carried out under different conditions than indicated here without sacrifice of safety. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that study sections give consideration to such procedures which may allow change in the containment levels recommended here.

It is recommended that all publications dealing with recombinant DNA work include a description of the physical and biological containment procedures practiced, to aid and forewarn others who might consider repeating the work.

II. CONTAINMENT

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs (4–17). The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which either limit the infectivity of a vector or vehicle (plasmid, bacteriophage or virus) to specific hosts, or its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying different combinations of the physical and biological barriers to a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the guidelines for research on the different kinds of recombinant DNA (Section III).

A. *Standard practices and training.* The first principle of containment is a strict adherence to good microbiological practices (4–13). Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. This should include at least training in aspectic techniques and instruction in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which an effective vaccine is available, all workers should be immunized. Serological monitoring, where appropriate, should be provided.

B. *Physical containment levels.* A variety of combinations (levels) of special practices, equipment, and laboratory installations that provide additional physical barriers can be formed. For example, 31 combinations are listed in "Laboratory Safety at the Center for Disease Control" (4); four levels are associated with the "Classification of Etiologic Agents on the Basis of Hazard" (5), four levels were recommended in the "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (3); and the National Cancer Institute uses three levels for research on oncogenic viruses (6). We emphasize that these are an aid to, and not a substitute for, good technique. Personnel must be competent in the effective use of all equipment needed for the required containment level as described below. We define only four levels of physical containment here, both because the accuracy with which one can presently assess the biohazards that may result from recombinant DNAs does not warrant a more detailed classification, and because additional flexibility can be obtained by combination of the physical with the biological barriers. Though different in detail, these four levels (P1<P2<P3<P4) approximate those given for human etiologic agents by the Center for Disease Control (i.e., classes 1 through 4; ref. 5), in the Asilomar summary statement (i.e., minimal, low, moderate, and high; ref. 3), and by the National Cancer Institute for oncogenic viruses (i.e., low, moderate, and high; ref. 6), as is indicated by the P-number or adjective in the following headings. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of hazardous organisms.

We anticipate, and indeed already know of, procedures (14) which enhance physical containment capability in novel ways. For example, miniaturization of screening, handling, and analytical procedures provides substantial containment of a given host-vector system. Thus, such procedures should reduce the need for the standard types of physical containment, and such innovations will be considered by the Recombinant DNA Molecule Program Advisory Committee.

The special practices, equipment and facility installations indicated for each level of physical containment are required for the safety of laboratory workers, other persons, and for the protection of the environment. Optional items have been excluded; only those items deemed absolutely necessary for safety are presented. Thus, the listed requirements present basic safety criteria for each level of physical containment. Other microbiological practices and laboratory techniques which promote safety are to be encouraged. Additional information giving further guidance on physical containment is provided in a supplement to the guidelines (Appendix D).

P1 Level (Minimal). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P1 level is a laboratory that possesses no special engineering design features. It is a laboratory commonly used for microorganisms of no or minimal biohazard under ordinary conditions of handling. Work in this laboratory is generally conducted on open bench tops. Special containment equipment is neither required nor generally available in this laboratory. The laboratory is not separated from the general traffic patterns of the building. Public access is permitted.

The control of biohazards at the P1 level is provided by standard microbiological practices of which the following are examples: (i) Laboratory doors should be kept closed while experiments are in progress. (ii) Work surfaces should be decontaminated daily and following spills of recombinant DNA materials. (iii) Liquid wastes containing recombinant DNA materials should be decontaminated before disposal. (iv) Solid wastes contaminated with recombinant DNA materials should be decontaminated or packaged in a durable leakproof container before removal from the laboratory. (v) Although pipetting by mouth is permitted, it is preferable that mechanical pipetting devices be used. When pipetting by mouth, cotton-plugged pipettes shall be employed. (vi) Eating, drinking, smoking, and storage of food in the working area should be discouraged. (vii) Facilities to wash hands should be available. (viii) An insect and rodent control program should be provided. (ix) The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

P2 Level (Low). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P2 level is similar in construction and design to the P1 laboratory. The P2 laboratory must have access to an autoclave within the building; it may have a Biological Safety Cabinet.[1] Work which does not produce a considerable aerosol is conducted on the open bench. Although this laboratory is not separated from the general traffic patterns of the building, access to the laboratory is limited when experiments requiring P2 level physical containment are being conducted. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

The P2 laboratory is commonly used for experiments involving microorganisms of low biohazard such as those which have been classified by the Center for Disease Control as Class 2 agents (5).

The following practices shall apply to all experiments requiring P2 level physical containment: (i) Laboratory doors shall be kept closed while experiments are in progress. (ii) Only persons who have been advised of the potential biohazard shall enter the laboratory. (iii) Children under 12 years of age shall not enter the laboratory. (iv) Work surfaces shall be decontaminated daily and immediately following spills of recombinant DNA materials. (v) Liquid wastes of recombinant DNA materials shall be decontaminated before disposal. (vi) Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. Packaged materials shall be disposed of by incineration or sterilized before disposal by other methods. Contaminated materials that are to be processed and reused (i.e., glassware) shall be decontaminated before removal from the laboratory. (vii) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used. (viii) Eating, drinking, smoking, and storage of food are not permitted in the working area. (ix) Facilities to wash hands shall be available within the laboratory. Persons handling recombinant DNA materials should be encouraged to wash their hands frequently and when they leave the laboratory. (x) An insect and rodent control program shall be provided. (xi) The use of laboratory gowns, coats, or uniforms is required. Such clothing shall not be worn to the lunch room or outside the building. (xii) Animals not related to the experiment shall not be permitted in the laboratory. (xiii) Biological Safety Cabinets[1] and/or other physical containment equipment shall be used to minimize the hazard of aerosolization of recombinant DNA materials from operations or devices that produce a considerable aerosol (e.g., blender, lyophilizer, sonicator, shaking machine, etc.). (xiv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

[1] Footnotes at end of article.

P3 Level (Moderate). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P3 level has special engineering design features and physical containment equipment. The laboratory is separated from areas which are open to the general public. Separation is generally achieved by controlled access corridors, air locks, locker rooms or other double-doored facilities which are not available for use by the general public. Access to the laboratory is controlled. Biological Safety Cabinets[1] are available within the controlled laboratory area. An autoclave shall be available within the building and preferably within the controlled laboratory area. The surfaces of walls, floors, bench tops, and ceilings are easily cleanable to facilitate housekeeping and space decontamination.

Directional air flow is provided within the controlled laboratory area. The ventilation system is balanced to provide for an inflow of supply air from the access corridor into the laboratory. The general exhaust air from the laboratory is discharged outdoors and so dispersed to the atmosphere as to prevent reentry into the building. No recirculation of the exhaust air shall be permitted without appropriate treatment.

No work in open vessels involving hosts or vectors containing recombinant DNA molecules requiring P3 physical containment is conducted on the open bench. All such procedures are confined to Biological Safety Cabinets.[1]

The following practices shall apply to all experiments requiring P3 level physical containment: (i) The universal biohazard sign is required on all laboratory access doors. Only persons whose entry into the laboratory is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the potential biohazards before entry and they shall comply with posted entry and exit procedures. Children under 12 years of age shall not enter the laboratory. (ii) Laboratory doors shall be kept closed while experiments are in progress. (iii) Biological Safety Cabinets[1] and other physical containment equipment shall be used for all procedures that produce aerosols of recombinant DNA materials (e.g., pipetting, plating, fluming, transfer operations, grinding, blending, drying, sonicating, shaking, etc.). (iv) The work surfaces of Biological Safety Cabinets[1] and other equipment shall be decontaminated following the completion of the experimental activity contained within them. (v) Liquid wastes containing recombinant DNA materials shall be decontaminated before disposal. Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. Packaged material shall be sterilized before disposal. Contaminated materials that are to be processed and reused (i.e., glassware) shall be sterilized in the controlled laboratory area or placed in a durable leak-proof container before removal from the controlled laboratory area. This container shall be sterilized before the materials are processed. (vii) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used. (viii) Eating drinking, smoking, and storage of food are not permitted in the laboratory. (ix) Facilities to wash hands shall be available within the laboratory. Persons shall wash hands after experiments involving recombinant DNA materials and before leaving the laboratory. (x) An insect and rodent control program shall be provided. (xi) Laboratory clothing that protects street clothing (i.e., long sleeve solid-front or wrap-around gowns, no-button or slipover jackets, etc.) shall be worn in the laboratory. FRONT-BUTTON LABORATORY COATS ARE UNSUITABLE. Gloves shall be worn when handling recombinant DNA materials. Provision for laboratory shoes is recommended. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry. (xii) Raincoats, overcoats, topcoats, coats, hats, caps, and such street outerwear shall not be kept in the laboratory.

(xiii) Animals and plants not related to the experiment shall not be permitted in the laboratory. (xiv) Vacuum lines shall be protected by filters and liquid traps. (xv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xvi) If experiments of lesser biohazard potential are to be conducted in the same laboratory concurrently with experiments requiring P3 level physical containment they shall be conducted only in accordance with all P3 level requirements. (xvii) Experiments requiring P3 level physical containment can be conducted in laboratories where the directional air flow and general exhaust air conditions described above cannot be achieved, provided that this work is conducted in accordance with all other requirements listed and is contained in a Biological Safety Cabinet[1] with attached glove ports and gloves. All materials before removal from the Biological Safety Cabinet[1] shall be sterilized or transferred to a non-breakable, sealed container, which is then removed from the cabinet through a chemical decontamination tank, autoclave, ultraviolet air lock, or after the entire cabinet has been decontaminated.

P4 Level (High). Experiments involving recombinant DNA molecules requiring physical containment at the P4 level shall be confined to work areas in a facility of the type designed to contain microorganisms that are extremely hazardous to man or may cause serious epidemic disease. The facility is either a separate building or it is a controlled area, within a building, which is completely isolated from all other areas of the building. Access to the facility is under strict control. A specific facility operations manual is available. Class III Biological Safety Cabinets[1] are available within work areas of the facility.

A P4 facility has engineering features which are designed to prevent the escape of microorganisms to the environment (14, 15, 16, 17). These features include:

removal from these cabinets, shall be sterilized or transferred to a non-breakable sealed container, which is then removed from the system through a chemical decontaminated tank, autoclave, or after the entire system has been decontaminated.

(vii) No materials shall be removed from the facility unless they have been sterilized or decontaminatel in a manner to prevent the release of agents requiring P4 physical containment. All wastes and other materials and equipment not damaged by high temperautre or steam shall be sterilized in the double-door autoclave. Biological materials to be removed from the facility shall be transferred to a non-breakable sealed container which is then removed from the facility through a chemical decontamination tank or a chamber designed for gas sterilization. Other materials which may be damaged by temperature or steam shall be sterilized by gaseous or vapor methods in an air lock or chamber designed for this purpose. (viii) Eating, drinking, smoking, and storage of food are not permitted in the facility. Foot-operated water fountains located in the facility corridors are permitted. Separate potable water piping shall be provided for these water fountains. (ix) Facilities to wash hands shall be available within the facility. Persons shall wash hands after experiments. (x) An insect and rodent control program shall be provided. (xi) Animals and plants not related to the experiment shall not be permitted in the facility. (xii) If a central vacuum system is provided, each vacuum outlet shall be protected by a filter and liquid trap in addition to the branch line HEPA filter mentioned above. (xiii) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xiv) If experiments of lesser biohazard potential are to be conducted in the facility concurrently with experiments requiring P4 level containment, they shall be confined in Class I or Class II Biological Safety Cabinets [1] or isolated by other physical containment equipment. Work surfaces of Biological Safety Cabinets [1] and other equipment shall be decontaminated following the completion of the experimental activity contained within them. Mechanical pipetting devices shall be used. All other practices listed above with the exception of (vi) shall apply.

C. *Shipment.* To protect product, personnel, and the environment, all recombinant DNA material will be shipped in containers that meet the requirements issued by the U.S. Public Health Service (Section 72.25 of Part 72, Title 42, Code of Federal Regulations), Department of Transportation (Section 173.387(b) of Part 173, Title 49, Code of Federal Regulations) and the Civil Aeronautics Board (C.A.B. No. 82, Official Air Transport Restricted Articles Tariff No. 6-D) for shipment of etiologic agents. Labeling requirements specified in these Federal regulations and tariffs will apply to all viable recombinant DNA materials in which any portion of the material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25. Additional information on packing and shipping is given in a supplement to the guidelines (Appendix D, part X).

D. *Biological containment levels.* Biological barriers are specific to each host-vector system. Hence the criteria for this mechanism of containment cannot be generalized to the same extent as for physical containment. This is particularly true at the present time when our experience with existing host-vector systems and our predictive knowledge about projected systems are sparse. The classification of experiments with recombinant DNAs that is necessary for the construction of the experimeneal guidelines (Section III) can be accomplished with least confusion if we use the host-vector system as the primary element and the source of the inserted DNA as the secondary element in the classification. It is therefore convenient to specify the nature of the biological containment under host-vector headings such as those given below for *Escherichia coli* K-12.

III. EXPERIMENTAL GUIDELINES

A general rule that, though obvious, deserves statement is that the level of containment required for any experiment on DNA recombinants shall never be less than that required for the most hazardous component used to construct and clone the recombinant DNA (i.e., vector, host, and inserted DNA). In most cases the level of containment will be greater, particularly when the recombinant DNA is formed from species that ordinarily do not exchange genetic information. Handling the purified DNA will generally require less stringent precautions than will propagating the DNA. However, the DNA itself should be handled at least as carefully as one would handle the most dangerous of the DNAs used to make it.

The above rule by itself effectively precludes certain experiments—namely, those in which one of the components is in Class 5 of the "Classification of Etiologic Agents on the Basis of Hazard" (5), as these are excluded from the United States by law and USDA administrative policy. There are additional experiments which may engender such serious biohazards that they are not to be performed at this time. These are considered prior to presentation of the containment guidelines for permissible experiments.

A. *Experiments that are not to be performed.* We recognize that it can be argued that certain of the recombinants placed in this category could be adequately contained at this time. Nonetheless, our estimates of the possible dangers that may ensue if that containment fails are of such a magnitude that we consider it the wisest policy to at least defer experiments on these recombinant DNAs until there is more information to accurately assess that danger and to allow the construction of more effective biological barriers. In this respect, these guidelines are more stringent than those initially recommended (1).

The following experiments are not to be initiated at the present time: (i) Clon-

[1] See footnotes at end of article.

ing of recombinant DNAs derived from the pathogenic organisms in Classes 3, 4, and 5 of "Classification of Etiologic Agents on the Basis of Hazard" (5), or oncogenic viruses classified by NCI as moderate risk (6), or cells known to be infected with such agents, regardless of the host-vector system used. (ii) Deliberate formation of recombinant DNAs containing genes for the biosynthesis of potent toxins (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.). (iii) Deliberate creation from plant pathogens of recombinant DNAs that are likely to increase virulence and host range. (iv) Deliberate release into the environment of any organism containing a recombinant DNA molecule. (v) Transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

In addition, at this time large-scale experiments (e.g., more than 10 liters of culture) with recombinant DNAs known to make harmful products are not to be carried out. We differentiate between small- and large-scale experiments with such DNAs because the probability of escape from containment barriers normally increases with increasing scale. However, specific experiments in this category that are of direct societal benefit may be excepted from this rule if special biological containment precautions and equipment designed for large-scale operations are used, and provided that these experiments are expressly approved by the Recombinant DNA Molecule Program Advisory Committee of NIH.

B. *Containment guidelines for permissible experiments.* It is anticipated that most recombinant DNA experiments initiated before these guidelines are next reviewed (i.e., within the year) will employ *E. coli* K–12 host-vector systems. These are also the systems for which we have the most experience and knowledge regarding the effectiveness of the containment provided by existing hosts and vectors necessary for the construction of more effective biological barriers.

For these reasons, *E. Coli* K–12 appears to be the system of choice at this time, although we have carefully considered arguments that many of the potential dangers are compounded by using an organism as intimately connected with a man as is *E. Coli*. Thus, while proceeding cautiously with *E. Coli*, serious efforts should be made toward developing alternate host-vector systems; this subject is discussed in considerable detail in Appendix A.

We therefore consider DNA recombinants in *E. coli* K–12 before proceeding to other host-vector systems.

1. *Biological containment criteria using E. coli K–12 host-vectors—EK1 host-vectors.* These are host-vector systems that can be estimated to already provide a moderate level of containment, and include most of the presently available systems. The host is always *E. coli* K–12, and the vectors include nonconjugative plasmids (e.g., pSC101, ColE1 or derivatives thereof (19–26)) and variants of bacteriophage λ (27–29).

The *E. coli* K–12 nonconjugative plasmid system is taken as an example to illustrate the approximate level of containment referred to here. The available data from experiments involving the feeding of bacteria to humans and calves (30–32) indicate that *E. coli* K–12 did not usually colonize the *normal* bowel, and exhibited little, if any, multiplication while passing through the alimentary tract even after feeding high doses (i.e., 10^9 to 10^{10} bacteria per human or calf). However, general extrapolation of these results may not be warranted because the implantation of bacteria into the intestinal tract depends on a number of parameters, such as the nature of the intestinal flora present in a given individual and the physiological state of the inoculum. Moreover, since viable *E. coli* K–12 can be found in the feces after humans are fed 10^9 bacteria in broth (30) or 3×10^8 bacteria protected by suspension in milk (31), transductional and conjugational transfer of the plasmid vectors from *E. coli* K–12 to resident bacteria in the fecal matter before and after excretion must also be considered.

The nonconjugative plasmid vectors cannot promote their own transfers, but require the presence of a conjugative plasmid for mobilization and transfer to other bacteria. When present in the same cell with derepressed conjugative plasmids such as F or R*1drd19*, the nonconjugative ColE1, ColE1-*trp* and pSC101 plasmids are transferred to suitable recipient strains under ideal laboratory conditions at frequencies of about 0.5, 10^{-4} to 10^{-5}, and 10^{-6} per donor cell, respectively. These frequencies are reduced by another factor of 10^2 to 10^4 if the conjugative plasmid employed is repressed with respect to expression of donor fertility.

The experimental transfer system which most closely resembles nonconjugative plasmid transfer in nature is a triparental mating. In such matings, the bacterial cell possessing the nonconjugative plasmid must first acquire a conjugative plasmid from another cell before it can transfer the nonconjugative plasmid to a secondary recipient. With ColE1, the frequencies of transfer are 10^{-2} and 10^{-4} to 10^{-6} when using conjugative plasmid donors possessing derepressed and repressed plasmids, respectively. Mobilization of ColE1-*trp* and pSC101 under similar laboratory conditions is so low as to be usually undetectable (33). Since most conjugative plasmids in nature are repressed for expression of donor fertility, the frequency at which nonconjugative plasmids are mobilized and transferred by this sequence of events *in vivo* is difficult to estimate. However, in calves fed on an antibiotic-supplemented diet, it has been estimated that such triparental nonconjugative R plasmid transfer occurs at frequencies of no more than 10^{-10} to 10^{-12} per 24 hours per calf (32). In terms of considering other means for plasmid transmission in nature, it should be noted that transduction does operate *in vivo* for *Staphylococcus aureus* (34) and probably for *E. coli* as well. However, no data are available to indicate the frequencies of plasmid transfer *in vivo* by either transduction or transformation.

These observations indicate the low probabilities for possible dissemination of such plasmid vectors by accidental ingestion, which would probably involve only a few hundred or thousand bacteria provided that at least the standard practices (Section II–A above) are followed, particularly the avoidance of mouth pipetting. The possibility of colonization and hence of transfer are increased, however, if the normal flora in the bowel is disrupted by, for example, antibiotic therapy (35). For this reason, persons receiving such therapy must not work with DNA recombinants formed with any *E. coli* K–12 host-vector system during the therapy period and for seven days thereafter; similarly, persons who have achlorhydria or who have had surgical removal of part of the stomach or bowel should avoid such work, as should those who require large doses of antacids.

The observations on the fate of *E. coli* K–12 in the human alimentary tract are also relevant to the containment of recombinant DNA formed with bacteriophage λ variants. Bacteriophage can escape from the laboratory either as mature infectious phage particles or in bacterial host cells in which the phage genome is carried as a plasmid or prophage. The fate of *E. coli* K–12 host cells carrying the phage genome as a plasmid or prophage is similar to that for plasmid-containing host cells as discussed above. The survival of the λ phage genome when released as infectious particles depends on their stability in nature, their infectivity and on the probability of subsequent encounters with naturally occurring λ-sensitive *E. coli* strains. Although the probability of survival of λ and its infection of resident intenstinal *E. coli* in animals and humans has not been measured, it is estimated to be small given the high sensitivity of λ to the low pH of the stomach, the insusceptibility to λ infection of smooth *E. coli* cells (the type that normally resides in the gut), the infrequency of naturally occurring λ-sensitive *E. coli* (36) and the failure to detect infective λ particles in human feces after ingestion of up to 10^{11} λ particles (37). Moreover, λ particles are very sensitive to desiccation.

Establishment of λ as a stable lysogen is a frequent event (10^0 to 10^{-3}) for the *att*$^+$ *int*$^+$ *cI*$^+$ phage so that this mode of escape would be the preponderant laboratory hazard; however, most EK1 λ vectors currently in use lack the *att* and *int* functions (27–29) thus reducing the probability of lysogenization to about 10^{-2} to 10^{-6} (38–40). The frequency for the conversion of λ to a plasmid state for persistence and replication is also only about 10^{-6} (41). Moreover, the routine treatment of phage lysates with chloroform (42) should eliminate all surviving bacteria including lysogens and λ plasmid carriers. Lysogenization could also occur

when an infectious λ containing cloned DNA infects a λ-sensitive cell in nature, and recombines with a resident lambdoid prophage. Although λ-sensitive *E. coli* strains seem to be rare, a significant fraction do carry lambdoid prophages (43–44) and thus this route of escape should be considered.

While not exact, the estimates for containment afforded by using these host-vectors are at least as accurate as those for physical containment, and are sufficient to indicate that currently employed plasmid and λ vector systems provide a moderate level of biological containment. Other nonconjugative plasmids and bacteriophages that, in association with *E. coli* K–12 can be estimated to provide the same approximate level of moderate containment are included in the EK1 class.

EK2 host-vectors. These are host-vector systems that have been genetically constructed and shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. The genetic modifications of the *E. coli* K–12 host and/or the plasmid or phage vector should not permit survival of a genetic marker carried on the vector, preferably a marker within an inserted DNA fragment, in other than specially designed and carefully regulated laboratory environments at a frequency greater than 10^{-8}. This measure of biological containment has been selected because it is a measurable entity. Indeed, by testing the contributions of preexisting and newly introduced genetic properties of vectors and hosts, individually or in various combinations, it should be possible to estimate with considerable precision, that the specially designed host-vector system can provide a margin of biological containment in excess of that required. For the time being, no host-vector system will be considered to be a bona fide EK2 host-vector system until it is so certified by the NIH Recombinant DNA Molecule Program Advisory Committee.

For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate the vector and/or a cloned DNA fragment under non-permissive conditions designed to represent the natural environment either by survival of the original host or as a consequence of transmission of the vector and/or a cloned DNA fragment by transformation, transduction or conjugation to a host with properties common to those in the natural environment.

In terms of potential EK2 plasmid-host systems, the following types of genetic modifications should reduce survival of cloned DNA. *The examples given are for illustrative purposes and should not be construed to encompass all possibilities.* The presence of the non-conjugative plasmids ColE1-*trp* and pSI01 in an *E. coli* K–12 strain possessing a mutation eliminating host-controlled restriction and modification (*hsdS*) results in about 10^3-fold reduction in mobilization to restriction-proficient recipients. The combination of the *dapD8*, *ΔbioH-asd*, *Δgal-chl*^{v} and *rfb* mutations in *E. coli* K–12 results in no detectable survivors in feces of rats following feeding by stomach tube of 10^{10} cells in milk and similarly leads to complete lysis of cells suspended in broth medium lacking diaminopimelic acid. *E. coli* K–12 strains with *ΔthyA* and *deoC* (*dra*) mutations undergo thymineless death in growth medium lacking thymine and give a 10^5-fold reduced survival during passage through the rat intestine compared to wild-type *thy*$^{+}$ *E. coli* K–12. (However, the *ΔthyA* mutation alone or in combination with a *deoB*(*drm*) mutation only reduces *in vivo* survival by a factor of 10^2.) Other host mutations, as yet untested, that might further reduce survival of the plasmid-host system or reduce plasmid transmission are: the combination *polA*(TS) *recA*(TS) *ΔthyA* which might interfere with ColE1 replication and lead to DNA degradation at body temperatures; Con^{-} mutations that reduce the ability of conjugative plasmids to enter the plasmid-host complex and thus should reduce mobilization of the cloned DNA to other strains; and mutations that confer resistance to known transducing phages. Mutations can also be introduced into the plasmid to cause it to be dependent on a specific host, to make its replication thermosensitive and/or to endow it with a killer capability such that all cells (other than its host) into which it might be transferred will not survive.

In the construction of EK2 plasmid-host systems it is important to use the most stable mutations available, preferably deletions. Obviously, the presence of all mutations contributing to higher degrees of biological containment must be verified periodically by appropriate tests. In testing the level of biological containment afforded by a proposed EK2 plasmid-host system, it is important to design relevant tests to evaluate the survival of the vector and/or a cloned DNA fragment under conditions that are possible in nature and that are also most advantageous for its perpetuation. For example, one might conduct a triparental mating with a primary donor possessing a derepressed F-type or I-type conjugative plasmid, the safer host with *ΔbioH-asd*, *dapD8*, *Δgal-chl*^{v}, *rfb*, *ΔthyA*, *deoC*, *trp* and *hsdS* mutations and a plasmid vector carrying an easily detectable marker such as for ampicillin resistance or an inserted gene such as *trp*$^{+}$, and a secondary recipient that is Su^{+} *hsdS trp* (i.e., permissive for the recombinant plasmid). Such matings would be conducted in a medium lacking diaminopimelic acid and thymine and survival of the Ap^{r} or *trp*$^{+}$ marker in any of the three strains followed as a function of time. Survival of the vector and/or a cloned marker by transduction could also be evaluated by introducing a known generalized transducing phage into the system. Similar experiments should also be done using a secondary recipient that is restrictive for the plasmid vector as well as with primary donors possessing repressed conjugative plasmids with incompatibility group properties like those commonly found in enteric microorganisms. Since a common route of escape of plasmid-host systems in the laboratory might be by accidental ingestion, it is suggested that the same types of experiments be conducted in suitable animal-model systems. In addition to these tests on survival of the vector and/or a cloned DNA fragment, it would be useful to determine the survival of the host strain under nongrowth conditions such as in water and as a function of drying time after a culture has been spilled on a lab bench.

For EK2 host-vector systems in which the vector is a phage, no more than one in 10^4 phage particles should be able to perpetuate itself and/or a cloned DNA fragment under non-permissive conditions designed to represent the natural environment either (a) as a prophage or plasmid in the laboratory host used for phage propagation or (b) by surviving in natural environments and transferring itself and/or a cloned DNA fragment to a host (or its resident lamboid prophage) with properties common to those in the natural environment.

In terms of potential EK2 λ-host systems, the following types of genetic modification should reduce survival of cloned DNA. *The examples given are for illustrative purposes and should not be construed to encompass all possibilities.* The probability of establishing λ lysogeny in the normal laboratory host should be reduced by removal of the phage *att* site, the Int function, the repressor gene(s) and adding virulence-enhancing mutations. The frequency of plasmid formation, although normally already less than 10^{-8}, could be further reduced by defects in the *pa-Q* region, including mutations such as *vir-s*, *cro*(TS), *cI*^{17}, *rI*^{s}, *O*(TS), *P*(TS), and *nin*. Moreover, chloroform treatment used routinely following cell lysis would reduce the number of surviving cells, including possible lysogens or plasmid carriers, by more than 10^6. The host may also be modified by deletion of the host *att* site and inclusion of one or more of the mutations described above for plasmid-host systems to further reduce the chance of formation and survival of any lysogen or plasmid carrier cell.

The survival of escaping phage and the chance of encountering a sensitive host in nature are very low, as discussed for EK1 systems. The infectivity of the phage particles could be further reduced by introducing mutations (e.g., suppressed ambers) which would make the phage particles extremely unstable except under special laboratory conditions (e.g., high concentrations of salts or putrescine). Another means would be to make the phage itself a two-component system, by eliminating the tail genes and reproducing the phage as heads packed with DNA; when necessary and under specially controlled conditions, these heads could be made infective by adding tail preparations. An additional safety factor in this regimen is the extreme instability of the heads, unless they are stored in 10mM putrescine, a condition easy to obtain in the laboratory but not in nature. The propagation of the es-

caping phage in nature could further be blocked by adding various conditional mutations which would permit growth only under special laboratory conditions or in a special permissive laboratory host with suppressor or *gro*-type (*mop, dnaB, rpoB*) mutations. An additional safety feature would be the use of an r⁻m⁻ (*hsdS*) laboratory host, which produces phage with unmodified DNA which should be restricted in r⁺m⁺ bacteria that are probably prevalent in nature. The likelihood of recombination between the λ vector and lambdoid prophages which are present in some *E. coli* strains might be reduced by elimination of the Red function and the presence of the recombination-reducing Gam function together with mutations contributing to the high lethality of the λ phage. However, these second-order precautions might not be relevant if the stability and infectivity of the escaping λ particles are reduced by special mutations or by propagating the highly unstable heads.

Despite multiple mutations in the phage vectors and laboratory hosts, the yield of phage particles under suitable laboratory conditions should be high (10^{10}-10^{11} particles/ml). This permits phage propagation in relatively small volumes and constitutes an additional safety feature.

The phenotypes and genetic stabilities of the mutations and chromosome alterations included in these λ-host systems indicate that containment well in excess of the required 10^{-6} or lower survival frequency for the λ vector with or without a cloned DNA fragment should be attained. Obviously the presence of all mutations contributing to this high degree of biological containment must be verified periodically by appropriate tests. Laboratory tests should be performed with the bacterial host to measure all possible routes of escape such as the frequency of lysogen formation, the frequency of plasmid formation and the survival of the lysogen or carrier bacterium. Similarly, the potential for perpetuation of a cloned DNA fragment carried by infectious phage particles can be tested by challenging typical wild-type *E. coli* strains or a λ-sensitive nonpermissive laboratory K-12 strain, especially one lysogenic for a lambdoid phage.

In view of the fact that accurate assessment of the probabilities for escape of infectious λ-grown on r⁻ m⁻ Su⁺ hosts is dependent upon the frequencies of r⁺, Su⁺, and λ-sensitive strains in nature, investigators need to screen *E. coli* strains for these properties. These data will also be useful in predicting frequencies of successful escape of plasmid cloning vectors harbored in r⁻ m⁻ Su⁺ strains.

When any investigator has obtained data on the level of containment provided by a proposed EK2 system, these should be reported as rapidly as possible to permit general awareness and evaluation of the safety features of the new system. Investigators are also encouraged to make such new safer cloning systems generally available to other scientists. NIH will take appropriate steps to aid in the distribution of these safer vectors and hosts.

EK3 host-vectors. These are EK2 systems for which the specified containment shown by laboratory tests has been independently confirmed by appropriate tests in animals, including humans or primates, and in other relevant environments in order to provide additional data to validate the levels of containment afforded by the EK2 host-vector systems. Evaluation of the effects of individual or combinations of mutations contributing to the biological containment should be performed as a means to confirm the degree of safety provided and to further advance the technology of developing even safer vectors and hosts. For the time being, no host-vector system will be considered to be a bona fide EK3 host-vector system, until it is so certified by the NIH Recombinant DNA Molecule Program Advisory Committee.

2. *Classification of experiments using the E. coli K-12 containment systems.* In the following classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimums. Higher levels of biological containment (EK3 > EK2 > EK1) are to be used if they are available and are equally appropriate for the purposes of the experiment.

(a) *Shotgun Experiments.* These experiments involve the production of recombinant DNAs between the vector and the total DNA or (preferably) any partially purified fraction thereof from the specified cellular source.

(i) *Eukaryotic DNA recombinants—Primates.* P3 physical containment + an EK3 host-vector, or P4 physical containment + an EK2 host-vector, except for DNA from uncontaminated embryonic tissue or primary tissue cultures therefrom, and germ-line cells for which P3 physical containment + an EK2 host-vector can be used. The basis for the lower estimated hazard in the case of DNA from the latter tissues (if freed of adult tissue) is their relative freedom from horizontally acquired adventitious viruses.

Other mammals. P3 physical containment + an EK2 host-vector.

Birds. P3 physical containment + an EK2 host-vector.

Cold-blooded vertebrates. P2 physical containment + an EK2 host-vector except for embryonic or germ-line DNA which require P2 physical containment + an EK1 host-vector. If the eukaryote is known to produce a potent toxin, the containment shall be increased to P3 + EK2.

Other cold-blooded animals and lower eukaryotes. This large class of eukaryotes is divided into the following two groups:

(*1*) Species that are known to produce a potent toxin or are known pathogens (i.e., an agent listed in Class 2 of ref. 5 or a plant pathogen) or are known to carry such pathogenic agents must use P3 physical containment + an EK2 host-vector. Any species that has a demonstrated capacity for carrying particular pathogenic agents is included in this group unless it has been shown that those organisms used as the source of DNA do not contain these agents; in this case they may be placed in the second group.

(*2*) The remainder of the species in this class can use P2 + EK1. However, any insect in this group should have been grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA.

Plants. P2 physical containment + an EK1 host-vector. If the plant carries a known pathogenic agent or makes a product known to be dangerous to any species, the containment must be raised to P3 physical containment + an EK2 host-vector.

(ii) *Prokaryotic DNA recombinants—Prokaryotes that exchange genetic information with E. coli.*[4] The level of physical containment is directly determined by the rule of the most dangerous component (see introduction to Section III). Thus P1 conditions can be used for DNAs from those bacteria in Class 1 of ref. 5 ("Agents of no or minimal hazard ***.") which naturally exchange genes with *E. coli;* and P2 conditions should be used for such bacteria if they fall in Class 2 of ref. 5 ("Agents of ordinary potential hazard * * *."), or are plant pathogens or symbionts. EK1 host-vectors can be used for all experiments requiring only P1 physical containment; in fact, experiments in this category can be performed with *E. coli* K-12 vectors exhibiting a lesser containment (e.g., conjugative plasmids) than EK1 vectors. Experiments with DNA from species requiring P2 physical containment which are of low pathogenicity (for example, enteropathogenic *Escherichia coli, Salmonella typhimurium,* and *Klebsiella pneumoniae*) can use EK1 host-vectors, but those of moderate pathogenicity (for example, *Salmonella typhi, Shigella dysenteriae* type I, and *Vibrio cholerae*) must use EK2 host-vectors.[3] A specific example of an experiment with a plant pathogen requiring P2 physical containment + an EK2 host-vector would be cloning the tumor gene of *Agrobacterium tumefaciens.*

Prokaryotes that do not exchange genetic information with E. coli. The minimum containment conditions for this class consist of P2 physical containment + an EK2 host-vector or P3 physical containment + an EK1 host-vector, and apply when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal. Experiments with DNAs from pathogenic species (Class 2 ref. 5 plus plant pathogens) must use P3 + EK2.

(iii) *Characterized clones of DNA recombinants derived from shotgun experiments.* When a cloned DNA recombinant has been rigorously characterized[4] and there is sufficient evidence that it is free of harmful genes,[4] then experiments involving this recombinant DNA can be carried out under P1 + EK1 conditions if the inserted DNA is from a

See footnotes at end of article.

species that exchanges genes with *E. coli*, and under P2 + EK1 conditions if not.

(*b*) *Purified cellular DNAs other than plasmids, bacteriophages, and other viruses.* The formation of DNA recombinants from cellular DNAs that have been enriched[5] by physical and chemical techniques (i.e., not by cloning) and which are free of harmful genes can be carried out under lower containment conditions than used for the corresponding shotgun experiment. In general, the containment can be decreased one step in physical containment (P4→P3→P2→P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (EK3→EK2→EK1) while maintaining the specified physical containment,—provided that the new condition is not less than that specified above for characterized clones from shotgun experiments (Section (a)—iii).

(*c*) *Plasmids, bacteriophages, and other viruses.* Recombinants formed between EK-type vectors and other plasmid or virus DNAs have in common the potential for acting as double vectors because of the replication functions in these DNAs. The containment conditions given below apply only to propagation of the DNA recombinants in *E. coli* K-12 hosts. They do not apply to other hosts where they may be able to replicate as a result of functions provided by the DNA inserted into the EK vectors. These are considered under other host-vector systems.

(i) *Animal viruses.* P4+EK2 or P3EK3 shall be used to isolate DNA recombinants that include *all or part* of the genome of an animal virus. This recommendation applies not only to experiments of the "shotgun" type but also to those involving partially characterized subgenomic segments of viral DNAs (for example, the genome of defective viruses, DNA fragments isolated after treatment of viral genomes with restriction enzymes, etc). When cloned recombinants have been shown by suitable biochemical and biological tests to be free of harmful regions, they can be handled in P3+EK2 conditions. In the case of DNA viruses, harmless regions include the late region of the genome; in the case of DNA copies of RNA viruses, they might include the genes coding for capsid proteins or envelope proteins.

(ii) *Plant viruses.* P3+EK1 or P2+EK2 conditions shall be used to form DNA recombinants that include *all or part* of the genome of a plant virus.

(iii) *Eukaryotic organelle DNAs.* The containment conditions given below apply only when the organelle DNA has been purified[4] from isolated organelles. Mitochondrial DNA from primates: P3+EK1 or P2+EK2. Mitochondrial or chloroplast DNA from other eukaryotes: P2+EK1. Otherwise, the conditions given under shotgun experiments apply.

(iv) *Prokaryotic plasmid and phage DNAs—Plasmids and phage from hosts that exchange genetic information with E. coli.* Experiments with DNA recombinants formed from plasmids or phage genomes that have not been characterized with regard to presence of harmful genes or are known to contribute significantly to the pathogenicity of their normal hosts must use the containment conditions specified for shotgun experiments with DNAs from the respective host. If the DNA recombinants are formed from plasmids or phage that are known not to contain harmful genes, or from purified[4] and characterized plasmid or phage DNA segments known not to contain harmful genes, the experiments can be performed with P1 physical containment + an EK1 host-vector.

Plasmids and phage from hosts that do not exchange genetic information with E. coli. The rules for shotgun experiments with DNA from the host apply to their plasmids or phages. The minimum containment conditions for this category (P2+EK2, or P3+EK1) can be used for plasmid and phage, or for purified[4] and characterized segments of plasmid and phage DNAs, when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal.

NOTE.—Where applicable, cDNAs(i.e., complementary DNAs) synthesized *in vitro* from cellular or viral RNAs are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under (a), shotgun experiments; cDNAs formed from purified and characterized RNAs are included under (b); cDNAs formed from viral RNAs are included under (c); etc.

3. *Experiments with other prokaryotic host-vectors.* Other prokaryotic host-vector systems are at the speculative, planning, or developmental stage, and consequently do not warrant detailed treatment here at this time. However, the *containment criteria for different types* of DNA recombinants formed with *E. coli* K-12 host-vectors can, with the aid of some general principles given here, serve as a guide for containment conditions with other host-vectors when appropriate adjustment is made for their different habitats and characteristics. The newly developed host-vector systems should offer some distinct advantage over the *E. coli* K-12 host-vectors—for instance, thermophilic organisms or other host-vectors whose major habitats do not include humans and/or economically important animals and plants. In general, the strain of any prokaryotic species used *as the host is to conform to the definition* of Class 1 etiologic agents given in ref. 5 (i.e., "Agents of no or minimal hazard. * * *"), and the plasmid or phage vector should not make the host more hazardous. Appendix A gives a detailed discussion of the *B. subtilis* system, the most promising alternative to date.

At the initial stage, the host-vector must exhibit at least a moderate level of biological containment comparable to EK1 systems, and should be capable of modification to obtain high levels of containment comparable to EK2 and EK3. The type of confirmation test(s) required to move a host-vector from an EK2-type classification to an EK3-type will clearly depend upon the preponderant habitat of the host-vector. For example, if the unmodified host-vector propagates mostly in, on, or around higher plants, but not appreciably in warm-blooded animals, modification should be designed to reduce the probability that the host-vector can escape to and propagate in, on, or around such plants, or transmit recombinant DNA to other bacterial hosts that are able to occupy these ecological niches, and it is these lower probabilities which must be confirmed. The following principles are to be followed in using the containment criteria given for experiments with *E. coli* K-12 host-vectors as a guide for other prokaryotic systems. Experiments with DNA from prokaryotes (and their plasmids or viruses) are classified according to whether the prokaryote in question exchanges genetic information with the host-vector or not, and the containment conditions given for these two classes with *E. coli* K-12 host-vectors applied. Experiments with recombinants between plasmid or phage vectors and DNA that extends the range of resistance of the recipient species to therapeutically useful drugs must use P3 physical containment + a host-vector comparable to EK1 or P2 physical containment + a host-vector comparable to EK2. Transfer of recombinant DNA to plant pathogens can be made safer by using nonreverting, doubly auxothrophic, non-pathogenic variants. Experiments using a plant pathogen that affects an element of the local flora will require more stringent containment than if carried out in areas where the host plant is not common.

Experiments with DNAs from eukaryotes (and their plasmids or viruses) can also follow the criteria for the corresponding experiments with *E. coli* K-12 vectors if the major habitats of the given host-vector overlap those of *E. coli*. If the host-vector has a major habitat that does not overlap those of *E. coli* (e.g., root nodules in plants), then the containment conditions for some eukaryotic recombinant DNAs need to be increased (for instance, higher plants and their viruses in the preceding example), while others can be reduced.

4. *Experiments with eukaryotic host-vectors—(a) Animal host-vector systems.* Because host cell lines generally have little if any capacity for propagation outside the laboratory, the primary focus for containment is the vector, although cells should also be derived from cultures expected to be of minimal hazard. Given good microbiological practices, the most likely mode escape of recombinant DNAs from a physically contained laboratory is carriage by humans; thus vectors should be chosen that have little or no ability to replicate in human cells. To be used as a vector in a eukaryotic host, a DNA molecule needs to display all of the following properties:

(1) It shall not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture.

(2) Its functional anatomy should be known—that is, there should be a clear

See footnotes at end of article.

idea of the location within the molecule of:

(a) The sites at which DNA synthesis originates and terminates,

(b) The sites that are cleaved by restriction endonucleases,

(c) The template regions for the major gene products.

(3) It should be well studied genetically. It is desirable that mutants be available in adequate number and variety, and that quantitative studies of recombination have been performed.

(4) The recombinant must be defective, that is, its propagation as a virus is dependent upon the presence of a complementing helper genome. This helper should either (a) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector to that particular cell line) or (b) consist of a defective genome or an appropriate conditional lethal mutant virus (in which case the experiments would be done under nonpermissive conditions), making vector and helper dependent upon each other for propagation. However, if none of these is available, the use of a non-defective genome as helper would be acceptable.

Currently only two viral DNAs can be considered as meeting these requirements: these are the genomes of polyoma virus and SV40.

Of these, polyoma virus is highly to be preferred. SV40 is known to propagate in human cells, both *in vivo* and *in vitro*, and to infect laboratory personnel, as evidenced by the frequency of their conversion to producing SV40 antibodies. Also, SV40 and related viruses have been found in association with certain human neurological and malignant diseases. SV40 shares many properties, and gives complementation, with the common human papova viruses. By contrast, there is no evidence that polyoma infects humans, nor does it replicate to any significant extent in human cells *in vitro*. However, this system still needs to be studied more extensively. Appendix B gives further details and documentation.

Taking account of all these factors:

(1) *Polyoma Virus.* (a) Recombinant DNA molecules consisting of defective polyoma virus genomes plus DNA sequences of any nonpathogenic organism, including Class 1 viruses (5), can be propagated in or used to transform cultured cells. P3 conditions are required. Appropriate helper virus can be used if needed. Whenever there is a choice, it is urged that mouse cells, derived preferably from embryos, be used as the source of eukaryotic DNA. Polyoma virus is a mouse virus and recombinant DNA molecules containing both viral and cellular sequences are already known to be present in virus stocks grown at a high multiplicity. Thus, recombinants formed *in vitro* between polyoma virus DNA and mouse DNA are presumably not novel from an evolutionary point of view.

(b) Such experiments are to be done under P4 conditions if the recombinant DNA contains segments of the genomes of Class 2 animal viruses (5). Once it has been shown by suitable biochemical and biological tests that the cloned recombinant contains only harmless regions of the viral genome (see Section IIIB-2-c-1) and that the host range of the polyoma virus vector has not been altered, experiments can be continued under P3 conditions.

(2) *SV40 Virus.*

(a) Defective SV40 genomes, with appropriate helper, can be used as a vector for recombinant DNA molecules containing sequences of any non-pathogenic organism or Class I virus (5), (i.e., a shotgun type experiment). P4 conditions are required. Established lines of cultured cells should be used.

(b) Such experiments are to be carried out in P3 (or P4) conditions if the non-SV40 DNA segment is (a) a purified " segment of prokaryotic DNA lacking toxigenic genes, or (b) a segment of eukaryotic DNA whose function has been established, which does not code for a toxic product, and which has been previously cloned in a prokaryotic host-vector system. It shall be confirmed that the defective virus-helper virus system does not replicate significantly more efficiently in human cells in tissue culture than does SV40, following infection at a multiplicity of infection of one or more helper SV40 viruses per cell.

(c) A recombinant DNA molecule consisting of defective SV40 DNA lacking substantial segments of the late region, plus DNA from non-pathogenic organisms or Class I viruses (5), can be propagated as an autonomous cellular element in established lines of cells under P3 conditions provided that there is no exogenous or endogenous helper, and that it is demonstrated that no infectious virus particles are being produced. Until this has been demonstrated, the appropriate containment conditions specified in 2. a. and 2. b. shall be used.

(d) Recombinant DNA molecules consisting of defective SV40 DNA and sequences from non-pathogenic prokaryotic or eukaryotic organisms or Class I viruses (5) can be used to transform established lines of non-permissive cells under P3 conditions. It must be demonstrated that no infectious virus particles are being produced; rescue of SV40 from such transformed cells by co-cultivation or transfection techniques must be carried out in P4 conditions.

(3) Efforts are to be made to ensure that all cell lines are free of virus particles and mycoplasma.

Since SV40 and polyoma are limited in their scope to act as vectors, chiefly because the amount of foreign DNA that the normal virions can carry probably cannot exceed 2×10^6 daltons, the development of systems in which recombinants can be cloned and propagated purely in the form of DNA, rather than in the coats of infectious agents is necessary. Plasmid forms of viral genomes or organelle DNA need to be explored as possible cloning vehicles in eukaryotic cells.

(b) *Plant host-vector systems.* For cells in tissue cultures, seedlings, or plant parts (e.g. tubers, stems, fruits, and detached leaves) or whole mature plants of small species (e.g., *Arabidopsis*) the P1-P4 containment conditions that we have specified previously are relevant concepts. However, work with most plants poses additional problems. The greenhouse facilities accompanying P2 laboratory physical containment conditions can be provided by: (i) insect-proof greenhouses, (ii) appropriate sterilization of contaminated plants, pots, soil, and runoff water, and (iii) adoption of the other standard practices for microbiological work. P3 physical containment can be sufficiently approximated by confining the operations with whole plants to growth chambers like those used for work with radioactive isotopes, provided that (i) such chambers are modified to produce a negative pressure environment with the exhaust air appropriately filtered, (ii) that other operations with infectious materials are carried out under the specified P3 conditions, and (iii) to guard against inadvertent insect transmission of recombinant DNA, growth chambers are to be routinely fumigated and only used in insect proof rooms. The P2 and P3 conditions specified earlier are therefore extended to include these cases for work on higher plants.

The host cells for experiments on recombinants DNAs may be cells in culture, in seedling or plant parts. Whole plants or plant parts that cannot be adequately contained shall not be used as hosts for shotgun experiments at this time, and attempts to infect whole plants with recombinant DNA shall not be initiated until the effects on host cells in culture, seedlings or plant parts have been thoroughly studied.

Organelle or plasmid DNAs or DNAs of viruses of restricted host range may be used as vectors. In general, similar criteria for selecting host-vectors to those given in the preceding section on animal systems are to apply to plant systems.

DNA recombinants formed between the initial moderately contained vectors and DNA form cells of species in which the vector DNA can replicate, require P2 physical containment. However, if the source of the DNA is itself pathogenic or known to carry pathogenic agents, or to produce products dangerous to plants, or if the vector is an unmodified virus of unrestricted host range, the experiments shall be carried out under P3 conditions.

Experiments on recombinant DNAs formed between the above vectors and DNAs from other species can also be carried out under P2 if that DNA has been purified[4] and determined not to contain harmful genes. Otherwise, the experiments shall be carried out under P3 conditions if the source of the inserted DNA is not itself a pathogen, or known to carry such pathogenic agents, or to produce harmful products—and under P4 conditions if these conditions are not met.

The development and use of host-vector systems that exhibit a high level of biological containment permit a decrease or one step in the physical con-

See footnotes at end of article.

tainment specified above (P4→P3→P2→P1).

(c) *Fungal or similar lower eukaryotic host-vector systems.* The containment criteria for experiments on recombinant DNAs using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, in that the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. We therefore consider that the containment guidelines given for experiments with *E. coli* K–12 and other prokaryotic host-vectors (Sections IIIB–1 and –2, respectively) provide adequate direction for experiments with these lower eukaryotic host-vectors. This is particularly true at this time since the development of these host-vectors is presently in the speculative stage.

IV. ROLES AND RESPONSIBILITIES

Safety in research involving recombinant DNA molecules depends upon how the research team applies these guidelines. Motivation and critical judgment are necessary, in addition to specific safety knowledge, to ensure protection of personnel, the public, and the environment.

The guidelines given here are to help the principal investigator determine the nature of the safeguards that should be implemented. These guidelines will be incomplete in some respects because all conceivable experiments with recombinant DNAs cannot now be anticipated. Therefore, they cannot substitute for the investigator's own knowledgeable and discriminating evaluation. Whenever this evaluation calls for an increase in containment over that indicated in the *guidelines, the investigator has a responsibility* to institute such an increase. In contrast, the containment conditions called for in the guidelines should not be decreased without review and approval at the institutional and NIH levels.

The following roles and responsibilities define an administrative framework in which safety is an essential and integrated function of research involving recombinant DNA molecules.

A. *Principal Investigator.* The principal investigator has the primary responsibility for: (i) Determining the real and potential biohazards of the proposed research, (ii) determining the appropriate level of biological and physical containment, (iii) selecting the microbiological practices and laboratory techniques for handling recombinant DNA materials, (iv) preparing procedures for dealing with accidental spills and overt personnel contamination, (v) determining the applicability of various precautionary medical practices, serological monitoring, and immunization, when available, (vi) securing approval of the proposed research prior to initiation of work, (vii) submitting information on purported EK2 and EK3 systems to the NIH Recombinant DNA Molecule Program Advisory Committee and making the strains available to others, (viii) reporting to the institutional biohazards committee and the NIH Office of Recombinant DNA Activities new information bearing on the guidelines, such as technical information relating to hazards and new safety procedures or innovations, (ix) applying for approval from the NIH Recombinant DNA Molecule Program Advisory Committee for large scale experiments with recombinant DNAs known to make harmful products (i.e., more than 10 liters of culture), and (x) applying to NIH for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes.

Before work is begun, the principal investigator is responsible for: (1) Making available to program and support staff copies of those portions of the approved grant application that describe the biohazards and the precautions to be taken, (ii) advising the program and support staff of the nature and assessment of the real and potential biohazards, (iii) instructing and training this staff in the practices and techniques required to ensure safety, and in the procedures for dealing with accidentally created biohazards, and (iv) informing the staff of the reasons and provisions for any advised or requested precautionary medical practices, vaccinations, or serum collection.

During the conduct of the research, the *principal investigator is responsible for:* (i) Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed, (ii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities and the institutional biohazards committee any serious or extended illness of a worker or any accident that results in (a) inoculation of recombinant DNA materials through cutaneous penetration, (b) ingestion of recombinant DNA materials, (c) probable inhalation of recombinant DNA materials following gross aerosolization, or (d) any incident causing serious exposure to personnel or danger of environmental contamination, (iii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities and the institutional biohazards committee any problems pertaining to operation and implementation of biological and physical containment safety practices and procedures, or equipment or facility failure, (iv) correcting work errors and conditions that may result in the release of recombinant DNA materials, and (v) ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., genotypic and phenotypic characteristics, purity, etc.).

B. *Institution.* Since in almost all cases, NIH grants are made to institutions rather than to individuals, all the responsibilities of the principal investigator listed above are the responsibilities of the institution under the grant, fulfilled on its behalf by the principal investigator. In addition, the institution is responsible for establishing an institutional biohazards committee[1] to: (i) Advise the institution on policies, (ii) create and maintain a central reference file and library of catalogs, books, articles, newsletters, and other communications as a source of advice and reference regarding, for example, the availability and quality of the safety equipment, the availability and level of biological containment for various host-vector systems, suitable training of personnel and data on the potential biohazards associated with certain recombinant DNAs, (iii) develop a safety and operations manual for any P4 facility maintained by the institution and used in support of recombinant DNA research, (iv) certify to the NIH on applications for research support and annually thereafter, that facilities, procedures, and practices and the training and expertise of the personnel involved *have been reviewed and approved by the* institutional biohazards committee.

The biohazards committee must be sufficiently qualified through the experience and expertise of its membership and the diversity of its membership to ensure respect for its advice and counsel. Its membership should include individuals from the institution or consultants, selected so as to provide a diversity of disciplines relevant to recombinant DNA technology, biological safety, and engineering. In addition to possessing the professional competence necessary to assess and review specific activities and facilities, the *committee should possess or have available* to it, the competence to determine the acceptability of its findings in terms of applicable laws, regulations, standards of practices, community attitudes, and health and environmental considerations. Minutes of the meetings should be kept and made available for public inspection. The *institution is responsible for* reporting names of and relevant background information on the members of its biohazards committee to the NIH.

C. *NIH Initial Review Groups (Study Sections).* The NIH Study Sections, in addition to reviewing the scientific merit of each grant application involving recombinant DNA molecules, are responsible for: (i) Making an independent evaluation of the real and potential biohazards of the proposed research on the basis of these guidelines, (ii) determining whether the proposed physical containment safeguards certified by the *institutional biohazards committee* are appropriate for control of these biohazards, (iii) determining whether the proposed biological containment safeguards are appropriate, (iv) referring to the NIH Recombinant DNA Molecule Program Advisory Committee or the NIH Office of Recombinant DNA Activities those problems pertaining to assessment of biohazards or safeguard determination that cannot be resolved by the Study Sections.

The membership of the Study Sections will be selected in the usual manner. Biological safety expertise, however, will be available to the Study Sections for consultation and guidance.

D. *NIH Recombinant DNA Molecule Program Advisory Committee.* The Recombinant DNA Molecule Program Advisory Committee advises the Secretary, Department of Health, Education, and

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Welfare, the Assistant Secretary for Health, Department of Health, Education, and Welfare, and the Director, National Institutes of Health, on a program for the evaluation of potential biological and ecological hazards of recombinant DNAs (molecules resulting from different segments of DNA that have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of their host's genome), on the development of procedures which are designed to prevent the spread of such molecules within human and other populations, and on guidelines to be followed by investigators working with potentially hazardous recombinants.

The NIH Recombinant DNA Molecule Program Advisory Committee has responsibility for: (i) Revising and updating guidelines to be followed by investigators working with DNA recombinants, (ii) for the time being, receiving information on purported EK2 and EK3 systems and evaluating and certifying that host-vector systems meet EK2 or EK3 criteria, (iii) resolving questions concerning potential biohazard and adequacy of containment capability if NIH staff or NIH Initial Review Group so request, and (iv) reviewing and approving large scale experiments with recombinant DNAs known to make harmful products (e.g., more than 10 liters of culture).

E. *NIH Staff.* NIH Staff has responsibility for: (i) Assuring that no NIH grants or contracts are awarded for DNA recombinant research unless they (a) conform to these guidelines, (b) have been properly reviewed and recommended for approval, and (c) include a properly executed Memorandum of Understanding and Agreement, (ii) reviewing and responding to questions or problems or reports submitted by institutional biohazards committees or principal investigators, and disseminating findings, as appropriate, (iii) receiving and reviewing applications for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes, (iv) referring items covered under (ii) and (iii) above to the NIH Recombinant DNA Molecule Program Advisory Committee, as deemed necessary, and (v) performing site inspections of all P4 physical containment facilities, engaged in DNA recombinant research, and of other facilities as deemed necessary.

V. FOOTNOTES

[1] Biological Safety Cabinets referred to in this section are classified as *Class I, Class II* or *Class III* cabinets. A *Class I* cabinet is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency or high efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is used in three operational modes; (1) with an 8 inch high full width open front, (2) with an installed front closure panel (having four eight inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm length rubber gloves. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all Biohazard Safety Cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air filtered through HEPA filters. Exhaust air is filtered through HEPA filters or incinerated before being discharged to the outside environment.

[2] Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection and/or conjugation with transfer of phage, plasmid and/or chromosomal genetic information.

[3] The inserts which constitute Class 2 of ref. 5 ("Agents of ordinary potential hazard * * *.") represent a broad spectrum of otiologic agents which possess different levels of virulence and degrees of communicability. We think it appropriate for our specific purpose to further subdivide the agents of Class 2 into those which we believe to be of relatively low pathogenicity and those which are moderately pathogenic. The several specific examples given may suffice to illustrate the principle.

[4] The terms "characterized" and "free of harmful genes" are unavoidably vague. But, in this instance, before containment conditions lower than the ones used to clone the DNA can be adopted, the investigator must obtain approval from the National Institutes of Health. Such approval would be contingent upon data supporting: (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or which code for toxic substances), (b) the relation between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable), and (c) maintenance of the biological properties of the vector.

[5] A DNA preparation is defined as enriched if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation. The reason for lowering the containment level when this degree of enrichment has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more that 10^2-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

[6] The DNA preparation is defined as purified if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

[7] In special circumstances, in consultation with the NIH Office of Recombinant DNA Activities, an area biohazards committee may be formed, composed of members from the institution and/or other organizations beyond its own staff, as an alternative when additional expertise outside the institution is needed for the indicated reviews.

VI. REFERENCES

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APPENDIX A—STATEMENT ON THE USE OF "Bacillus subtilis" IN RECOMBINANT MOLECULE TECHNOLOGY

Unquestionably, *Escherichia coli* is the most well characterized unicellular organism. Years of basic research have enabled investigators to develop a well characterized genetic map, to obtain detailed knowledge of virulent and temperate bacteriophages, and to explore the physiology, genetics, and regulation of plasmids. More recently, the development of DNA-mediated transformation has permitted exogenous fragments or molecules of DNA to be incorporated into the genome or to reside as self-replicating units. The discovery of transformation of *Bacillus subtilis* by Spizien (1) stimulated the development of analternative model system. The purpose of this report is to summarize the current status of this genetic system and to describe the actual and potential vectors and vehicles available for recombinant molecule technology.

A. CURRENT KNOWLEDGE OF THE CHROMOSOMAL ARCHITECTURE AND MECHANISMS OF GENETIC EXCHANGE IN *B. "SUBTILIS"*

Two mechanisms of genetic exchange have been utilized to establish the linkage map of *B. subtilis*, DNA-mediated transformation (capable of transferring approximately 1% of the genome) and transduction with bacteriophage PBS1 (capable of transferring 5–8% of the chromosome). Recent detailed genetic studies with PBS1 by Lepesant-Kejzlarová *et al.* (2) have resulted in the development of a circular genetic map for this organism. The current edition of the map (3) contains 198 loci. Biophysical analyses have established that the chromosome is circular (4) and replicates bidirectionally (5).

Transformation with purified fragments of DNA is a highly efficient process in *B. subtilis* with frequencies of 1 to 4% usually attained for any auxotrophic or antibiotic resistance markers. Frequencies of approximately 10% transformation can be achieved with DNA prepared from gently lysed L-forms or protoplasts (6). These large fragments of DNA are readily incorporated by the recipient cell. Generalized transduction occurs with bacteriophages SP10 (7), PBS1 (8), and SPP1 (9), while a low frequency of specialized transduction has been reported with bacteriophage ϕ105 (10).

Although transformation is most efficient in homologous crosses (*B. subtilis* into *B. subtilis*), it has also been possible to exchange DNA among closely related species (11). The most extensively studied members of the *B. subtilis* genospecies include *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, and *B. globigii* (refer to reference 12 for a review and references 13–15 for examples of this heterologous exchange). This exchange occurs even

though there is a surprisingly wide discrepancy between DNA—DNA hybridization among these organisms (16). Even though the frequency of transformation is low in the heterologous cross [e.g., *B. amyloliquefaciens* (donor)/*B subtilis* (recipient)], the newly acquired DNA from *B. amyloliquefaciens* in the *B. subtilis* background can be readily transferred at high efficiencies to other recipient strains of *B. subtilis* (14). Therefore, the extremely high frequency of transformation permits the recognition and selection of rare events.

B. CURRENT AND POTENTIAL VECTORS FOR RECOMBINANT MOLECULE EXPERIMENTS

Lovett and coworkers have recently described cryptic plasmids in *B. pumilus* (17) and *B. subtilis* (18). Of these organisms, *B. subtilis* ATCC 7003 appears to be the most useful since it carries one to two copies of a plasmid with a molecular weight of 46×10^6. This strain is als closely related to *B. subtilis* 168. Another strain of *B. subtilis* (ATCC 15841) contains 16 copies of a plasmid with a molecular weight of 4.8×10^6. Currently it is not known whether genetic markers can be readily introduced into these plasmids. To date it has not been possible to readily stabilize plasmids derived from *B. pumilus* in *B. subtilis* even with heavy selective pressure (P. Lovett, personal communication).

Two temperate bacteriophages are under development as vectors in *B. subtilis*, ϕ3T and SPO2. Lysogeny of thymine auxotrophs (strains carrying *thyA thyB*) by bacteriophage ϕ3T results in "conversion" to a Thy$^+$ phenotype. The attachment site for this bacteriophage and the bacteriophage gene for thymidylate synthetase (*thyP*) map between the bacterial *thyA* and *thyB* loci in the terminal region of the chromosome of *B. subtilis* (19). The viral genome is readily cleaved by the site-specific endonuclease, Bam I (20), to produce 6 fragments (one of which carries the *thyP* gene). The *thyP* carrying gene can be integrated into the bacterial genome in the absence of the intact viral genome. Because deletions are available that include the *thyP* region, it is theoretically possible to introduce *thyP* at many sites on the chromosome. The *thyP* gene can be readily purified for insertion into plasmids or utilized as a scafold to integrate other heterologous DNA into the chromosome of *B. subtilis*. Alternatively, it is possible to purify fragments of the chromosome by gel electrophoresis (21, 22), for insertion into bacteriophage ϕ3T or SPO2. At present, unfortunately, only the former carries a selective marker, i.e., the gene for thymidylate synthetase, *thyP*.

C. DEVELOPMENT OF VEHICLES

B. subtilis is a Gram-positive sporulating rod that usually inhabits soil. Although it can exist on cutaneous surfaces of man (23) and experimental animals, it rarely produces disease. To develop a suitable vehicle it is imperative to have a host that is asporogenic. The most appropriate deletion mutation is deletion 2ϕ (cit D). In addition to a deficiency in sporulation this mutant rapidly lyses when it has reached the end of its growth cycle. Presumably this is due to the failure to inactivate one of the autolytic enzymes (24). Through the introduction of a D-alanine requirement (34 ug/ml) it is possible to block transport of compounds that are transported by active transport (25, 26). The further introduction of thymine auxotrophy (defects in the *thyA thyB* loci) will enable the strain to survive only with a plasmid vector carrying the purified *thyP* gene from bacteriophage ϕ3T or a defective bacteriophage ϕ3T carrying the *thyP* gene but attached to the chromosome at an alternative site (due to the presence of deletion 2ϕ in the host). We have recently isolated temperature-sensitive *thyP* mutants. If we can isolate a temperature-dependent lysogen that will grow only at 48°C it should be possible to make an unusual vehicle.

D. SITE-SPECIFIC ENDONUCLEASES

Recently two restriction modification systems have been observed between *B. subtilis* 168 and other bacilli. Trautner *et al.* have isolated an effective system that inhibits infection of the R strain of *B. subtilis* by bacteriophage SPP1 propagated on *B. subtilis* 168 (27). The site-specific nuclease recognizes the sequence GGCC. Young, Radnay, and Wilson observed a restriction modification system between *B. amyloliquefaciens* and *B. subtilis* 168 (28). The endonuclease from *B. amyloliquefaciens* (20) recognizes the sequence GGATCC (29). More recently, two CCTAGG additional enzymes have been isolated from *B. globigii* (30). The recognition sequence is not known.

E. ADVANTAGES AND LIABILITIES OF THE *B. "SUBTILIS* SYSTEM"

a. Advantages

1. *B. subtilis* is nonpathogenic. Asporogenic deletion mutants are available to preclude the problem of persistence through sporulation.

2. The circular chromosomal map is well defined. At least 190 loci have been positioned.

3. The organism is commercially important in the fermentation industry.

4. Large numbers of organisms can be disposed of readily with minimal environmental impact.

5. Unlike *E. coli*, it lacks endotoxin in the cell wall. Therefore the cells can be used as a single cell protein source.

6. The frequency of transformation is very high, facilitating the detection of rare events.

7. A unique bacteriophage, ϕ3T, exists that carries a gene that can be readily purified for "scaffolding" experiments.

b. Disadvantages

1. The knowledge of genetics and physiology of plasmids and viruses is primitive compared with *E. coli*.

2. High-frequency, specialized transduction is not available as a means of gene enrichment.

Based on its promise, it seems appropriate, and not chauvinistic, to urge development of this system.

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APPENDIX B.—POLYOMA AND SV40 VIRUS

Polyoma virus is a virus of mice, and infection of wild mouse populations is a common event, for the virus has often been isolated from a high proportion of healthy adult animals, both wild and laboratory bred, of many colonies (Gross, L., Proc. Soc. Exp. Biol. *88*, 362–368, 1955; Rowe, W. P., Bact. Rev. *25* 18–31, 1961). As far as is known the virus almost never causes a disease in these animals. However, when large quantities of the virus are inoculated into newborn or suckling mice or hamsters, a variety of solid tumors is induced (Gross, L., Oncogenic Viruses, Second Edition, Pergamon Press, NY).

Polyoma virus grows lytically in mouse cells in tissue culture. Thus mouse cells in culture are probably transformed only by virus particles that contain certain kinds of defective genomes. Cells of other rodent species, however, can be transformed by polyoma virus particles that contain complete genomes (Folk, W., J. Virol., *11*, 424–431, 1973). The virus does not replicate to a significant extent in human cells in tissue culture (Eddy, B. E., Virol. Monogr., *7*, 1–14, 1969; Pollack, R. E. Salas, J., Wang, R., Kusano, T., and Green, H., J. Cell Physiol. *77*, 117–120, 1971). The resistance of the cells seems to be a consequence of the failure of the virus to absorb or uncoat. However even when naked viral DNA is introduced into the cells only an abortive cycle of replication ensues; early viral proteins are made, there is induction of cellular DNA synthesis, but no expression of late viral proteins is detectable (Gruen, R., Grassmann, M. and Grassmann, A., Virology, *58*, 290–293, 1974).

There is no evidence that polyoma virus can infect humans (Hartley, J., Huebner, R., Parker, J. and Rowe, W. P., unpublished data). Thus no antibodies to the virus have been detected in people living in buildings that are infested with virus-infected mice, nor in laboratory workers who have been exposed to the virus for a number of years.

At most, a small segment of polyoma virus DNA shows weak homology with a portion of the late region of SV40 DNA (Ferguson, J. and Davis, R. W., J. Bol. Biol., *94*, 135–150, 1975). However, there appears to be no genetic interaction between the two viruses and there is no immunological cross-reaction between the gene products of the two viruses.

SV40 causes persistent but apparently harmless infections of the kidneys of virtually all adult rhesus monkeys (Hsiung, G. D., Bact. Revs. *32*, 185–205, 1968), it causes tumors when injected into newborn hamsters (Girardi, A. J., Sweet, B. H., Slotnick, V. B. and Hilleman, M. R., Proc. Soc. Exp. Biol. Med., *105*, 420–427, 1964) and transforms cells of several mammalian species (including human). SV40 is able to infect human since antibodies to the virus are found in a small proportion of the human population (Shah, K. V., Goverdhan, M. K. and Ozer, H. L., Am. J. Epid. *93*, 291–298, 1970) and serum conversions have been noted in many laboratory personnel who have been exposed to the virus (Horvath, L. B., Acta Microbiol. Acta Sci. Hung. *12*, 201–206, 1965).

Isolations of SV40 have been reported from humans, twice from patients suffering from the rare demyelinating disease, progressive multifocal leukoencephalopathy (Weiner, L., Herndon, R., Narayon, O., Johnson, R. T., Shah, K., Rubinstein, L. G., Preziosi, T. J. and Conley, F. K., New England J. Med. *286*, 385–390, 1972) and apparently from a tumor of a person with metastatic melanoma (Sotnikov, F., Shahbenna, G. E. and Goicoa, M., Nature. *249*, 421–424, 1974). In other studies a non-structural antigen characteristic of papoviruses, T antigen, has been detected in the nuclei of cells cultured from 2 meningiomas, while another SV40-specific antigen, V antigen, has been found in the cells of a third tumor of the same type (Weiss, A. F., Portman, R., Fisher, H., Simon, J. and Zang, K. D., Proc. Nat. Acad. Sci. USA *72*, 609–613, 1975). Furthermore new papoviruses have been isolated from the brains of patients with PML (JC virus—Padgett, B. L., Walker, D. L., zuRhein, G. M., Eckroade, R. J. and Dessel, B. H., Lancet *1*, 1257–1260, 1971), from the urine of a patient carrying a renal allograft (BK virus—Gardner, S. D., Field, A. M., Coleman, D. V. and Hulme, B. Lancet *1*, 1253–1257, 1971); and from a reticulum cell sarcoma and the urine of patients with the sex-linked recessive disorder, Wiscott-Aldrich syndrome (Takemoto, K. K., Rabson, A. S., Mullarkey, M. F., Blaese, R. M. Garon, C. F. and Nelson, D. J., Nat. Cancer Inst., *53*, 1205–1207, 1974). All of these viruses which are distributed widely throughout human populations share antigenic and biological properties with SV40; the virus particles are identical in size and architecture (Madeley, C. R., In Virus Morphology, Churchill-Livingstone, London, M., Alberts, A. R. and Morris, R. P., Science *181*, 874–876, 1973; Shah, K. V., Daniel, R. W. and Strandberg, J., J. Nat. Cancer Inst. *54*, 945–950, 1975); BK virus causes transformation of hamster cells in culture (Major, E. D., and DiMayorca, G., Proc. Nat. Acad. Sci. US, *70*, 2910–2912, 1973; Portolani, M., Barbanti, A., Brodano, G. and LaPiaca, M. J., Virol. *15*, 420–423, 1975) and is able to complement the growth of certain temperature-sensitive mutants of SV40 (Mason, D. H. and Takemoto, K. K., submitted for publication).

FURTHER WORK

At present, a potential eukaryotic vector of choice is polyoma virus. And while available information indicates that it fulfills all the necessary criteria, we recommend that the following subjects be further investigated:

1. The molecular mechanism of resistance of human cells to the virus.

2. The extent of homology between polyoma virus DNA and the DNAs of human papoviruses.

3. The ability of human papoviruses to complement defective polyoma virus genomes.

Report of a working group consisting of: Dr. Bernard Fields, Harvard University School of Medicine; Dr. Thomas J. Kelly, Jr., Johns Hopkins University School of Medicine; Dr. Andrew Lewis, National Institute of Allergy and Infectious Diseases; Dr. Malcolm Martin, National Institute of Allergy and Infectious Diseases; Dr. Robert Martin, National Institute of Arthritis, Metabolism, and Digestive Diseases; Dr. Elmer Pfefferkorn, Dartmouth Medical School; Dr. Wallace P. Rowe, National Institute of Allergy and Infectious Diseases; Dr. Aaron Shatkin, Roche Institute of Molecular Biology; Dr. Marina Singer, National Cancer Institute. Rapporteur: Dr. Joe Sambrook, Cold Spring Harbor Laboratory.

APPENDIX C.—SUMMARY OF THE WORKSHOP ON THE DESIGN AND TESTING OF SAFER PROKARYOTIC VEHICLES AND BACTERIAL HOSTS FOR RESEARCH ON RECOMBINANT DNA MOLECULES

TORREY PINES INN, LA JOLLA, CALIFORNIA

The development of techniques for the cloning of DNA from both prokaryotic and eukaryotic organisms in bacteria has had great impact on research in biology and medicine and promises extraordinary social benefits. The biohazards involved in the use of this technology in many instances are very difficult to assess. For this reason codes of practice are being formulated in the United States and other countries for the conduct of those experiments that present a potential biohazard. One of the requirements for conducting certain cloning experiments is the use of safer vector (bacteriophage or plasmid) -host systems, i.e., vector-bacterium systems that have restricted capacity to survive outside of controlled conditions in the laboratory. Approximately sixty scientists from the United States and several foreign countries participated in a workshop on the Design and Testing of Safer Prokaryotic Vehicles and Bacterial Hosts for Research on Recombinant DNA Molecules at La Jolla, California, on 1 to 3 December, 1975. The workshop was sponsored by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases. The purposes of the meeting were the exchange of recent data on the development of safer prokaryotic host-vector systems, devising methods of testing the level of containment provided by these systems and exploring the various directions that future research should take in the construction of safer bacterial systems for the cloning of foreign DNA.

The first session of the workshop, chaired by W. Szybalski (University of Wisconsin), was devoted to bacteriophage vectors. Szybalski outlined the main safety features of the two-component, phage-bacterial system, in which the host bacteria offer the safety features of not carrying the cloned DNA, and the phage vectors cannot be propagated in the absence of an appropriate host. There are two primary escape routes for the clones of foreign DNA carried by the phage vector: (1) establishment of a stable prophage or plasmid in the laboratory host used for phage propagation, and subsequent escape of this self replicating lysogen or carrier system, and (2) escape of the phage vector which carries the cloned DNA and its subsequent productive encounter with a suitable host in the natural environment. The general consensus was that to ensure safety, both routes should be blocked by appropriate genetic modifications. For phage λ, route (1) can be blocked by phage mutations that interfere with lysogenization (*att*-, *int*-, *cI*-, *cIII*-, *vir*) *and plasmid formation* (N^s, *ninR*, *vS*, *ri*-, *cIV*, *Ote*, *crots*), and by mutations on the *Escherichia coli* host that affect these processes (*attB*-; *dnaAts*) and host survival. Route (2), (which is of low probability since λ phages do not survive well in natural environments (no λci phage was recovered after ingestion of 10^8-10^{10} particles), are killed by desiccation, and have a low chance to encounter a naturally sensitive host) can be blocked further by the following phage modifications: (a) mutations which result in extreme instability of the infectious phage particles under all conditions other than those specially designed for phage propagation in the laboratory (e.g., high concentrations of putrescine or some other compound); or (b) employing phage vectors in which the tail genes are deleted and which permit propagation of only the DNA-packed heads; only under laboratory conditions could such heads be made transiently infec-

tious by rejoining them with separately prepared tails. The high instability of the phage would minimize the possibility of transfer of the cloned genes into receptive bacteria found in nature. Moreover, the propagation of the phage can be blocked by many conditional mutations, which would be designed to block any secondary route of escape, mainly depending on transfer of the cloned DNA into another phage or bacterial host. It was recommended further that the vector be designed in such a manner as to permit easy insertion and monitoring of the foreign DNA and rapid assay of the safety features and give a high yield of cloned DNA (not less than 10^{14} molecules per ml). There also was general agreement that host-phage systems other than *E. coli* should be considered, especially those restricted to very rare and unusual environments. Also, plasmids derived from phage vectors and which give very high DNA yields while exhibiting safety features, e.g., *kdv*erots, should be considered as vehicles for cloned DNA.

Szybalski and S. Bromer (Cambridge University) stressed that research on recombinant DNA molecules may lend itself to very simple and inexpensive mechanical containment, e.g., a small sealed glove box, since all the vectors that carry such recombinant molecules possibly can be both created and destroyed in such a box, while development of special methods might permit study of many properties of the recombinant DNA, *without ever removing it from the box.*

These safety features were reflected in the subsequent presentations. F. Blattner and W. Williams (University of Wisconsin) described four specially constructed λ-φ80 phages which incorporate many of these safety features, and which they named Charon phages, for the mythical boatman of the river Styx. Some of these highly contained phages give yields of over 10^{13} particles/ml. R. Davis, J. Cameron and K. Struhl (Stanford University) found that λ phages that carry foreign DNA never grow as well as the parental vector, which would select against their survival in nature. They also reported that the eukaryotic genes could be expressed in *E. coli*, partially compensating for deficiencies in the histidine pathway or in *polA* or *fg* functions. These investigators surveyed over 1000 strains of *E. coli* isolated in the natural environment and did not find a single strain that could support propagation of the *kdv* vector.

V. Bode (Kansas State University) discussed the possibility of growing tail-free λ heads. Such heads, which are packed with DNA, are very fragile, unless stored in 0.01 M putrescine buffer. Head yields close to 10^{14}/ml could easily be attained and, when required, heads could be quantitatively rejoined with separately supplied tails under special laboratory conditions. W. Arber, D. Scandella and J. Elliott (University of Basel) described bacterial host mutants that *permit efficient infection only by phages with a* full complement of DNA. This permits selecting for vectors that carry long fragments of foreign DNA.

K. Matsubara, T. Mukai and Y. Takagi (University of Osaka and Kyushu University), and G. Hobom and P. Phillipsen (University of Freiburg and Stanford University) described various defective λ plasmids (*kdv*) that could be used as efficient vectors. Matsubara has shown that temperature-sensitive *cro* mutations permit obtaining between 1000 and 8000 cloned molecules per cell and at the same time result in killing of the carrier cells at body temperature. The mutations *Ots* and *Pts* were also evaluated as safety features. Phillipsen described many new *kdv* plasmids created by cutting λ DNA with *Hin*dIII and *Bam*I restriction endonucleases followed by ligation. The final talk by F. Young, G. Wilson and M. Williams (University of Rochester) summarized the progress on the development of safer *Bacillus subtilis* host mutants and phages, especially φ3, as vectors. New restriction nucleases, Bgl-1 and Bgl-2, were also described.

The morning session on bacteriophage vectors was followed by a session on plasmid vectors that was chaired by D. Helinski (University of California, San Diego). Helinski presented the following properties as highly desirable characteristics of a safer plasmid vehicle: (a) non-conjugative; (b) non-mobilizable or poorly mobilizable by a conjugative plasmid; (c) possesses little or no extraneous genetic information; (d) poorly recombines or does not recombine with the chromosome of the host cell; (e) provides no selective advantage to the host cell or the selective property is conditional; and (f) possesses mutations that restrict its maintenance to a specific host, prevent replication at mammalian body temperature and/or provide the plasmid with the capability of killing any cell to which it might be transmitted other than the host cell. V. Herchfield (University of California, San Diego) described the properties of a variety of derivatives of the ColE1 plasmid and the broad-host range, P-type plasmid, RK2. One of the ColE1 derivatives, ColE1-*trp*, constructed in collaboration with C. Yanofsky and N. Franklin (Stanford University) provides the means to use the tryptophan genes of *E. coli* as a selective marker in transformation with recombinant DNA in situations where it is desirable to avoid antibiotic resistance genes. In addition, Hershfield described collaborative work with H. Boyer that resulted in the development of a mini-ColE1 plasmid and derivatives of this plasmid (mini-ColE1-*kan* and mini-ColE1-*trp*) as cloning vehicles. Finally, she described the temperature-sensitivity properties of *trp* and *kan* derivatives of a temperature sensitive replication mutant of ColE1 isolated by J. Collins (Molecular biology Institute, Stockholm) and hybrid ColE1 plasmids carrying the EcoRI generated Ots fragment of bacteriophage λ-*trp*61.

J. Carbon (University of California, Santa Barbara) described a replica plating method that greatly facilitates the detection of *E. coli* clones bearing ColE1 plasmids. The procedure, which utilizes the F' plasmid to promote the transfer of a hybrid ColE1 plasmid to a suitable auxotrophic recipient, was successful in identifying clones bearing hybrid plasmids carrying a number of different regions of the *E. coli* chromosome. The contributions of A. J. Clark and collaborators (University of California, Berkeley) were relevant to the problem of the mobilization and subsequent transfer of non-conjugative plasmids carrying a number of potentially hazardous mature. Clark described the variations in transmission frequencies between the nonconjugative plasmids pSC101, pML31, pSC138 and a number of pSC101 hybrids containing various *EcoR*I fragments of F when the conjugal transfer of these plasmids was promoted by several different conjugative plasmids.

I. C. Gunsalus and collaborators (University of Illinois) and A. Chakrabarty (General Electric Research and Development Center) described the properties of a variety of plasmids isolated from *Pseudomonas putida*. These contributions were followed by a discussion on the merits of developing plasmid-host systems involving *Pseudomonas strains* that naturally exhibit unusual growth requirements. Similar studies with plasmids isolated from *Bacillus megaterium* by B. Carlton (University of Georgia) from *B. subtilis* by P. Lovett (University of Maryland) and other naturally occurring *Bacillus* species by W. Goebel and K. Bernhard (Microbiology *Institute*, Wurzburg) were discussed and their further development as plasmid-host cloning systems was explored. It was clear from these presentations that considerable progress has been made recently in the identification and characterization of a variety of plasmid elements that occur naturally in *Pseudomonas* and *Bacillus* species. Several of the plasmids described show considerable promise as plasmid cloning systems involving a host other than *E. coli*.

A third session on the ecology and epidemiology of vector-host systems was chaired by S. Falkow (University of Washington). This workshop emerged, in part, from expressed fears that microorganisms containing cloned fragments of foreign DNA potentially pose a threat to health or disrupt the normal ecological chain in some manner. Consequently, this session was devoted to a review of currently available information on the ecology and epidemiology of *E. coli* and related bacterial species since it was recognized that *E. coli* K–12 would be the prokaryotic host most commonly employed in the cloning of DNA molecules in the immediate future. F. Orskov (Escherichia Reference Center, Copenhagen) reviewed the state of *E. coli* serotyping and what has been learned about the distribution of *E. coli* types in health and disease. Only certain *E. coli* types are generally recognized as good colonizers of the human gut and such strains *come from a handful of the 180 well defined* O (lipopolysaccharide) antigen types and invariably posses K (acidic polysaccharide capsule) antigens. Some serotypes apparently have become disseminated worldwide and possibly represent the proliferation of a bacterial clone because of, as yet unknown, selective pressures. In contrast, *E. coli* K–12 has no detectable O or K antigens and is considered to be rough. This may account, at least in part, for its demonstrable poor ability to colonize the human or animal gut. However, R. Freter (University of Michigan) point out that we still remain largely ignorant of the factors which control intestinal *E. coli* populations. Freter also noted that while adherence to the mucosal surface of the small intestine is important in the pathogenesis of *E. coli* diarrheal disease, the "normal" long-lasting symbiotic relationship between a mammalian host and bacterium is established in the cecum and colon. It is in these locations that factors come into play to determine whether an *E. coli* strain passing through the intestine will become successfully implanted or whether it will be quickly eliminated in the feces.

The factors controlling implantation include competition for substrates, inhibitors and the physiological state of the organism when it reaches the large bowel. For example, ingested *E. coli* previously grown under usual laboratory conditions fare poorly while cells of the same strain "pre-adapted" in Eh, pH, etc., often colonize well. Freter has developed a continuous flow culture model which may be useful in studying the mechanisms of implantation. Falkow reviewed the pathogenicity of *E. coli*. *E. coli* causes diarrheal disease either by direct invasion of the bowel epithelium or by elaboration of enterotoxin(s). While invasive *E. coli* appear to owe their pathogenicity to a constellation of at least five unlinked chromosomal gene clusters, toxigenic *E. coli* species generally owe their pathogenicity to the possession of two species, Ent and K. The introduction of Ent and K plasmids may be sufficient to convert a normal wild-type *E. coli* into a strain now capable of causing overt clinical disease. However, the introduction of these plasmids into *E. coli* K–12 sublines had no discernible effect on their ability to cause disease, although the K–12 strains could now better colonize calves. Despite the observation that

fold and 10⁸-fold reductions, respectively, in strain titers in feces. Strains with *ΔthyA* mutations also exhibited thymineless death in *in vitro* tests. Since strains with the *dapD8* allele can revert to Dap⁺ strains were constructed with both the *dapDs and ΔbioH-asd mutations*. These strains have not been observed to revert to Dap⁺ but can survive passage through the rat intestine and in growth media lacking diaminopimelic acid but containing NaCl and 0.5% usable carbon sources. This survival was due to the production of the mucopolysaccharide, colanic acid, which permits many of the cells to grow and survive as spheroplasts. A *Δgal-chl*⁻ mutation (also deletes *kat*, *bio* and *uvrB* genes) was introduced which blocks colanic acid biosynthesis and leads to no detectable survivors in media lacking diaminopimelic acid or following passage through the rat intestine. The *dapD8 ΔbioH-asd Δgal-chl*⁻ strains are more readily lysed, transform at higher frequencies and are conjugation-defective in matings with donors possessing conjugative plasmids in the P, W and C incompatibility groups but Con⁺ as recipients for F, I and T group plasmids when compared to the dap⁺ *gal*⁺ parent strain. Strains with *endA mutations* were also observed to exhibit increased transformation frequencies. Attempts to introduce temperature-sensitive *polA* alleles into strains to block replication of ColE1 cloning vectors at elevated temperatures and to cause DNA degradation at elevated temperatures in the presence of *recA* and *ΔthyA* alleles often do not have the same properties in the constructed strains as in the strains in which the allele was originally induced. Many mutations causing a Con⁻ phenotype have been investigated, but many of these revert or do not exhibit a Con⁻ phenotype in matings with donors possessing conjugative plasmids of the incompatibility groups commonly found in enteric microorganisms. Some Con⁻ mutants exhibit increased sensitivity to bile salts; thus, the mutant described by Bukhari may also exhibit a Con⁻ phenotype. All of the strains constructed by the Curtiss group are SuII⁺ and most have mutations abolishing restriction alone or both restriction and modification. Thus, sufficient information is now known to construct a usable safer *E. coli* K-12 host. *Curtiss and collaborators* are now introducing the *ΔthyA* and *dna* mutations into their *dapD8 ΔbioA-asd Δgal-chl⁻-uvrB hsr nalr* (for use in monitoring) Su⁺ λ⁺ Φ80⁺ strain to accomplish this objective.

The final session involved a general discussion of some of the major points raised previously in the workshop. There was general agreement at this session that both plasmid-host and phage-host systems have been developed that should meet the criteria of an EK2 system specified by the National Institutes of Health guidelines for research on recombinant DNA molecules. Additional testing is required to confirm the EK2 properties of these available systems, but it is anticipated that these vector-host systems will meet these tests.

Dr. Donald R. Helinski, University of California, San Diego.

Dr. Stanley Falkow, University of Washington.

Dr. Roy Curtiss III, University of Alabama.

Dr. Waclaw Szybalski, University of Wisconsin.

APPENDIX D.—SUPPLEMENTARY INFORMATION ON PHYSICAL CONTAINMENT

I. Biological Safety Cabinets.
Table I.
II. Universal Biohazard Warning Symbol.
III. Laboratory Techniques for Biohazard Control:
A. Pipetting.
B. Syringes & Needles.
C. Opening Culture Plates, Tubes, Bottles, and Ampoules.
D. Centrifuging.
E. High-Speed Centrifuges.
F. Blenders, Ultrasonic Disintegrators, Colloid Mills, Ball Mills, Jet Mills, Grinders, Mortar and Pestle.
G. Miscellaneous Precautions & Recommendations.
IV. Personal Hygiene Habits, & Practices.
V. Care & Use of Laboratory Animals:
A. Care & Handling.
B. Cages Housing Infected Animals.
C. General Guidelines that Apply to Animal Room Maintenance.
D. Necropsy Rules for Infected Animals.
VI. Decontamination & Disposal:
A. Introduction.
B. Decontamination Methods.
C. Laboratory Spills.
D. Disposal.
E. Characteristics of Chemical Decontaminants in Common Use in Laboratory Operations.
F. Properties of Some Common Decontaminants.
G. Vapors and Gases.
H. Residual Action of Decontaminants.
I. Selecting Chemical Decontaminants for Research on Recombinant DNA Molecules.
Table II.
VII. Housekeeping:
A. Introduction.
B. Floor Care.
C. Dry Sweeping.
D. Vacuum Cleaning.
E. Selection of a Cleaning Solution.
F. Wet Mopping—Two-Bucket Method.
G. Alternative Floor Cleaning Method for Animal Care Areas and Areas with Monolithic Floors.
VIII. Clean-Up of Biohazardous Spills:
A. Biohazardous Spill in a Biological Safety Cabinet.
B. Biohazard Spill Outside a Biological Safety Cabinet.
C. Radioactive Biohazard Spill Outside a Biological Safety Cabinet.
IX. A Secondary Reservoir and Filtration Apparatus for Vacuum Systems.
X. Packaging and Shipping:
A. Introduction.
B. Packaging of Recombinant DNA Materials.
C. Labeling of Packages Containing Recombinant *DNA Materials*.
Table III.
Table IV.
XI. Training Aids, Materials and Courses:
A. Slide-Tape Cassettes.
B. Films.
C. Courses.
XII. Outline of a Safety and Operation Manual for a P4 Facility.
References.

I. BIOLOGICAL SAFETY CABINETS

Biological Safety Cabinets suitable for confining operations involving recombinant DNA molecules are described below:

1. *Class I.* A ventilated cabinet for personnel protection only, with an unrecirculated inward flow of air away from the operator. The exhaust air from this cabinet may be filtered through a high-efficiency or high-efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is suitable for research work with the Center for Disease Control (CDC) classes of etiologic agents 1, 2 and 3 where no product protection is required. This cabinet may be used in three operational modes: (i) with an eight-inch high, full-width open front; (ii) with an installed front closure panel (having four, eight-inch diameter openings) without gloves; and (iii) with an installed front closure panel equipped with arm length rubber gloves. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

2. *Class II.* A ventilated cabinet for personnel and product protection having an

open front with inward air flow for personnel protection, and HEPA-filtered recirculated mass air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. Two models of this cabinet are available, Type 1 and Type 2.

(i) *Type 1.* The Type 1 recirculates approximately 70% of the air. The exhaust air from this cabinet may discharge into the laboratory or be diverted out of the laboratory. This cabinet is suitable for CDC classes of etiologic agents 1, 2, and 3. Vapors or gases which are hazardous from a toxic, radioactive, or flammability standpoint should not be used in this cabinet because of the high quantity of recirculated air.

(ii) *Type 2.* The Type 2 cabinet recirculates approximately 30% of the air. The exhaust air from this cabinet is normally ducted out of the laboratory through a HEPA filter and, occasionally, an activated charcoal filter depending on the operation. The cabinet may be used with gases or vapors that are hazardous from a toxic, radioactive, or flammability standpoint. However, any consideration of use of such materials should be evaluated carefully from the standpoint of build-up to dangerous levels and problems of decontamination of the cabinet. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

3. *Class III.* A closed front ventilated cabinet of gas-tight construction providing total protection for personnel and product from contaminants exterior to the cabinet. The cabinet is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is HEPA-filtered. Exhaust air is HEPA-filtered or incinerated to protect the environment. This cabinet, fitted with arm length rubber gloves, provides the highest containment of these three classes of cabinets and is utilized for all activities involving high risk agents (i.e., CDC etiologic agents, class 4). See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

The integrity of any cabinet depends on initial and periodic evaluation to meet established performance tests. Table I outlines the minimum performance required to assure that the cabinets will provide protection of personnel and the environment.

TABLE I.—*Biological safety cabinets, safety performance requirements, and specifications, June 1976*

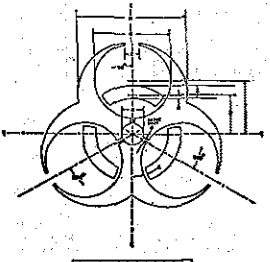
Cabinet	Use classification		Performance requirements				Exhaust filter efficiency (percent)
	DNA[1]	CDC[2]	Face velocity (linear feet per minute)	Exhaust air (cubic feet per minute) 4-ft hood	8-ft hood	Leak tightness	
Class I	P1-P3	1-3	75	200	300	Not applicable	99.97
Class II, type 1	P1-P2	1-3	75	280	400	One light; leak rate <1 by 10^{-4} cm^3/s at 2-in water gage pressure.	99.97
Class II, type 2	P1-P3	1-3	100	280	360	Pressure hold; no soap bubble at 2-in water gage pressure.	99.97
Class III	P4	4	(3)	(3)	(3)	One light; leak rate <1 by 10^{-4} cm^3/s at 3-in water gage pressure.	99.97

[1] For work with recombinant DNA molecules.
[2] Center for Disease Control (U.S. Public Health Service).
[3] Not applicable.
[4] Based on 1 vol. of air change each 3 min, in the absence of unusual heat or moisture that would require more air changes.

II. UNIVERSAL BIOHAZARD WARNING SYMBOL (11)

The biological hazard warning symbol (biohazard symbol) specified herein shall be used to signify the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals or combinations thereof which contain or are contaminated with viable hazardous agents.

The biohazard symbol shall be designed and proportioned as illustrated here:



The symbol shall be as prominent as practical, and of a size consistent with the size of the equipment or material to which it is affixed, provided the proportions shown above are maintained, and, in any case, that the symbol can be easily seen from as many directions as possible.

Except when circumstances do not permit, the symbol shall be oriented with one of the three open circles pointed up and the other two forming a base.

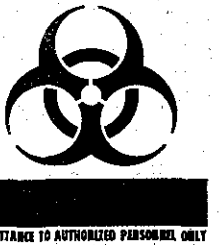
The symbol color shall be a fluorescent orange or orange-red color.* Background color is optional as long as there is sufficient contrast for the symbol to be clearly defined.



Revised 2-1-68
*Munsell 2.5R 6/14 fluorescent orange-red. Use in effect as an example, not as requirement.

The biohazard symbol shall be used or displayed only to signify the actual or potential presence of biological hazard.

Appropriate wording may be used in association with the symbol to indicate the nature or identity of the hazard, name of individual responsible for its control, precautionary information, etc., but never should this information be superimposed on the symbol.



III. LABORATORY TECHNIQUES FOR BIOHAZARD CONTROL

A. Pipetting

1. No infectious or toxic materials should be pipetted by mouth (2, 3, 4).

2. No infectious mixture should be prepared by bubbling expiratory air through a liquid with a pipette (2, 3, 4).

3. No infectious material should be blown out of pipettes (2, 3, 4).

4. Pipettes used for the pipetting of infectious or toxic materials should be plugged with cotton (2, 3, 4).

5. Contaminated pipettes should be placed horizontally in a pan containing enough suitable disinfectant to allow complete immersion (2, 4, 4). They should not be placed vertically in a cylinder.

6. The pan and pipettes should be autoclaved as a unit and replaced by a clean pan with fresh disinfectant (2, 3, 4).

7. Infectious material should not be mixed by alternate suction and expulsion through a pipette (2, 3, 4).

8. Mark-to-mark pipettes are preferable to other types, as they do not require expulsion of the last drop (5).

9. Discharge should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle whenever possible—not dropped from a height (5).

10. A disinfectant-wetted towel over the immediate work surface is useful in some cases to minimize the splash from accidental droppage (9).

B. Syringes and Needles (9)

1. To lessen the chance of accidental injection, aerosol production or spills, avoid unnecessary use of the syringe and needle. For instance:

(i) Use the needle for parenteral injections but use a blunt needle or a cannula on the syringe for oral or intranasal inoculations.

(ii) Do not use a syringe and needle as a substitute for a pipette in making dilutions of dangerous fluids.

2. Use the syringe and needle in a Biological Safety Cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.

3. Examine glass syringes for chips and cracks, and needles for barbs and plugs.

Note: This should be done prior to sterilization before use.

4. Use needle-locking (Luer-Lok® type) syringes only, and be sure that the needle is locked securely into the barrel. A disposable syringe-needle unit (where the needle is an integral part of the unit) is preferred.

5. Wear surgical or other type rubber gloves for all manipulations with needles and syringes.

6. Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.

7. Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with the proper disinfectant, or into a small bottle of sterile cotton.

8. Do not use the syringe to expel forcefully *a stream of infectious fluid into an* open vial or tube for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the needle is held below the surface of the fluid in the tube.

9. If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in transfer of infectious material to the fingers.

10. When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with the proper disinfectant. If there is danger of the disinfectant contaminating sensitive experiments, a sterile dry pledget may be used and discarded immediately into disinfectant solution.

11. Inoculate animals with the hand "behind" the needle to avoid punctures.

12. Be sure the animal is properly restrained prior to the inoculation, and be on the alert for any unexpected movements of the animal.

13. Before and after injection of an animal, swab the site of injection with a disinfectant.

14. Discard syringes into a pan of disinfectant without removing the needle. The syringe first may be filled with disinfectant by immersing the needle and slowly withdrawing the plunger, and finally removing the plunger and placing it separately into the disinfectant. The filling action clears the needle and dilutes the contents of the syringe. Autoclave syringes and needles in the pan of disinfectant.

15. Use separate pans of disinfectant for disposable and nondisposable syringes and needles to eliminate a sorting problem in the service area.

16. Do not discard syringes and needles into pans containing pipettes or other glassware that must be sorted out from the syringes and needles.

C. Opening Culture Plates, Tubes, Bottles, and Ampoules

1. Plates, tubes and bottles of fungi may release spores in large numbers when opened. Such cultures should be manipulated in a Biological Safety Cabinet (6, 15).

2. In the absence of definite accidents or obvious spillage, it is not certain that opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents, some infections have occurred by this means and are represented in the 60% for which *no known act or accident* is ascribable (3).

3. Water of syneresis in petri dish cultures is usually infected and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when this film is broken by opening the plate. Vented plastic petri dishes where the lid touches the rim at only three points are less likely to offer this hazard (8, 19).

4. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent, dispersal. If plates are obviously wet they should be opened in the Biological Safety Cabinet (8).

5. Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of infected liquid which may collect between the rim and the liner is broken during removal of the closure (8).

6. Dried, infected culture material may also collect at or near the rim or neck of culture tubes and may be dispersed into the air when disturbed (18). Containers of dry powdered hazardous materials (e.g., Class 3 fungal agents in the spore phase of growth) should be opened only in a Biological Safety Cabinet (8, 14).

7. When the neck of an ampoule containing liquid is broken after nicking with a file, the snapping action creates aerosols. The following methods have been recommended:

(i) After nicking the ampoule with a file, wrap the ampoule in disinfectant-wetted cotton before breaking. Wear gloves (2).

(ii) The bottom of the ampoule should be held in several layers of tissue paper to protect the hands, and a file mark made at the neck. A hot glass rod should be carefully applied to the mark. The glass will crack, allowing air to enter the ampoule and equalize the pressures. After a few seconds the ampoule should be wrapped in a few layers of tissue and broken along the crack. The tissues and ampoule neck can then be discarded into disinfectant, and the contents of the ampoule removed with a syringe. If the ampoule contains dried cultures, about 0.5 cm³ of broth should be added slowly to avoid blowing dried material out. The contents may then be mixed without bubbling and withdrawn into a culture tube (8).

(iii) The researcher uses an intense, but tiny, gas-oxygen flame and heats the tip of the hard glass ampoule until the expanding *internal air pressure blows a bubble. After* allowing this to cool, he breaks the bubble while holding it in a large low temperature flame: this immediately incinerates any infectious dust which may come from the ampoule when the glass is broken (16). Preliminary practice with a simulant ampoule of the same type actually in use is necessary to develop a technique that will not cause explosion of the ampoule.

(iv) A simple device has been recommended consisting of a sleeve of rubber tubing into which the ampoule is inserted before it is broken (17, 18).

D. Centrifuging

1. A safety centrifuge cabinet or safety centrifuge cup (3, 7, 8, 14, 22) may be used to house or safeguard all centrifuging of infectious substances. When bench type centrifuges are used in a Biological Safety Cabinet, the glove panel should be in place with the glove ports covered. The centrifuge operation creates air currents that may cause escape of agent from an open cabinet (2, 3, 4, 13).

2. In some situations, in the absence of O-ring cap sealed trunnion cups, specimens can be enclosed in sealed plastic bags before centrifugation (12).

3. Before centrifuging, inspect tubes for cracks, inspect the inside of the trunnion cup for rough walls caused by erosion or adhering matter, and carefully remove bits of glass from the rubber cushion (4, 10).

4. A germicidal solution should be added between the tube and trunnion cup to disinfect the materials in case of accidential breakage. This practice also provides an excellent cushion against shocks that might otherwise break the tube (4, 10).

5. Avoid decanting centrifuge tubes. If you must do so, afterwards wipe off the outer rim with a disinfectant; otherwise the infectious fluid will spin off as an aerosol (4, 10).

6. Avoid filling the tube to the point that the rim, cap or cotton plug ever becomes wet with culture (4, 10).

7. Screw caps, or caps which fit over the rim outside the centrifuge tube are safer than plug-in closures. Some fluid usually collects between a plug-in closure and the rim of the tube. Even screw-capped bottles are not without risk, however; if the rim is soiled some fluid will escape down the outside of the tube. Screw-capped bottles may jam in the bucket, and removing them is hazardous. Propping such bottles higher in the bucket with additional rubber buffers is mechanically unsound (8).

8. Kitchen foil is often used to cap centrifuge tubes. This creates more risk than the screw cap. Foil caps often become detached in handling and centrifuging (8).

9. The balancing of buckets is often mismanaged. Care must be taken to ensure that matched sets of trunnions, buckets and plastic inserts do not become mixed. If the components are not inscribed with their weights by the manufacturer, colored stains can be applied to avoid confusion. When the tubes are balanced, the buckets, trunnions and inserts should be included in the procedure; and care must be taken to ensure that the centers of gravity of the tubes are equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20 g of mercury and 20 g of water respectively will balance perfectly on the scales; but their performance in motion is totally different, leading to violent vibration with all its attendant hazards (5).

10. Fill and open centrifuge tubes or trunnion cups in a Biological Safety Cabinet (10).

E. High-Speed Centrifuges (22)

1. In high-speed centrifuges the bowl is connected to a vacuum pump. If there is a breakage or accidental dispersion of infected particles the pump and the oil in it will become contaminated. A high efficiency filter should be placed between the centrifuge and the pump (8).

2. High speed rotor heads are prone to metal fatigue, and where there is a chance that they may be used on more than one machine each rotor should be accompanied by its own log book indicating the number of hours run at top or de-rated speeds. Failure to observe this precaution can result in dangerous and expensive disintegration. Frequent inspection, cleaning and drying are important to ensure absence of corrosion or other traumata which may lead to creeping cracks. Rubber O-rings and tube closures must be examined for deterioration and be kept lubricated with the material recommended by the makers. Where tubes of different materials are provided (e.g., celluloid, polypropylene, stainless steel), care must be taken that the tube closures designed specifically for the type of tube in use are employed. These caps are often similar in appearance, but are prone to leakage if applied to tubes of the wrong material. When properly designed tubes and rotors are well maintained and handled, leaking should never occur (5).

3. Cleaning and disinfection of tubes, rotors and other components requires considerable care. It is unfortunate that no single process is suitable for all items, and the various manufacturers' recommendations must be followed meticulously if fatigue, distortion and corrosion are to be avoided. This is not the place to catalogue recommended methods, but one less well appreciated fact is worthy of mention. Celluloid (cellulose nitrate) centrifuge tubes are not only highly inflammable and prone to shrinkage with age and distortion on boiling, but can be-

have as high explosive in an autoclave (5). Large-scale zonal centrifugation requires special attention (11).

F. Blenders, Ultrasonic Disintegrators, Colloid Mills, Ball Mills, Jet Mills, Grinders, Mortar and Pestle.

All these devices release considerable aerosols during their operation. For maximum protection to the operator during the blending of infectious materials, the following practices should be observed:

1. Operate blending and cell-disruption and grinding equipment in a Biological Safety Cabinet (9).

2. Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl (9).

3. In the absence of a leak-proof rotor, inspect the rotor bearing at the bottom of the blender bowl for leakage prior to operation. Test it in a preliminary run with sterile saline or methylene blue solution prior to use with infected material (9).

4. Sterilize the device and residual infectious contents promptly after use. Use a towel moistened with disinfectant over the top of the blender (9).

5. Glass blender bowls are undesirable for use with infectious material because of potential breakage. If used, they should be covered with a polypropylene jar to prevent dispersal of glass (8).

6. A new machine, the Colworth Stomacker (England), in which material is homogenized in a plastic bag in a closed container, would appear to be safer than some of the other blenders (8).

7. A heat-sealed flexible plastic film enclosure for a grinder or blender can be used, but it must be opened in a Biological Safety Cabinet (7).

8. Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal efforts on the product (7).

9. Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.

10. Clinical or other laboratories handling human blood should be aware of the aerosols produced by the microhaematocrit centrifuge, the autoanalyzer stirrer, and the microtonometer, inasmuch as it seems that airborne transmission of infectious hepatitis may occur in the laboratory (20).

G. Miscellaneous Precautions and Recommendations

1. Water baths and Warburg baths used to inactive, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended (4, 10).

2. Deepfreeze, liquid nitrogen, and dry ice chests and refrigerators should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deepfreezes should be properly labelled. Security measures should be commensurate with the hazards (4, 10, 21).

3. Freeze-dried culture ampoules should always be opened in a Biological Safety Cabinet. The ampoule should be wrapped in a disinfectant-soaked swab before breaking it open to minimize the risk of cutting the hands, and to a lesser extent of releasing aerosol of dried material. Whenever possible, ampoules should be filled with dry nitrogen after freeze-drying, thus avoiding implosion that may occur during the sealing as well as opening of evacuated ampoules. The whole process of freeze-drying itself should be performed in a Biological Safety Cabinet. Filtration of the effluent air from the vacuum pump is desirable either up (preferably), or down stream of the pump (5).

4. Ensure that all virulent fluid cultures or visible powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, nonbreakable leak-proof containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel (4, 10).

5. All inoculated petri plates or other inoculated solid media should be transported and incubated in leak-proof pans or leak-proof containers (4, 10).

6. Care must be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as noninfectious until culture or other tests have proved their sterility (4, 10).

7. Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.

8. No person should work alone on an extremely hazardous operation (4, 10).

IV. PERSONAL HYGIENE, HABITS, AND PRACTICES

Personal hygienic practices in the laboratory are directed, in most part, toward the prevention of occupationally acquired physical injury or disease. To a less obvious extent, they can raise the quality of the laboratory work by reducing the possibilities for contamination of experimental materials. The principles of hazards unrecognized in another, and for many of the recommended precautions and practices are obvious. In some instances, amplification will permit a better review of the applicability to any one specific laboratory.

Consequently, what might be forbidden in one laboratory might be only discouraged in another, and for many is permissible in a third. Nevertheless, adherence to safe practices that become habitual, even when seemingly not essential, provides a margin of safety in situations where the hazard is unrecognized. The history of occupational injury is replete with examples of hazards unrecognized until too late. The following guidelines, recommendations, and comments are presented with this in mind:

1. Food, candy, gum, and beverages for human consumption will be stored and consumed only outside the laboratory (5, 10).

2. Foot-operated drinking fountains should be the sole source of water for drinking by human occupants of the laboratory (27).

3. Smoking is not permitted in the laboratory or animal quarters. Cigarettes, pipes, and tobacco will be kept only in clean areas (5, 10, 26).

4. Shaving and brushing of teeth are not permitted in the laboratory. Razors, toothbrushes, toiletry supplies, and cosmetics are permissible only in clean change rooms or other clean areas, and should never be used until after showering or thorough washing of the face and hands (27).

5. A beard may be undesirable in the laboratory in the presence of actual or potential airborne contamination, because it retains particulate contamination more persistently than clean-shaven skin. A clean-shaven face is essential to the adequate facial fit of a face mask or respirator when the work requires respiratory protection (10, 27, 31).

6. Develop the habit of keeping hands away from mouth, nose, eyes, face, and hair. This may prevent self-inoculation (10, 27).

7. For product protection, persons with long hair should wear a suitable hair net or head cover that can be decontaminated. This has long been a requirements in hospital operating rooms and in the manufacture of biological pharmaceutical products. A head cover also will protect the hair from fluid splashes, from swinging into Bunsen flames and petri dishes, and will reduce facial contamination caused by habitual repetitive manual adjustment of the hair (5).

8. Long-flowing hair and loose-flapping clothing are dangerous in the presence of open flame or moving machinery. Rings and wrist watches also are a mechanical hazard during operation of some types of machines (5, 10).

9. Contact lenses do not provide eye protection. The capillary space between the contact lenses and the cornea may trap any material present on the surface of the eye. Caustic chemicals trapped in this space cannot be washed off the surface of the cornea. If the material in the eye is painful or the contact lens is displaced, muscle spasms will make it very difficult, if not impossible to remove the lens. For this reason, contact lenses must not be worn by persons exposed to caustic chemicals unless safety glasses with side shields, goggles, or plastic face masks are also worn to provide full protection. It is the responsibility of supervisors to identify employees who wear contact lenses (25, 26).

10. Personal items, such as coats, hats, storm rubbers or overshoes, umbrellas, purses, etc., do not belong in the laboratory. These articles should be kept elsewhere (25).

11. Plants, cut flowers, an aquarium, and pets of any kind are undesirable sources of yeast, molds, and other potential microbial contaminants of biological experimental materials (25).

12. Books and journals returnable to the institutional library should be used only in the clean areas as much as possible (10, 27).

13. When change rooms with showers are provided, the employer should furnish skin lotion (27).

14. When employees are subject to potential occupational infection, the shower and/or face/hand-washing facilities should be provided with germicidal soap (8, 27).

15. Personal cloth handkerchiefs should not be used in the laboratory. Cleansing tissue should be available instead.

16. Hand washing for personal protection:

(i) This should be done promptly after removing protective gloves. Tests show it is not unusual for microbial or chemical contamination to be present despite use of gloves, due to unrecognized small holes, abrasions, tears, or entry at the wrist.

(ii) Throughout the day, at intervals dictated by the nature of the work, the hands should be washed. Presence of a wrist watch discourages adequate washing of the wrist (10, 25).

(iii) Hands should be washed after removing soiled protective clothing, before leaving laboratory area, before eating, and before smoking. The provision of hand cream by the employer encourages these practices (5, 8, 10).

(iv) A disinfectant wash or dip may be desirable in some cases, but its use must not be carried to the point of causing roughening, desiccation or sensitization of the skin.

17. Anyone with a fresh or healing cut, abrasion, or skin lesion should not work with infective material unless the injured area is completely protected (8, 25).

18. Persons vaccinated for smallpox may be shedders of vaccinia virus during the phase of cutaneous reaction. Therefore, vaccination requires permission of the appropriate supervisor, because two weeks' absence may be necessary before returning to work with

normal cell cultures or with susceptible animals, especially the normal mouse colony (25).

19. The surgeon's mask of gauze or filter paper is of little value for personal respiratory protection (29). It is designed to prevent escape of droplets from the nose or mouth (29G). If biohazards demand respiratory protection, then nothing but a full face respirator or ventilated hood will suffice. A half-mask respirator does not protect the eyes, which are an unevaluated avenue of infection through the conjunctiva and the nasolacrimal duct (5, 8).

20. Nonspecific contamination by environmental organisms from humans, animals, equipment, containers for specimens or supplies, and outside air is a complication that may affect or invalidate the results of an experiment. The human sources of this contamination are evaluated as follows:

(i) Sneezing, coughing and talking (23A, 24A). Sneezing, variously reported to generate as many 32,000 or 1,000,000 droplets below 100 microns in diameter; coughing, which produces fewer and larger droplets; and talking, which has been reported to average only 250 droplets when speaking 100 words, show great differences between persons in regard to the number of microorganisms aerosolized. As a general rule, it may be said that these actions by normal healthy persons may play a less important role in transmission or airborne infection to humans or experimental materials than does liberation of microorganisms from human skin.

(ii) Dispersal of bacteria from human skin. There is a tremendous variation in the number of bacteria shed from the skin by a clothed subject. For instance, in one study, the number varied from 6,000 to 60,000 per minute (23C). These bacteria were released on skin scales which were of a size that could penetrate the coarse fabric used for the laboratory and surgical clothing in the test (23D). Dispersal of skin bacteria was several times greater from below the waist than from upper parts of the body (24D). Effective reduction is accomplished by use of closely-woven or impervious clothing fitted tightly at the neck, wrists, and ankles to prevent the clothing from acting as a bellows that disperses air carrying skin scales laden with bacteria (23B). Such clothing sometimes is too warm to work in. It was found that a significant reduction in dispersal of bacteria occurred with the wearing of close-fitting and closely-woven underpants beneath the usual laboratory clothing (23D). The purpose of this summary is to alert laboratory personnel to the existence of this source of contamination (9).

(iii) Prolific dispersal of bacteria occurs from infected abrasions, small pustles, boils, and skin disease (23F, 24B). Washing the lesions with germicidal soap will greatly decrease the number of organisms on the skin and dispersal into the air. Healthy nasal carriers who generate aerosolized staphylococci usually can be identified by the presence of heavy contamination of their fingers, face, and hair (23E). This point may be useful in investigating the source of staphylococcal contamination of cell lines.

(iv) Footwear. In moderate and high risk situations, shoes reserved for only laboratory use have been recommended as a precaution against transporting spilled infectious agents outside the laboratory. However, in experiments during which reduction of potential contamination of experimental materials is important, laboratory-only shoes can reduce the microbial load brought into the laboratory each day by street shoes. Shoes are efficient transporters. In one study, there were 4 to 850 times as many bacteria per square centimeter on the laboratory footwear as on the floor itself (30).

V. CARE AND USE OF LABORATORY ANIMALS (10,32-37)

A. Care and Handling

1. Special attention must be given to the humane treatment of all laboratory animals in accordance with the Animal Welfare Act of 1970. The implementing rules and regulations appear in the Code of Federal Regulations (CFR) Title 9, Chapter 1, Subchapter A, parts 1, 2, 3. Recommended provisions and practices that meet the requirements of the Act have been published by the U.S. Public Health Service (32).

2. There are specific minimum requirements (33) concerning the caging, feeding, watering, and sanitation for dogs, cats, guinea pigs, hamsters, rabbits, and nonhuman primates. To meet these requirements, the animal room supervisor must have a copy of 9 CFR Chapter 1, Subchapter A, Parts 1, 2, 3.

3. Each laboratory should establish procedures to ensure the use of animals that are free of disease prejudicial to the proposed experiments and free from carriers of disease or vectors, such as ectoparasites, which endanger other experimental animals or personnel (10).

B. Cages Housing Infected Animals (10)

1. Careful handling procedures should be employed to minimize the dissemination of dust from cage refuse and animals.

2. Cages should be sterilized by autoclaving. Refuse, bowls and watering devices should remain in the cage during sterilization.

3. All watering devices should be of the "non-drip" type.

4. Cages should be examined each morning and at each feeding time so that dead animals can be removed.

5. Heavy gloves should be worn when feeding, watering, handling, or removing infected animals. Bare hands should NEVER be placed in the cage to move any object therein.

6. When animals are to be injected with biohazardous material, the animal caretaker should wear protective gloves and the laboratory workers should wear surgeons gloves. Animals should be properly restrained to avoid accidents that might result in disseminating biohazardous material, as well as to prevent injury to the animal and to personnel.

7. Animals exposed to biohazardous aerosols should be housed in ventilated cages, in gas-tight cabinet systems, or in rooms designed for protection of personnel by use of ventilated suits.

8. Animals inoculated by means other than by aerosol should be housed in equipment suitable for the level of risk involved.

9. Infected animals to be transferred between buildings should be placed in ventilated cages or other aerosol-proof containers.

10. The oversize canine teeth of large monkeys present a particular biting hazard; these are important in the potential transmission of naturally-occurring, and very dangerous, monkey virus infections. Such teeth should be blunted or surgically removed by a veterinarian.

11. Presently available epidemiological evidence indicates that infectious hepatitis may be transmitted from nonhuman primates (typically chimpanzees) to man. Newly imported animals may be naturally infected with this disease, and persons in close contact with such animals may become infected. After six months residence in this country, chimpanzees apparently no longer transmit the disease. A record should be maintained for each newly imported animal. A sign should be posted at rooms housing these animals to warn that the animals are potentially infectious.

C. General Guidelines that Apply to Animal Room Maintenance (10)

1. Doors to animal rooms should be kept closed at all times except for necessary entrance and exit.

2. Unauthorized persons should not be permitted to enter animal rooms.

3. A container of disinfectant should be kept in each animal room for disinfecting gloves and hands, and for general decontamination, even though no infectious animals are present. Hands, floors, walls, and cage racks should be washed with an approved disinfectant at the recommended strength as frequently as the supervisor directs.

4. Floor drains in animal rooms, as well as floor drains throughout the building should be flooded with water or disinfectant periodically to prevent backup of sewer gases.

5. Shavings or other refuse on floors should not be washed down the floor drain because such refuse clogs the sewer lines.

6. An insect and rodent control program should be maintained in all animal rooms and in animal food storage areas.

7. Special care should be taken to prevent live animals, especially mice, from finding their way into disposable trash.

D. Necropsy Rules for Infected Animals (10)

1. Necropsy of infected animals should be carried out by trained personnel in Biological Safety Cabinets with the hinged glass panel down. The glove port panel with or without attached gloves, and a respirator should be used at the discretion of the supervisor.

2. Surgeons gowns should be worn over laboratory clothing during necropsies.

3. Rubber gloves should be worn when performing necropsies.

4. The fur of the animal should be wetted with a suitable disinfectant.

5. Small animals should be pinned down or fastened on wood or metal in a metal tray.

6. Upon completion of necropsy, all potentially biohazardous material should be placed in suitable containers and sterilized immediately.

7. Contaminated instruments should be placed in a horizontal bath containing a suitable disinfectant.

8. The inside of the Biological Safety Cabinets and other potentially contaminated surfaces should be disinfected with a suitable germicide.

9. Grossly contaminated rubber gloves should be cleaned in disinfectant before removal from the hands, preparatory to sterilization.

10. Dead animals should be placed in proper leak-proof containers, autoclaved and properly tagged before being placed outside for removal and incineration.

VI. DECONTAMINATION AND DISPOSAL (7, 10, 38-42)

A. Introduction

Available data on the efficacy of various decontaminants for etiologic agents indicate that no major surprises will be forthcoming regarding the susceptibility of organisms containing recombinant DNA molecules. In the absence of adequate information, tests to determine the efficacy of candidate decontaminants should be conducted with the specific agent of interest. The goal of decontamination is not only the protection of personnel and the environment from exposure to infectious agents, but also the prevention of contamination of experimental materials by a variable, persistent, and unwanted background of microorganisms. This additional factor should be considered in selecting decontamination materials and methods.

B. Decontamination Methods

Physical and chemical means of decontamination fall into four main categories: Heat; Liquid Decontaminants; Vapors and Gases; and UV Radiation.

1. *Heat.* The application or heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121 C under pressure in the autoclave is the most convenient method of rapidly achieving *sterility. Dry* heat at *160 to 170 C for* periods to 2 to 4 hours is suitable for destruction of viable agents on impermeable nonorganic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat in the decontamination of microorganisms and also serves as an efficient means for disposal.

2. *Liquid Decontaminants.* In general, the liquid decotaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems.

There are many misconceptions concerning the use of liquid decontaminants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, time of contact, pH, concentration, and the *presence and state of* dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in effectiveness of decontamination. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants when the end result must be sterility.

There are many liquid decontaminants available under a wide variety of trade names. In general, these can be categorized as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketons, alcohols and amines. Unfortunately, the more active the decontaminant the more likely it is that the decontaminant will possess undesirable characteristics, such as the possession of corrosive propertie. None is equally useful or effective under all conditions.

3. *Vapors and Gases.* A variety of vapors and gases possess decontamination properties. The most useful of these are formaldehyde and ethylene oxide. When these can be employed in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas decontaminants are primarily useful in decontaminating: (i) Biological Safety Cabinets and associated effluent air-handling systems and air filters; (ii) bulky or stationary equipment that resists penetration by *liquid surface decontaminants;* (iii) instruments and optics that might be damaged by other decontamination methods; and (iv) rooms and buildings and associated airhandling systems.

4. *Radiation.* The usefulness of ultraviolet (UV) irradiation as a decontaminant is limited by its low penetrating power. No information is available regarding the effectiveness of UV irradiation for decontaminating microorganisms containing recombinant DNA molecules. Dependence on UV must be based on the results of experiments imitating particular anticipated environmental conditions and applications. Ultraviolet light is generally of limited application and is primarily useful in air locks and animal holding areas for controlling low levels of airborne contaminants.

No one procedure or material will solve all decontamination problems. The only method of assuring the efficacy of selected methodologies is to critically examine the results obtained in practical tests with the microorganism(s) of interest.

C. Laboratory Spills

A troublesome problem that may occur in the laboratory is the decontamination of an overt biological spill. The occurrence of a spill poses less of a problem if it occurs in a Biological Safety Cabinet provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid decontaminant and a thorough wipe down of the internal surfaces of such cabinetry will usually be effective for decontaminating the work ozne but gaseous decontaminants would be required to rid the interior sections of the cabinet of contaminants. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be *lost to the experiment.*

The greater problem arises if the incident occurs in the open laboratory. All laboratory protocols should be designed to prevent such occurrences. The first action in the event of an overt laboratory spill is evacuation of the affected area to minimize the exposure of personnel involved. Next, the spill area must be isolated to prevent exposure of personnel and experimental materials beyond those involved in the immediate area of the spill. The procedures adopted must be rapidly effective and must not create additional aerosol or foster mechanical transfer of materials to unaffected areas. Personnel carrying out the procedures must be provided with protective clothing and equipment, including respiratory protection. Consideration must be given to the safe disposal of all materials and liquids resulting from cleanup procedures. Reentry of personnel to the area should be avoided until it can be reasonably established that the area has been effectively *decontaminated. Further specific details are* provided in Section VIII.

D. Disposal

Decontamination and disposal in infectious disease laboratories are closely interrelated acts in which decontamination constitutes the introductory phase of disposal. All materials and equipment used in research on recombinant DNA molecules will ultimately be disposed of; however, in the sense of daily use, only a portion of these will require actual removal from the laboratory complex or on-site destruction. The remainder will be recycled for use either within the same laboratory or in other laboratories that may or may not engage in DNA recombinant research. Examples of the latter that immediately come to mind are: reusable laboratory glassware, instruments used in necropsy of infected animals, and laboratory clothing. Disposal should therefore be interpreted in the broadest sense of *the word, rather than in the restrictive sense* of dealing solely with a destructive process.

The principal questions to be answered prior to disposal of any objects or materials from laboratories dealing with potentially infectious microorganisms or animal tissues are:

1. Have the objects or materials been effectively decontaminated by an approved procedure?

2. If not, have the objects or materials been packaged in an approved manner for immediate on-site incineration or transfer to another laboratory?

3. Does disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to personnel either:

(i) Those carrying out the immediate disposal procedures or

(ii) Those who might come into contact with the objects or materials outside the laboratory complex?

Laboratory materials requiring disposal will normally occur as liquid, solid, and animal room wastes. The volume of these can become a major problem when there is the requirement that all wastes be decontaminated prior to disposal. It is most evident that a significant portion of this problem can be eliminated if the kinds of materials initially entering the laboratory are reduced in any case, and wherever possible, materials not essential to the research should be retained in the nonresearch areas for disposal by conventional methods. Examples are the packaging materials in which goods are delivered, disposable carton-cages for transport of animals, and large carboys or tanks of fluids which can be left outside and drawn from as required. Reduction of this bulk will free autoclaves and other decontamination and disposal processes within the laboratory for the more rapid and efficient handling of materials known to be contaminated.

Inevitably, disposal of materials raises the question, "How can we be sure that the materials have been treated adequately to assure that their disposal does not constitute a hazard?" In the small laboratory, the problem is often solved by requiring each investigator decontaminate all contaminated materials not of immediate use at the end of each day and place them in suitable containers for routine disposal. In larger laboratories where the mass of materials for disposal becomes much greater and sterilization and decontamination bottlenecks occur, materials handling and disposal will likely be the chore of personnel not engaged in the actual research. In either situation, a case can be made for establishing a positive method of designating the state of materials to be disposed of. This may consist of a tagging system stating that the materials are either *sterile or contaminated.*

Disposal of materials from the laboratory and animal holding areas will be required for research projects ranging in size from an individual researcher to those involving large numbers of researchers of many disciplines. Procedures and facilities to accomplish this will range from the simplest to the most elaborate. The primary consideration in any of these is to dispel the notion that laboratory wastes can be disposed of in the same manner and with as little thought as household wastes. Selection and enforcement of safe procedures for disposal of laboratory materials are of no less importance than the consideration given to any other methodology for the accomplishment of research objectives.

Materials of disaimilar nature will be common in laboratories studying recombinant DNA molecules. Examples are combinations of common flammable solvents, chemical carcinogens, radioactive isotopes, *and concentrated viruses or nucleic acids.* These may require input from a number of disciplines in arriving at the most practical approach for their decontamination.

E. Characteristics of Chemical Decontaminants in Common Use in Laboratory Operations

Every person actively working with viable microorganisms, no matter how remote the field of specialization, will, from time to time, find it necessary to decontaminate by chemical methods work areas and materials, equipment, and specialized instruments. Chemical decontamination is necessary because the use of pressurized steam, the most rapid and reliable method of sterilization, is not normally feasible for decontaminating large spaces, surfaces, and stationary equipment. Moreover, high temperatures and moisture often damage delicate instruments,

particularly those having complex optical and electronic components.

Chemicals with decontaminant properties are, for the most part, available as powders, crystals, and liquid concentrates. These may be added to tap water for application as surface decontaminants, and some, when added in sufficient quantity, find use as decontaminants of bulk liquid wastes. Chemical decontaminants that are gaseous at room temperatures are useful as space-penetrating decontaminants. Others become gases at reasonably elevated temperatures and can act as either aqueous surface or gaseous space-penetrating decontaminants.

Inactivation of microorganisms by chemical decontaminants may occur in one or more of the following ways:

1. Coagulation and denaturation of protein.
2. Lysis.
3. Binding to enzymes, or inactivation of an essential enzyme by either oxidation, binding, or destruction of enzyme substrate.

The relative resistance to the action of chemical decontaminants can be substantially altered by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay systems employed.

There are dozens of decontaminants available under a wide variety of trade names. In general, these decontaminants can be classified as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the decontaminant the more likely it will possess undesirable characteristics. For example, peracetic acid is a fast-acting, universal decontaminant. However, in the concentrated state it is a hazardous compound that can readily decompose with explosive violence. When diluted for use, it has a short half-life, produces strong, pungent, irritating odors, and is extremely corrosive to metals. Nevertheless, it is such an outstanding decontaminant that it is commonly used in germ-free animal studies despite these undesirable characteristics.

The halogens are probably the second most active group of decontaminants. Chlorine, iodine, bromine, and fluorine will rapidly kill bacterial spores, viruses, rickettsiae, and fungi. These decontaminants are effective over a wide range of temperatures. In fact, chlorine has been shown to be effective at −40 F. (On the other hand, phenols and formaldehyde have high temperature coefficients). The halogens have several undesirable features. They readily combine with protein, so that an excess of the halogen must be used if proteins are present. Also, the halogens are relatively unstable so that fresh solutions must be prepared at frequent intervals. Finally, the halogens corrode metals. A number of manufacturers of decontaminants have treated the halogens to remove some of the undesirable features. For example, sodium hypochlorite reacts with p-toluenesulfonamide to form Chloramine T, and iodine reacts with certain surface-active agents to form the popular iodophors. These "tamed" halogens are stable, non-toxic, odorless, and relatively noncorrosive to metals. However, the halogens are highly reactive elements, and, because they are reactive they are good germicides. When a halogen acts as a decontaminant, free halogen is the effective agent. Rising the pH or combining the halogen with other compounds to decrease the corrosive effect will also decrease the germicidal power. A trade-off situation occurs.

Ineffectiveness of a decontaminant is due primarily to the failure of the decontaminant to contact the microorganisms rather than failure of the decontaminant to act. If one places an item in a liquid decontaminant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. Also, if there are spots of grease, rust or dirt on the object, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful, and a decontaminant should have, and most do have, incorporated surface-active agents.

F. Properties of Some Common Decontaminants

1. *Alcohol.* Ethyl or isopropyl alcohol in a concentration of 70–85% by weight is often used. Alcohols denature proteins and are somewhat slow in their germicidal action. However, they are effective decontaminants against lipid-containing viruses.

2. *Ether and Chloroform.* These compounds are not ordinarily used as decontaminants, but they do demonstrate the fact that lipid-containing viruses are inactivated by these organic solvents, whereas non-lipid-containing viruses are quite resistant.

3. *Formaldehyde.* Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37% concentration referred to as formalin or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures and the pungent, irritating odors make formaldehyde solutions difficult to use in the laboratory. Formaldehyde vapor generated from formaldehyde solution is an effective space decontaminant for decontaminating rooms or buildings, but in the vapor state with water it tends to polymerize out on surfaces to form paraformaldehyde, which is persistent and unpleasant. Formaldehyde gas can be liberated by heating paraformaldehyde to depolymerize it. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than formaldehyde released in the vapor state.

4. *Phenol.* Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against some viruses, rickettsiae, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.

5. *Quaternary Ammonium Compounds or Quats.* After 30 years of testing and use, there is still a considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses. The Quats will attach to protein so that dilute solutions of Quats will quickly lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The Quats have the advantages of being nontoxic, odorless, nonstaining, noncorrosive to metals, stable, and inexpensive.

6. *Chlorine.* This halogen is a universal decontaminant active against all microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25 percent available chlorine or 52,500 ppm. If one dilutes them to 1 to 100, the solution will contain 525 ppm of available chlorine, and, if a nonionic detergent such as Naccanol is added in a concentration of about 0.7 percent, a very good decontaminant is created.

7. *Iodine.* The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants used in the laboratory is the iodophors, and Wescodyne is perhaps the most popular. The range of dilution of Wescodyne recommended by the manufacturer is 1 oz. in 5 gal. of water giving 25 ppm of available iodine to 3 oz. in 5 gal. giving 175 ppm. At 75 ppm, the concentration of free iodine is .0075 percent. This small amount can be rapidly taken up by any extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For bacterial spores, a dilution of 1 to 40 giving 750 ppm is recommended by the manufacturer. For washing the hands, it is recommended that Wescodyne be diluted 1 to 10 or 10% in 50% ethyl alcohol (a reasonably good decontaminant itself) which will give 1,500 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

G. Vapors and Gases

The use of formaldehyde as a vapor or gas has already been discussed. Other chemical decontaminants which have been used this way included ethylene oxide, peracetic acid, beta-propiolactone (BPL), methyl bromide, and ethylene amine. When these can be used in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can be obtained. Residues from ethylene oxide must be removed by aeration; but otherwise it is convenient to use, versatile, and nonreactive. Peracetic acid is corrosive for metals and rubber. BPL in the vapor form acts rapidly against bacteria, rickettsiae, and viruses. It has a half-life of 3.5 hours when mixed with water, is easily neutralized with water, and lends itself to removal by aeration. The National Institutes of Health does not recommend BPL as a decontaminant because it has been identified as a suspect carcinogen.

H. Residual Action of Decontaminants

As noted in the preceding discussion of decontaminant properties, many of the chemical decontaminants often have residual properties that may be considered a desirable feature in terms of aiding in the control of background contamination. One is cautioned, however, to consider residual properties carefully. Ethylene oxide used to sterilize laboratory shoes can leave residues which cause skin irritation. Animal cell cultures, as well as viruses of interest, are also inhibited or inactivated by the decontaminants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in liquid decontaminant prior to autoclaving and cleaning should receive particular attention in rinse cycles. Similarly, during general area decontamination with gases or vapors, it may be necessary to protect new and used clean items by removing them from the area or by enclosing

them in gastight bags or by insuring adequate aeration following decontamination.

I. Selecting Chemical Decontaminants for Research on Recombinant DNA Molecules

No single chemical decontaminant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical decontaminants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

1. What is the target microorganism(s)?
2. What decontaminants in what form are known to, or can be expected to, inactivate the target microorganisms(s)?
3. What degree of inactivation is required?
4. In what menstruum is the microorganism suspended; i.e., simple or complex, on solid or porous surfaces, and/or airborne?
5. What is the highest concentration of cells anticipated to be encountered?
6. Can the decontaminant either as an aqueous solution, a vapor, or a gas reasonably be expected to contact the microorganisms, and can effective duration of contact be maintained?
7. What restrictions apply with respect to compatibility of materials?
8. Does the anticipated use situation require immediate availability of an effective concentration of the decontaminant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the infectious disease laboratory is the microorganism under active investigation. Laboratory preparations or infectious agents usually have titers grossly in excess of those normally observed in nature. The decontamination of these high-titer materials presents certain problems. Maintenance systems for bacteria or viruses are specifically selected to preserve viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interferences with the desired action of decontaminants may require the use of decontaminant concentrations and contact times in excess of those shown to be effective in the test tube. Similarly, a major portion of decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these survivors. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting decontaminants and procedures for their use.

Microorganisms exhibit a range of resistance to chemical decontaminants. In terms of practical decontamination, most vegetative bacteria, fungi and lipid-containing viruses, are relatively susceptible to chemical decontamination. The non-lipid-containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Spore forms are the most resistant.

A decontaminant selected on the basis of its effectiveness against microorganisms on any range of the resistance scale will be effective against microorganisms lower on the scale. Therefore, if decontaminants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other microorganisms generated by laboratory operations, even in high concentrations, would also be inactivated.

An additional area that must be considered and for which there is little definitive information available is the "inactivation" of nucleic acid. Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Strong oxidizers, strong acids and bases, and either gaseous or aqueous formaldehyde should react readily with nucleic acids. Their ability to destroy the nucleic acid being studied, however, should be confirmed in the experimenter's laboratory. Because of innate differences in the chemistry of RNA and DNA the effectiveness of a decontaminant for one cannot be extrapolated to the other. For example, RNA molecules are susceptible to mild alkaline hydrolysis by virtue of the free hydroxyl group in the 2′ position, whereas DNA molecules are not susceptible to mild alkaline hydrolysis.

Table II summarizes pertinent characteristics and potential applications for several categories of chemical decontaminants most likely to be used in the biological laboratory. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstruums. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. The efficacy of any of the decontaminants should be conclusively determined by individual investigators. It is readily evident that each of the decontaminants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of decontaminants.

Appendix B

TABLE II

SUMMARY OF PRACTICAL DECONTAMINANTS FOR USE IN THE LABORATORY

DECONTAMINANTS		PRACTICAL REQUIREMENTS				INACTIVATES					IMPORTANT CHARACTERISTICS											POTENTIAL APPLICATION											PROPRIETARY DECONTAMINANTS EXAMPLES
TYPE	CHEMICAL	CONCENTRATION (PERCENT, APPROXIMATE)	CONTACT TIME (MINIMUM) LIQUID	CONTACT TIME (MINIMUM) GAS	TEMP., °C	VEG. BACT.	LIPID VIRUS	NONLIPID VIRUS	SPORES	CORROSIVE TO METAL	CORROSIVE TO RUBBER	CORROSIVE TO PLASTIC	FIXATIVE	CLEARING	CAUSTIC	OXIDIZING AGENT	REDUCING AGENT	TOXIC	IRRITANT	CORROSIVE TO SKIN	CORROSIVE TO EYES	NON-POROUS	POROUS SURFACES	SURFACE DISINFECTION	SURFACE DISINFECTION (SPILLS)	FUMIGATION	EQUIPMENT DISINFECTION (IMMERSION)	EQUIPMENT DISINFECTION (SURFACE WIPING)	EQUIPMENT DISINFECTION (INTERNAL SURFACES)	LABORATORY WASTE DISINFECTION	LABORATORY WASTE DISINFECTION (SOLIDS)	LABORATORY WASTE DISINFECTION (LIQUIDS)	
ENZYME	Conc. Amon. Chlor.	1%	10	N.E.		+	+	-	-				+	+			+	+		+		+	/	/				/					5-11, CID, SH-40C, 51-52, SILKO-QUAT
	Phenolic Chlor.	1%	10	N.E.		+	+	+	-			+		+			+	+		+		+	/	/				/					512-WHEN, 4256, SILKO-40C, O-SYN
	Calcium Chlor.	5%	10	30		+	+	+	+				+	+			+	+		+		+	/	/				/					CHLORINE Y, CLOROX, PUREX
	Iodophor	2%	10	30		+	+	+	+	+				+	+		+	+		+		+	/	/				/					51-256, IODOPH, BETADINE, HEXIDINE
	Alcohol, Vinyl	60%	10	N.E.		+	+	+	-	+										+		+	/	/				/					
	Alcohol, Isopropyl	91%	10	N.E.		+	+	+	-	+										+		+	/	/				/					
	Formaldehyde	8%	30	-		+	+	+	+	+				+						+		+	/	/				/					STORE
	Glutaraldehyde	2%	10	30		+	+	+	+	+			+	+						+		+	/	/				/					CIDEX
GAS	Ethylene Oxide	450/L	60	60	37	30	+	+	+	+	N.A.			+	+					+	+	+	+	+			+				+		QUIKSEM, OXYSHIELD
	Beta-Propiolactone	2g/L	60	30	37-55	+	+	+	+	+	N.A.			+	+					+	+	+	+	+			+				+		

N.A. = Not Applicable. N.E. = Not Established.

1. Variable activity dependent on strain.
2. Exhibit flammable or explosive in 3% (by or flammable hydrocarbon air flow, at concentrations of 2 to 7% by volume in air, mixed-component in open flame.
3. Protection from light required.
4. Refer to manufacturer's literature and/or health hazard.
5. Exact limitations practical limitations all products available, individual laboratory (as necessary) on any apply endorsement or rejection of any product by the FDA.
6. Refers to effluvium and dumb laboratory.

VII. HOUSEKEEPING

A. Introduction

Well-defined housekeeping procedures and schedules are essential in reducing the risks of working with etiologic agents and in protecting the integrity of the research program. This is particularly true in the biological laboratory operating under less than total containment concepts and in all areas used for the housing of animals, whether or not they have been intentionally infected. A well-conceived and well-executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that promotes the efficient use of decontaminants in the event of the inadvertent release of a harmful agent. Less immediately evident are the benefits of establishing, among personnel of widely varying levels of education, an appreciation of the nature and sources of biological contamination.

Housekeeping is an omnibus term that can be interpreted as broadly or as narrowly as one chooses. It can be seen that many of the

procedures found under special headings, such as decontamination, disposal, and animal care, are, in reality, specific instructions for safely accomplishing otherwise routine housekeeping chores. In these safety suggestions for research on recombinant DNA molecules, it has been elected to address specifically only tasks of a janitorial nature under the subject of housekeeping.

The objectives of housekeeping in the biological laboratory are to:

1. Provide an orderly work area conducive to the accomplishment of the research program.

2. Provide work areas devoid of physical hazards.

3. Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems being researched.

4. Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.

5. Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of organic debris that (i) may harbor microorganisms that are a potential threat to the integrity of the biological systems under investigation, (ii) may enhance the survival of microorganisms inadvertently released in experimental procedures, (iii) may retard penetration of decontaminants, (iv) may be transferable from one area to another on clothing and shoes, (v) may, with sufficent buildup, become a biohazard as a consequence of secondary aerosolization by personnel and air movement, and (vi) may cause allergenic sensitization of personnel, e.g., to animal danders.

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other areas in the laboratory have the constant potential for creation of significant quantities of contaminated organic debris than do animal care facilities.

In all laboratories, efforts to achieve total decontamination and to conduct a major cleanup of the biological complex are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding.

The following checklist outlines a portion of the items requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed manner in which housekeeping in the biological laboratory complex must be viewed.

Administration Areas
Aisles
Animal Food Storage
Animal Bedding Storage
Biological Safety Cabinets
Bench Tops and Other Work Surfaces
Ceilings
Change Rooms
Cleaning Solution Disposal
Cages and Cage Racks
Dry Ice Chests
Deep Freeze Chests
Entry and Exit Ways
Equipment Storage
Floors
Glassware
General Laboratory Equipment Cleanup
Hallways
Incubators
Instruments
Insect and Rodent Control
Light Fixtures
Mechanical Equipment Areas
Mops
Pipes—Wall and Ceiling Hung
Refrigerators
Showers
Supply Storage
UV Lamps
Vacuum Cleaners
Waste Accumulations
Waste Water Disposal
Others

Housekeeping in the laboratory is one of the avenues that leads to accomplishing the research program safely. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research program and special hazards of the research environment. The recommended approach to housekeeping is the assignment of housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need be accomplished. He should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by an alien cleanup crew, delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements, and the location of concentrated biological preparations and contaminated equipment used in their preparation and application will be known.

B. Floor Care

Avoidance of dry sweeping and dusting will reduce the formation of nonspecific environmental aerosols. Wet mopping or vacuum cleaning with a high-efficiency particulate air (HEPA) filter on the exhaust is recommended.

Careful consideration must be given to design and quality in the selection of cleaning equipment and materials and in their use to prevent the substitution of one hazard for another.

In the absence of overt hazardous spills, the cleaning process commonly will consist of an initial vacuuming to remove all gross particulate matter and a follow-up wet mopping with a solution of chemical decontaminant containing a detergent. Depending on the nature of the surfaces to be cleaned and availability of floor drains, removal of residual cleaning solutions can be accomplished by a number of methods. Among these are: pickup with a partially dry mop, pickup with a wet vacuum that has an adequately filtered exhaust, or removal to a convenient floor drain by use of a floor squeegee.

After cleaning up a spill of infected material, the residual solution should not be discharged to a sanitary sewer until it has been autoclaved or given further chemical treatment, such as by the addition of sodium hypochlorite sufficient to provide a final concentration of 500 ppm chlorine. Most household bleaches are marketed with a chlorine content of 5.25%. These in a final dilution of 1:100, yield 525 ppm of available chlorine. After allowing a contact time of 15 minutes, these solutions may be flushed down any available drain. Chlorine solutions in these high concentrations may be too corrosive for general application to floors and equipment. In any event, if solutions are used in this way, after the contact time the area should be rinsed with water.

C. Dry Sweeping

While it is recommended that dry sweeping be minimized, this may be the only method available or practicable under certain circumstances. In such cases, sweeping compounds used with push brooms and dry-dust mop heads treated to suppress aerosolization of dust should be used.

Sweeping compounds available from the usual janitorial supply firms fall in three categories:

Wax-based compounds used on vinyl floors and waxed floor coverings.

Oil-based compounds for concrete floors.

Oil-based compounds with abrasives (such as sand) to achieve a dry scouring action where much soil is present.

Dry-dust mop heads can be purchased as treated disposable units or as reusable, washable heads that must be treated with appropriate sprays or by other means to improve their dust-capturing property.

D. Vacuum Cleaning

In the absence of a HEPA filter on the exhaust, the usual wet and dry industrial-type vacuum cleaner is a potent aerosol generator. The HEPA-filtered exhaust used in conjunction with a well-sealed vacuum unit, however, can negate this factor because of its ability to pass large volumes of exhaust air while retaining particles with a minimum efficiency of 99.97%. Wet and dry units incorporating a HEPA filter on the exhaust are available from a number of manufacturers.

There are no particular requirements with respect to the manner in which the dry vacuuming is accomplished other than to emphasize that the objective is to remove all debris and particulate matter. The manufacturer's directions adequately detail the frequency of bag changes, filter changes, and mechanical adjustments.

Dry material vacuum-collected during these floor-cleaning activities is potentially contaminated, but the nature of the risk is probably greater to the experiment than to the experimenter. It is wise to effect bag and filter changes and to clean out collection tanks in a manner that will avoid or minimize aerosolizing the contents of the vacuum cleaner.

A vacuum machine that collects debris in a disposable bag is preferable to machines that collect the major debris in a tank and on an exposed primary filter. Even though it may serve as a primary filter, the disposable bag must be removed with caution. A bellows effect may pump dust out of the bag if its intake opening is not sealed before moving it to a plastic bag for transfer out of the area. In any event, the outer surface of the disposable bag will probably bear some dust contamination, which also may occur on inner surfaces of the machine.

To avoid contaminating experimental materials, the emptying of vacuum collection tanks and changing of bags and filters are best done away from the immediate laboratory area, for example, in a small area that can be easily cleaned afterwards. The use of heavy rubber gloves is recommended when

removing wastes from tanks in case broken glass is present. After making the filter changes, all external surfaces of the immediate work area and the equipment should be wiped with a cloth moistened in decontaminant. The operator might plan for a change of laboratory clothing afterwards so as to minimize carrying contamination into other areas of the laboratory.

Avoid use of dry vacuum cleaning equipment in work with high risk agents in the open laboratory. Should it be necessary to use it, it is recommended that gaseous sterilization be used to minimize aerosolization of microorganisms before waste is emptied from the vacuum container. Because complete penetration of sterilizing gases into the collected dry dust may be a problem, all wastes should be placed in a plastic bag, which then is tightly closed and incinerated or disposed of in an approved manner.

When dry vacuum cleaning equipment has been used within a gastight safety cabinet system, it can be treated in an attached double-door autoclave (an autoclave equipped with an ethylene oxide gas sterilization system) to allow for removal and emptying of the collection tank.

If a wet vacuum is to be used for pickup of the detergent-germicide solution from the floor, the manufacturer's recommendations on filter life should be followed. In addition, the operation of the vacuum should be closely observed for evidence of operating changing indicating restricted airflow or, conversely, increased flow indicating filter failure. Liquids collected in the vacuum cleaner after floor mopping will contain decontaminant material. These liquids may be poured down a convenient floor drain, except in the case of cleanup wastes from an overt spill. The collected liquid should then be autoclaved or treated with chlorine solution before disposal.

Provisions should be made for regular decontamination of the entire vacuum cleaner with formaldehyde gas or vapor, or ethylene oxide. This should be done after use if the vacuum is used in any manner for cleanup of overt spills of infectious material.

E. Selection of a Cleaning Solution

The selection of a detergent-decontaminant combination for routine cleaning of the laboratory complex should be based on the requirements of the area of greatest potential for contamination by the widest spectrum of microorganisms. With rare exception, this will be identified as the animal holding area and the expected microorganisms. With rare exception, this will be identified as the animal holding area and the expected microorganisms may well include fungi, viruses, and the vegetative and spore forms of bacteria. A decontaminating solution for such a range of microorganisms would, however, be expensive and excessively corrosive for routine use. Except in those rare instances where it can be assumed that pathogenic spores are being shed by laboratory animals, the risks from the spores are more likely to affect the experiments than the personnel. The spores tend to be associated with organic debris from bedding and food, thus offering potential for removal or at least a large initial reduction in their numbers by vacuum cleaning. A wide range of cleaning solutions that are mildly sporicidal, reasonably residual, and are not destructive to the physical plant are available. Phenol derivatives in combination with a detergent have these characteristics and have been selected for routine use in a number of research facilities. There are numerous detergent-phenolic combinations available on the market. The phenols are one type of a broad spectrum of biocidal substances that includes the mercurials, quaternary ammonium compounds, chloride compounds, iodophores, alcohols, formaldehyde, glutaraldehyde, and combinations of alcohol with either iodine or formaldehyde. These have been discussed in Section VI.

The laboratory supervisor should make a selection from those types most readily available which meet the general criteria of effectiveness, residual properties, and low corrosiveness.

F. Wet Mopping—Two-Bucket Method

Wet mopping of floors in laboratory and animal care areas is, from a safety standpoint, most conveniently and efficiently accomplished using a two-bucket system. The principal feature of such a system is that fresh detergent-decontaminant solution is always applied to the floor from one bucket, while all spent cleaning solution wrung from the mop is collected in the second bucket. Compact wall-mounted double-bucket units with foot-operated wringers are available from most janitorial supply houses. A freshly laundered mop head of the cotton string type should be used daily. This requires that a mop with removable head be provided as opposed to a fixed-head type. In practice, the mop is saturated with fresh solution, very lightly wring into the second bucket and applied to the floor using a figure eight motion of the mop head. After every four or five strokes, the mop head is turned over and the process continued until an area of approximately 150 ft² has been covered. After allowing a contact time of five minutes, the solution is removed with either a wet vacuum cleaner with HEPA-filtered exhaust or with the wrung-out mop. The mopping is continued in 100 ft² increments until the total floor area has been covered. Floor-cleaning procedures are most effectively completed after the majority of the work force has departed and should progress from areas of least potential contamination to those of greatest potential. Before a mop head is sent to a laundry, it should be autoclaved. Spent cleaning fluids are disposed of by flushing down the drain.

If the cleanup follows an overt spill of infectious material, the spent cleaning solution, after removal from the floor, should be autoclaved or treated with chlorine solution. Chlorine (as household bleach) should be added to give 500 ppm and held for a contact time of 15 minutes before dumping in the sanitary sewer.

G. Alternative Floor Cleaning Method for Animal Care Areas and Areas with Monolithic Floors

The absence of permanently placed laboratory benches and fixed equipment, coupled with the mobility of modern cage racks, makes possible alternate floor-cleaning procedures in animal care facilities. As in all considerations of methodologies in biomedical laboratory facilities, it is necessary to assess the compatibility of procedures and facilities from the hazard point of view. The alternative floor-cleaning procedure to be discussed requires that floors are completely sealed or of monolithic construction so that liquid leakage to adjacent areas does not occur and that floor drains or wet vacuum cleaners are available.

Subsequent to the removal of all debris by dry vacuum, move the cage racks to one side of the room. Cover the floor of the remaining cleared portion of the room with detergent-decontaminant solution applied at a rate of approximately one gallon per 144 ft² from a one-gallon tank sprayer, using a setting of the nozzle which will cause the solution to flow on and not create a spray. The nozzle is placed close to the floor. Allow a fifteen-minute contact period; then push the cleaning solution to the floor drain with a large floor squeegee or pick it up with a wet vacuum. Allow the flow to air dry; move the cage racks into the cleaned area, and repeat the process for the remaining floor area. Floor drains in these areas should be rim-flush, at least six inches in diameter, and fitted with a screen or porous trap bucket to catch large debris that escapes the initial dry cleaning. Such screens and baskets should be emptied after treatment with a decontaminant. If space utilization does not require frequent floor washdown, pour a half-gallon of detergent-decontaminant solution into the drain each week to keep the trap in the waste line filled against backup of sewer gases.

VIII. CLEAN-UP OF BIOHAZARDOUS SPILLS (6, 9, 10)

A. Biohazards Spill in a Biological Safety Cabinet

Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.

1. Spray or wipe walls, work surfaces, and equipment with a 2% solution of an iodophor-decontaminant (Wescodyne or equivalent). A decontaminant detergent has the advantage of detergent activity, which is important because extraneous organic substances frequently interfere with the reaction between the microorganisms and the active agent of the decontaminant. Operator should wear gloves during this procedure.

2. Flood the top work surface tray, and, if a Class II cabinet, the drain pans and catch basins below the work surfaces with a decontaminant and allow to stand 10–15 minutes.

3. Remove excess decontaminant from the tray by wiping with a sponge or cloth soaked in a decontaminant. For Class II cabinets, drain the tray into the cabinet base, lift out tray and removable exhaust grille work, and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a decontaminant. Then replace in position and drain decontaminant from cabinet base into appropriate container and autoclave according to standard procedures. Gloves, cloth or sponge should be discarded in an autoclave pan and autoclaved.

B. Biohazard Spill Outside a Biological Safety Cabinet

1. Hold your breath, leave the room immediately, and close the door.

2. Warn others not to enter the contaminated area.

3. Remove and put into a container contaminated garments for autoclaving and thoroughly wash hands and face.

4. Wait 30 minutes to allow dissipation of aerosols created by the spill.

5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. For a high risk agent, a jumpsuit with tight-fitting wrists and use of a respirator should be considered.

6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.

7. Let stand 20 minutes to allow an adequate contact time.

8. Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

9. The dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable items with non autoclavable plastic bags should be avoided—separation of the plastic after autoclaving can be very difficult.

C. Radioactive Biohazard Spill Outside a Biological Safety Cabinet

In the event that a biohazardous spill also involves a radiation hazard, the clean-up procedure may have to be modified, depending on an evaluation of the risk assessment of relative biological and radiological hazard.

Laboratories handling radioactive substances must have the services of a designated radiation protection officer available for consultation.

The following procedure indicates suggested variations from the biohazard spill procedure (above) that should be considered when a radioactive biohazard spill occurs outside a Biological Safety Cabinet.*

1. Holding your breath, leave the room immediately and close the door.

2. Warn others not to enter the contaminated area.

3. Remove and put in a container contaminated garments for autoclaving and thoroughly wash hands and face.

4. Wait thirty minutes to allow dissipation of aerosols created by the spill.

**Before clean-up procedures begin, a radiation protection officer should survey the spill for external radiation hazard to determine the relative degree of risk.*

5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. (For a high risk agent, a jumpsuit with tight-fitting sleeves and a respirator should be considered).

6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.

7. Let stand 20 minutes to allow adequate disinfectant contact time.

8. **In most cases, the spill will involve ^{14}C or ^{3}H, which present no external hazard. However, if more energetic beta or gamma emitters are involved, care must be taken to prevent hand and body radiation exposure. The radiation protection officer must make this determination before the clean-up operation is begun.*

If the radiation protection officer approves, the biohazard-handling procedure may begin: Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

**If the radiation protection officer determines that radioactive vapors may be released and thereby contaminate the autoclave, the material must not be autoclaved. In that case, sufficient decontaminant solution to immerse the contents should be added to the waste container. The cover should be sealed with waterproof tape, and the container stored and handled for disposal as radioactive waste. Radioactive and biohazard warning symbols should be affixed to the waste container. As a general rule, autoclaving should be avoided.*

9. If autoclaving has been approved, the dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable items with plastic bags should be avoided—separation of the plastic after autoclaving can be very difficult.

**A final radioactive survey should be made of the spill area, dust pan, and squeegee with a Geiger counter, or a smear should be taken and counted in a liquid scintillation counter.*

IX. A SECONDARY RESERVOIR AND FILTRATION APPARATUS FOR VACUUM SYSTEMS

The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into collection vessels or reservoirs is a common procedure in many laboratories. To prevent the accidental contamination by aerosols or fluids of house vacuum systems or laboratory pumps, some investigators have installed side arm flasks containing cotton, sulfuric acid or decontaminant between the reservoir and the vacuum line. Cotton is not completely effective as a filtering agent, sulfuric acid will corrode pipes, and decontaminants may lose their inactivating ability upon standing. The introduction of a cartridge-type filter that is moisture resistant and has a rated capacity to remove particles 350 nm (0.35u) or larger in size provides an effective barrier to virus aerosols.

The secondary reservoir and filtration apparatus can be assembled from readily available units as shown in Figure 1. A length of plastic tubing ¼ inch I.D. X ½ inch wall is attached at one end of the reservoir and at the other end to the lower arm of a filtration and media storage flask. These flasks vary in capacity from 250 to 4000 ml, the choice of flask depending on available space and amount of fluid that could be accidentally aspirated. A second tube of the same dimensions is attached from the upper arm of the flask to the inlet port of the disposable filter assembly. The third tube is attached from the filter assembly to a vacuum source. The tubes are securely held to the filter by fittings supplied with the filter and the other tubing connections can be secured by worm drive hose clamps.

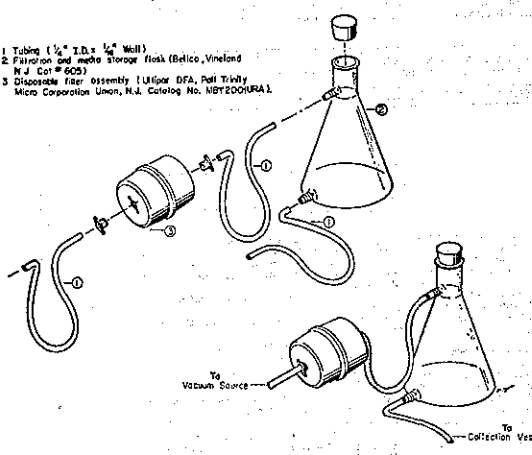
Ideally the flask should be placed higher than the reservoir of collection vessel. If fluid is accidentally drawn into the flask, the liquid can drain back into the reservoir by gravity if the connection at the vacuum line is broken. This prevents the loss of fluid which the investigator needs to retain.

Should the flask be used only for the recovery and storage of waste fluids, then the addition of a few grams of Dow Corning Antifoam A to the flask will reduce violent foaming of fluids aspirated into it. Such fluids can be decontaminated by introducing into the reservoir a final 5% concentration of an iodophor or other appropriate decontaminant, holding for 30 minutes and draining as above.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter and flask should be autoclaved before the filter is discarded. A new filter can then be installed and the assembly replaced.

*Changes in procedures have been starred and underlined.

Figure 1
A SECONDARY RESERVOIR AND FILTRATION APPARATUS



X. PACKAGING AND SHIPPING

A. Introduction

Federal regulations and carrier tariffs have been promulgated to ensure the safe transport of hazardous biological materials. The NIH Guidelines specify that all DNA recombinant materials will be packaged and shipped in containers that meet the requirements of these regulations and carrier tariffs. In addition when any portion of the recombinant DNA material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25 (which is included at the end of this section, page D-65) the labeling requirements in these regulations and carrier tariffs shall apply.

B. Packaging of Recombinant DNA Materials

1. Volume less than 50 ml.

Material shall be placed in a securely closed, watertight container [primary container (test tube, vial, etc.)] which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s).

Descriptions of this packaging method are given in Table III.

2. Volumes of 50 ml. or Greater.

Material shall be placed in a securely closed, watertight container (primary container) which shall be enclosed in a second, durable watertight container (secondary container). Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single secondary container. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength. A shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom, and sides between the secondary container and the outer shipping container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of materials which may be enclosed within a single outer shipping container should not exceed 4,000 ml.).

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

Descriptions of packages which comply with the regulations of the Department of Transportation (DOT) are given in Table IV.

C. Labeling of Packages Containing Recombinant DNA Materials

1. Materials which do not contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. Place only the address label on the outer shipping container, DO NOT USE THE LABEL FOR ETIOLOGIC AGENTS/BIOMEDICAL MATERIAL.

2. Materials which contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

Material data forms, letters, and other information identifying or describing the material should be placed around the *outside* of the secondary container. In addition to the address label, the label for Etiologic Agents/Biomedical Material must be affixed to the outer shipping container. This label is described in paragraph (c) (4) of 42 CFR 72.25.

3. Materials which contain any portion of a plant pest (plant pathogens) which are so defined by the Department of Agriculture (USDA).

Material data forms, letters, and other information identifying or describing the material should be placed around the *outside* of the secondary container. In addition to the address label, the shipping labels furnished by the USDA as part of the General, Courtesy, or Special Permits required for research with and shipment of such agents shall be affixed to the outer shipping container.

D. Additional Shipping Requirements and Limitations for Recombinant DNA Materials

1. Domestic Transportation.

Civil Aeronautics Board Rule No. 82 (Air Transport Association Restricted Articles Tariff 6-D) requires that a Shipper's Certificate, depicted below, be completed and affixed to all shipments which bear the ETIOLOGIC AGENT/BIOMEDICAL MATERIALS label required under the provisions of the Interstate Quarantine regulations [42 CFR Section 72.25(c)]. The Certificate must be completed in duplicate and affixed to the outer shipping container.

This is to certify that the contents of this consignment are properly classified, described by proper shipping name and are packed, marked and labelled and are in proper condition for carriage by air according to all applicable carrier and government regulations. (For international shipments add "and to the IATA Restricted Articles Regulations".) This consignment is within the limitations prescribed for: PASSENGER AIRCRAFT/CARGO ONLY (cross out nonapplicable).

Number of Packages	Specify Each Article Separately (Proper Shipping Name)	Classification	Net Quantity per Package
	ETIOLOGIC AGENT, n.o.s.	ETIO. AG.	

Shipper: _____

Date _____

(Signature of Shipper) _____

Shipments of recombinant DNA Materials exceeding 50 ml in volume and containing any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25 are restricted, by DOT regulations, to transport by cargo only aircraft. When the volume of a single primary container exceeds the 50 ml limitation, this restriction must be indicated on the Shipper's Certificate by crossing out "Passenger Aircraft".

When dry ice is used as a refrigerant an "ORM-Group A-DRY ICE LABEL" should be affixed to the outer shipping container. The amount of dry ice used and the date packed should be designated on the label.

2. International Transportation.

In addition to the packaging and labeling requirements of the regulations previously cited, international shipments of recombinant DNA materials in which any portion of the material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25 must have one or more of the following documents—depending on the country of destination:

(1) Parcel Post Customs Declaration (PS 2966) tag.

(2) Parcel Post Customs Declaration (PS 2966-A) label.

(3) International Parcel Post—Instructions Given by Sender (POD 2922) label.

(4) Dispatch note (POD 2972) tag.

(5) "Violet Label".

(6) Shipper's Certificate specified in the current International Air Transport Association Tariff.

Individual country requirements are listed in "International Postage Rates and Fees" (USPO Publication 51).

TABLE III.—*Description of packages for material in volume less than 50 ml.*

Volume (milliliter)	Primary container	Packing	Secondary container	Packing	Outer shipping container[1]
15 maximum	Sealed vial(s) or small glass test tube, screw cap or stopper, taped.[2]	Nonparticulate absorbent material at top, bottom, and sides that will completely absorb contents of the primary container(s).	Metal can 1-in diameter by 7-in outside dimensions metal screw cap.	None required	Fiberbody; metal screw cap, top and bottom; 1¾-in diameter by 7-to 7½-in outside dimensions.
50 or less	One 20 by 150 mm test tube, taped stopper or multiple small vials.[2]	do	Metal can 2¼-in diameter by 6¼-in high outside dimensions screw cap.	do	Fiberbody; metal screw cap, top and bottom; 3¼-in diameter by 7-to 7½ in outside dimensions.
Do	Plastic screw cap bottle or Pyrex glass with skirt rubber stopper.[3]	do	do	do	Do.
Do	Multiple watertight vials or tubes, taped stoppers.[2]	do	1 or more friction-seal tin cans 300 by 400 or larger.[4]	None required, but with the 300 by 400 cans or larger cans use sufficient nonparticulate shock-absorbent material to prevent rattling.	Fiberboard box.

[1] If materials are to be refrigerated, it is recommended that an overpack be used to contain the refrigerant and the sealed (original) outer shipping container. A leakproof outer container must be used for water ice. If dry ice is used the outer container must permit release of carbon dioxide. Interior supports must be provided to hold the container(s) in the original position(s) after wet or dry ice has dissipated.

[2] The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5- or 6-mil polyvinyl tubing, heat sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

[3] 610 by 708 and 304 by 305 are trade designations for outside dimensions of 6⅛-in diameter by 7⅛-in height, and 3⅛-in by 9⅝-in.

TABLE IV.—*Description of packages for material in volumes of 50 ml or greater*

Volume (milliliter)	Primary container	Packing	Secondary container	Packing		Outer shipping container	
				With refrigerant	Without refrigerant	With refrigerant	Without refrigerant
51 to 100	Plastic or Pyrex glass screw cap bottle, rubber or skirt rubber stopper, taped.[1]	Nonparticulate absorbent material at top, bottom, and sides that will completely absorb contents of the primary container(s).	Consists of metal container and outer container specified in table III.	Styrofoam box shock-absorbent insulation.	Shock absorbent material in volume at least equal to that between the primary and secondary container(s), at the top, bottom, and sides between the secondary container and the outer shipping container. The shock absorbent material shall be so placed that the secondary container(s) does not become loose inside the outer shipping container as the water ice or dry ice is dissipated.	Fiberboard box closely fitting the styrofoam box, taped shut.	Corrugated fiberboard or cardboard box, taped shut.
100 maximum	1 100-ml plastic screw cap narrow neck bottle or Pyrex glass, taped.[1]	do	No. 2 crimp seal tin can 404 by 700 or a 1-gal friction-seal tin can, 610 by 708, top soldered or clipped at 4 points.[2]	do	do	do	V3C cardboard box PS3 type, 9¾ in by 9¾ in by 11¼ in high outside dimensions, taped shut with 3 in type PS3 tape.
200 maximum	2 100-ml plastic screw cap bottles or Pyrex glass, taped.[1]	do	do[2]	do	do	do	Do.
250 maximum	1 250-ml plastic narrow mouth screw cap bottle or Pyrex glass skirted rubber stopper, taped.[1]	do	do[2]	do	do	do	Do.
300 maximum	2 250-ml plastic screw cap bottles or Pyrex glass bottles, taped.[1]	do	2-gal friction-seal tin can, 904 by 908, top soldered or clipped at 4 points.[2]	do	do	do	V3C cardboard box 12¼ in by 12¼ in by 10⅜ in high outside dimensions, taped shut with 3-in wide PS3 tape.
Do	300-ml Pyrex glass bottle, rubber-skirt stopper, taped, or 500-ml plastic bottle, narrow or wide mouth, screw cap, taped.[1]	do	No. 12 crimp seal tin can 603 by 810 2-gal friction-seal tin can, 804 by 908, top soldered or clipped at 4 points.[2]	do	do	do	V3C cardboard box 12¼ in by 12¼ in by 10⅜ in high outside dimensions, taped shut with 3 in wide PS3 tape. For the No. 12 can a cardboard box is OK taped shut.

[1] The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

[2] 610 by 708 and 804 by 908 are trade designations for outside dimensions of 6⅞₁₆ in diameter by 7⅝₁₆ in height, and 8¼ in by 9⅜ in.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE; PUBLIC HEALTH SERVICE; CENTER FOR DISEASE CONTROL; ATLANTA, GEORGIA 30333; TELEPHONE: (404) 633-3311, EXT. 3883

TITLE 42—PUBLIC HEALTH; CHAPTER I—PUBLIC HEALTH SERVICE, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE; SUBCHAPTER F—QUARANTINE, INSPECTION, LICENSING; PART 72—INTERSTATE QUARANTINE; SUBPART C—SHIPMENT OF CERTAIN THINGS

Section 72.25 of Part 72, Title 42, Code of Federal Regulations, is amended to read as follows:

§ 72.25 Etiologic agents.[1]

(a) *Definitions.* As used in this section:

(1) An "etiologic agent" means a viable microorganism or its toxin which causes, or may cause, human disease.

(2) "diagnostic specimen" means any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue, and tissue fluids being *shipped for purposes of diagnosis.*

(3) A "biological product" means a biological product prepared and manufactured in accordance with the provisions of 9 CFR Part 10, Licensed Veterinary Biological Products, 42 CFR Part 73, Licensed Human Biological Products, 21 CFR 130.3, *New drugs for investigational use in humans,* 9 CFR Part 103, Biological Products for Experimental Treatment of Animals, or 21 CFR 130.3(a), *New drugs for investigational use in animals,* and which, in accordance with such provisions may be shipped in interstate traffic.

(b) *Transportation; etiologic agent minimum packaging requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, including but not limited to, diagnostic specimens and biological products, containing, or reasonably believed by such person to contain an etiologic agent unless such material is packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.

(c) *Transportation; etiologic agents subject to additional requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, other than diagnostic specimens and biological products, containing, or reasonably believed by such person to contain, one or more of the following etiologic agents unless such material is packaged in accordance with the requirements specified in paragraph (b) of this section, and unless, in addition, such material is packaged and shipped in accordance with the requirements specified in subparagraphs (1)–(6) of this paragraph:

[1] The requirements of this section are in addition to and not in lieu of any other packaging or other requirements for the transportation of etiologic agents in interstate traffic prescribed by the Department of Transportation and other agencies of the Federal Government.

BACTERIAL AGENTS

Actinobacillus—all species.
Arizona hinshawii—all serotypes.
Bacillus anthracis.
Bartonella—all species.
Bordetella—all species.
Borrelia recurrentis, B. vincenti.
Brucella—all species.
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani.
Corynebacterium diphtheriae C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale.
Diplococcus (Streptococcus) pneumoniae.
Erysipelothrix insidiosa.
Escherichia coli, all enteropathogenic serotypes.
Francisella (Pasteurella) tularensis.
Haemophilus ducreyi, H. influenzae.
Herellea vaginicola.
Klebsiella—all species and all serotypes.
Leptospira interrogans—all serotypes.
Listeria—all species.
Mima polymorpha.
Moraxella—all species.
Mycobacterium—all species.
Mycoplasma—all species.
Neisseria gonorrhoeae, N. meningitidis.
Pasteurella—all species.
Pseudomonas pseudomallei.
Salmonella—all species and all serotypes.
Shigella—all species and all serotypes.
Sphaerophorus necrophorus.
Staphylococcus aureus.
Streptobacillus moniliformis.
Streptococcus pyogenes.
Treponema caretcum, T. pallidum, and T. pertenue.
Vibrio fetus, V. comma, including biotype El Tor, *and V. parahaemolyticus.*
Yersenia (Pasteurella) pestis.

FUNGAL AGENTS

Actinomycetes (including *Nocardia species, Actinomyces* species and *Arachnia propionica*).
Blastomyces dermatitidis.
Coccidioides immitis.
Cryptococcus neoformans.
Histoplasma capsulatum.
Paracoccidioides brasiliensis.

VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS

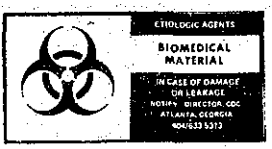
Adenoviruses—human—all types.
Arboviruses.
Coxiella burnetii.
Coxsackie A and B viruses—all types.
Cytomegaloviruses.
Dengue virus.
Echoviruses—all types.
Encephalomyocarditis virus.
Hemorrhagic fever agents, including *Crimean hemorrhagic fever (Congo), Junin,* and *Machupo viruses,* and others as yet undefined.
Hepatitis-associated antigen.
Herpesvirus—all members.
Infectious bronchitis-like virus.
Influenza viruses—all types.
Lassa virus.
Lymphocytic choriomeningitis virus.
Marburg virus.
Measles virus.
Mumps virus.
Parainfluenza viruses—all types.
Poliovirases—all types.
Poxviruses—all members.
Psittacosis-Ornithosis-Trachoma-Lymphogranuloma group of agents.
Rabies virus—all strains.
Reovirases—all types.
Respiratory syncytial virus.
Rhinoviruses—all types.
Rickettsia—all species.
Rubella virus.
Simian viruses—all types.
Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever,* and *Central European encephalitis viruses.*
Vaccinia virus.
Varicella virus.
Variola major and *Variola minor viruses.*
Vesicular stomatis virus.
Yellow fever virus.

(1) *Volume less than 50 ml.* Material shall be placed in a securely closed, watertight container (primary container (test tube, vial, etc.)) which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container consisnicted or corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

(2) *Volume 50 ml. or greater.* Packaging of material in volumes of 50 ml. or more shall include, in addition, a shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers at the top, bottom, and sides between the secondary container and the outer shipping container. Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single secondary container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of etiologic agent which may be enclosed within a single outer shipping container shall not exceed 4,000 ml.)

(3) *Dry ice.* If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be so placed that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

(4) *Labels.* The label for Etiologic Agents/ Biomedical Material, except for size and color, must be shown:



(i) The color of material on which the label is printed must be white and the symbol and printing in red.

(ii) The label must be a rectangle measuring 51 mm. (2 inches) high by 102.5 mm. (4 inches) long.

(iii) The red symbol measuring 38 mm. (1½ inches) in diameter must be centered in a white square measuring 51 mm. (2 inches) on each side.

(iv) Type size of the letters of label shall be as follows:

ETIOLOGIC AGENT	10 pt. rev.
BIOMEDICAL MATERIAL	14 pt.
IN CASE OF DAMAGE OR LEAKAGE	10 pt. rev.
NOTIFY DIRECTOR CDC ATLANTA, GA	8 pt. rev.
404 633 5313	10 pt. rev.

(5) *Damaged packages.* Carriers shall promptly, upon discovery of damage to the package that indicates damage to the primary container, isolate the package and notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633-5313), and the sender.

(6) *Registered mail or equivalent system.* Transportation of the following etiologic agents shall be by registered mail or an equivalent system which requires or provides for sending notification to the shipper immediately upon delivery:

Actinobacillus mallei.
Coccidioides immitis.
Francisella (Pasteurella) tularensis.
Hemorrhagic fever agents, including, but not limited to, *Crimean hemorrhagic fever (Congo), Junin, Machupo viruses.*
Herpesvirus simiae (B virus).
Histoplasma capsulatum.
Lassia virus.
Marburg virus.
Pseudomonas pseudomallei.
Tick-borne encephalitis virus complex. Including, but not limited to, *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever,* and *Central European encephalitis viruses. Variola minor* and *Variola major.*
Yersenia (Pasteurella) pestis.

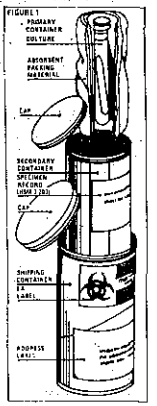
(d) *Notice of delivery; failure to receive.* When notice of delivery of agents containing, or suspected of containing, etiologic agents listed in paragraph (c) (6) of this section is not received by the sender within 5 days following anticipated delivery of the package, the shipper shall notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633-5313).

(e) *Requirements; variations.* The Administrator may approve variations from the requirements of this section if, upon review and evaluation, he finds that such variations provide protection at least equivalent to that provided by compliance with the requirements specified in this section and makes such findings a matter of official record.

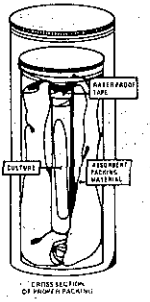
(Sec. 361, 58 Stat. 703; 42 U.S.C. 264)

[FR Doc.72-9887 Filed 6-29-72;8:45 am]

Effective July 30, 1972



PACKAGING AND LABELING OF ETIOLOGIC AGENTS



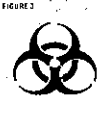
The Interstate Quarantine Regulations (42 CFR, Part 72.25, Etiologic Agents) was revised July 31, 1972 to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic.

Figures 1 and 2 diagram the packaging and labeling of etiologic agents in volumes of less than 50 ml. in accordance with the provisions of subparagraph (C) (1) of the cited regulation. Figure 3 illustrates the color and size of the label, described in subparagraph (C) (4) of the regulations, which shall be affixed to all shipments of etiologic agents.

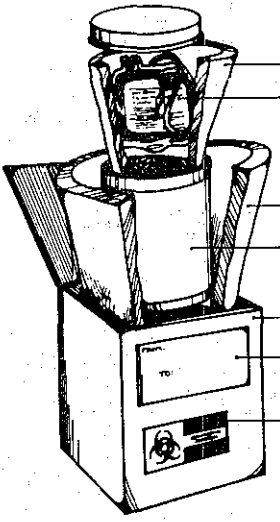
For further information on any provision of the regulation contact:

Center for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333
Telephone: 404 633-3311

FIGURE 3



PACKAGING AND LABELING OF ETIOLOGIC AGENTS



*NOTE: Single primary containers may not exceed 500 ml. of material. Two or more primary containers whose combined volumes do not exceed 500 ml. may be enclosed in a single, secondary container. The maximum volume of etiologic agent which may be enclosed in a single outer shipping container shall not exceed 4000 ml.

The Interstate Quarantine Regulations (42 CFR, Part 72.25, Etiologic Agents) was revised July 31, 1972, to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic. The illustration shows acceptable packaging and labeling of etiologic agents in accordance with subparagraphs (c) (2) and (4) of the cited regulation.

For further information on any provision of this regulation contact:

Center for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333

Telephone: 404-633-3311

XI. TRAINING AIDS, MATERIALS AND COURSES

A. Slide-Tape Cassettes

1. Assessment of Risk in the Cancer Virus Laboratory ($10).

2. Effective Use of The Laminar Flow Biological Safety Cabinet ($10).

3. Formaldehyde Decontamination of Laminar Flow Biological Safety Cabinets ($10).

4. Certification of Class II (Laminar Flow) Biological Safety Cabinets ($13).

5. Hazard Control in the Animal Laboratory ($10).

6. Basic Principles of Contamination Control (In preparation).

7. Selection of a Biological Safety Cabinet (In preparation). These slide tape cassettes are available for purchase from the National Audiovisual Center. The price for each is given above after the title. Send your order prepaid with a check or money order made payable to National Archives Trust Fund and mail to: Sales Branch, National Audiovisual Center (GSA), Washington, D.C. 20409.

8. Research Laboratory Safety. This slide tape cassette, stock number 176-79, is available for $75 from the National Safety Council, 425 North Michigan Avenue, Chicago, Illinois 60611.

B. Films

1. Air Sampling for Microbiological Particulates (M-926).

2. Handling the Laboratory Guinea Pig (T2619-X).

3. Handling the Laboratory Mouse (T2617-X).

4. Infectious Hazards of Bacteriological Techniques (M-382).

5. Laboratory Design for Microbiological Safety (M-1061).

6. Plastic Isolators: New Tools for Medical Research (M-599).

7. Safe Handling of Laboratory Animals (M-456).

8. Surface Sampling for Microorganisms (Rodac Method) (M-324).

9. Surface Sampling for Microorganisms (Swab Method) (M-925).

These films are available on loan without charge from: Media Resources Branch, National Medical Audiovisual Center (Annex), Station K, Atlanta, Georgia 30324.

The same films (except 2 and 3) can be rented or bought from: National Audiovisual Center (GSA) (Rental Branch) = (Sales Branch), Washington, D.C. 20409.

C. Courses

1. Biohazard and Injury Control in the Biomedical Laboratory. Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquiries to Dr. Donald Vesley, University of Minnesota, School of Public Health, 1325 Mayo Memorial Building, Minneapolis, Minnesota 55455. June 22–24, 1976, Los Angeles, CA; October 26–28, 1976, Boston, MA; December 7–9, 1976, Bethesda, MD.

2. Biohazard Containment and Control for Recombinant DNA Molecules. Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquiries as above. September 8–9, 1976, Stanford, CA; September 21–11, 1976; Cold Spring Harbor, NY.

3. Safety in Laboratory. Presented by National Institute of Occupational Safety and Health, Division of Training and Manpower Development, by special arrangement. Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226.

4. Laboratory Safety Management. Presented by the Laboratory and Training Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia. September 14–16, 1976, September 13–15, 1977.

XII. OUTLINE OF A SAFETY AND OPERATION MANUEL FOR A P4 FACILITY

A. Purpose

B. Policy

C. Responsibility and Authority

1. Management.
2. Supervisor.
3. Each Employee.
4. Facility Safety Officer.
5. Biohazard Safety Committee.

D. Facility Assignment Procedures

E. Reporting of Major and Minor Accidents and Injuries, Exposure to Toxic or Infectious Materials, Unsafe Conditions and Property Damages, and Rendering First-Aid

F. General Laboratory Safety

1. Fire.
2. Equipment.
3. Physical.
4. Chemical.
5. Radiological.

G. Safety Procedures Associated with Biohazard Activities of the Laboratory

1. Personnel Practices.
2. Operational Practices.

H. Medical Surveillance

I. Facility Operations

1. Personnel Access Procedures.
2. Access Procedures for Equipment Materials and Supplies.
3. Maintenance and Support.
4. Zone Classification.
5. Facility Monitoring Procedures.
6. Housekeeping.

J. Others

1. Packaging and Shipment of Biohazardous Materials.
2. Emergency Procedures.
3. Insect and Rodent Control.
4. Orientation and Training.

Appendix D was prepared by a Working Group Consisting of:

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1975



CLASSIFICATION OF ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE



CLASSIFICATION OF ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
OFFICE OF BIOSAFETY
ATLANTA, GEORGIA 30333

Prepared by the U.S. Public Health Service *Ad Hoc* Committee on the Safe Shipment and Handling of Etiologic Agents, with assistance from the staff of the Center for Disease Control.

CONTENTS

I. Introduction 1
II. Principles of Classification 2
III. Classification of Agents 4
Classification of Bacterial Agents 4
Classification of Fungal Agents 6
Classification of Parasitic Agents 6
Classification of Viral, Rickettsial, and Chlamydial Agents 6
IV. Level of Competence and Physical Containment Recommended for Each Class 9
V. General Safety 11
VI. Federal Regulations Covering Etiologic Agents 12

I. INTRODUCTION

This document provides a standard for evaluating the hazards associated with various etiologic agents and defines minimal safety conditions for their management without restricting or hampering bona fide microbiological investigations. Human etiologic agents are placed in four classes of increasing hazard. A fifth class, composed of animal agents excluded from the United States by law and United States Department of Agriculture (USDA) administrative policy, is described on page 2. The degree of hazard depends on the etiologic agent and the nature and kind of study in which it is being used. Aerosol studies, passage in animals, and infection of arthropod vectors markedly increase the hazard, whereas strict adherence to *in vitro* experiments decreases the hazard.

Other important factors must be considered when planning experiments with etiologic agents, particularly with those in Classes 3 and 4. These factors obviously vary from situation to situation, and it would be impossible to list all of them. Therefore, each investigator must use scientific judgment in interpreting the classification. For example, the public health risk accompanying animal infection or transmission experiments with yellow fever in southeastern states where the mosquito vector of the disease is prevalent would make them highly inadvisable except under the most rigidly controlled conditions, although a similar experiment in northern states, where natural vectors are lacking, could be conducted with far less risk.

Another factor that must be considered is whether the agent to be used naturally exists in the United States. This distinction is especially important when planning work with disease agents in

Classes 3 and 4 such as smallpox, which has been eradicated in this country. Experiments with such agents should be undertaken only when valid scientific consideration requires the use of the particular agents and no less hazardous agent can be substituted for it.

Anyone planning to work with etiologic agents should be aware of the animal agents in Class 5 which are excluded from the United States by law (virus of foot and mouth disease) and USDA administrative policy (African horse sickness virus, African swine fever virus, *Besnoitia besnoiti*, Borna disease virus, bovine infectious petechial fever, camel pox virus, ephemeral fever virus, fowl plague virus, goat pox virus, hog cholera virus, louping ill virus, lumpy skin disease virus, Nairobi sheep disease virus, Newcastle disease virus (Asiatic strains), *Mycoplasma mycoides* (contagious bovine pleuropneumonia), *Mycoplasma agalactiae* (contagious agalactia of sheep), *Rickettsia ruminatium* (heart water), Rift valley fever virus, rinderpest virus, sheep pox virus, swine vesicular disease virus, Teschen disease virus, *Trypanosoma vivax* (Nagana), *Trypanosoma evansi, Theileria parva* (East Coast fever), *Theileria annulata, Theileria lawrencei, Theileria bovis, Theileria hirci,* vesicular exanthema virus, Wesselsbron disease virus, *Zyonema farciminosum* (pseudofarcy)).

II. PRINCIPLES OF CLASSIFICATION

Members from those offices of the Public Health Service (PHS) and the USDA which have regulatory responsibility for quarantine and interstate shipment of etiologic agents participated in the development of the "Basis for Agent Classifications" which begins on page 3. Therefore, the principles expressed in the "Basis for Agent Classifications" are equally applicable to human and animal pathogens, although the specific list of agents, with the few exceptions noted, includes only human pathogens.

The least hazardous agents are in Class 1, and those requiring the greatest restrictions are in Class 4. Since the number of relatively or completely nonpathogenic agents is very large, listing all of

them in Class 1 would be impractical. Therefore, all agents which are not listed in Classes 2 through 4 belong in Class 1. Three special viruses in Class 1 are listed because the PHS *Ad Hoc* Committee on the Safe Shipment and Handling of Etiologic Agents considered them suitable for science experiments at a junior level. Newly recognized agents will be classified in later editions.

Basis for Agent Classifications

CLASS 1

Agents of no or minimal hazard under ordinary conditions of handling.

CLASS 2

Agents of ordinary potential hazard. This class includes agents which may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration but which are contained by ordinary laboratory techniques.

CLASS 3

Agents involving special hazard or agents derived from outside the United States which require a federal permit for importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment.

CLASS 4

Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This class includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

CLASS 5

Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy. (These agents are listed on page 2.)

Note: Federally licensed vaccines containing live bacteria or viruses are not subject to these classifications. These classifications are applicable, however, to cultures of the strains used for vaccine production, or further passages of the vaccine strains.

III. CLASSIFICATION OF AGENTS[1,2]

A. Classification of Bacterial Agents

CLASS 1

All bacterial agents not included in higher classes according to "Basis for Agent Classifications."

CLASS 2

Actinobacillus – all species except *A. mallei*, which is in Class 3
Arizona hinshawii – all serotypes
Bacillus anthracis
Bordetella – all species
Borrelia recurrentis, B. vincenti
Clostridium botulinum,
Cl. chauvoei, Cl. haemolyticum,
Cl. histolyticum, Cl. novyi,
Cl. septicum, Cl. tetani
Corynebacterium diphtheriae,
C. equi, C. haemolyticum,
C. pseudotuberculosis,
C. pyogenes, C. renale
Diplococcus (Streptococcus) pneumoniae

1. This classification does not include strictly animal pathogens.
2. A PHS permit is required to import any agent or to transfer within the United States any agent imported under permit.

Erysipelothrix insidiosa
Escherichia coli – all enteropathogenic serotypes
Haemophilus ducreyi, H. influenzae
Herellea vaginicola
Klebsiella – all species and all serotypes
Leptospira interrogans – all serotypes
Listeria – all species
Mima polymorpha
Moraxella – all species
Mycobacteria – all species except those listed in Class 3
Mycoplasma – all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae,* which are in Class 5 (page 2)
Neisseria gonorrhoeae, N. meningitidis
Pasteurella – all species except those listed in Class 3
Salmonella – all species and all serotypes
Shigella – all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pyogenes
Treponema carateum, T. pallidum, and *T. pertenue*
Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus*

CLASS 3

*Actinobacillus mallei**
Bartonella – all species
Brucella – all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocida type B ("buffalo" and other foreign virulent strains*)
*Pseudomonas pseudomallei**
Yersenia pestis

*USDA permit also required for import or interstate transport.

B. Classification of Fungal Agents

CLASS 1

All fungal agents not included in higher classes according to "Basis for Agent Classifications"

CLASS 2

Actinomycetes (including *Nocardia* species and *Actinomyces* species and *Arachnia propionica)*
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis

CLASS 3

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

C. Classification of Parasitic Agents

CLASS 1

All parasitic agents not included in higher classes according to "Basis for Agent Classification."

CLASS 2

Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

CLASS 3

Schistosoma mansoni

D. Classification of Viral, Rickettsial, and Chlamydial Agents

CLASS 1

Class 1 includes all viral, rickettsial, and chlamydial agents not included in higher classes according to "Basis for Agent Classification." Specifically listed are:

Influenza virus A/PR8/34

Newcastle virus – strains licensed for vaccine use in U.S.

Parainfluenza virus 3, SF4 Strain

(These viruses are included because the Committee agreed that they are suitable for science experiments at a junior level.)

CLASS 2

Adenoviruses – human – all types

Cache Valley virus

Coxsackie A and *B viruses*

Cytomegaloviruses

Echoviruses – all types

Encephalomyocarditis virus (EMC)

Flanders virus

Hart Park virus

Hepatitis-associated antigen material

Herpes viruses – except *Herpesvirus simiae* (Monkey B virus) which is in Class 4

Corona viruses

Influenza viruses – all types except A/PR8/34, which is in Class 1

Langat virus

Lymphogranuloma venereum agent

Measles virus

Mumps virus

Parainfluenza viruses – all types except Parainfluenza virus 3, SF4 strain, which is in Class 1

Polioviruses – all types, wild and attenuated

Poxviruses – all types except *Alastrim, Smallpox, Monkey pox,* and *Whitepox,* which, depending on experiments, are in Class 3 or Class 4
Rabies virus – all strains except *Rabies street virus,* which should be classified in Class 3 when inoculated into carnivores
Reoviruses – all types
Respiratory syncytial virus
Rhinoviruses – all types
Rubella virus
Simian viruses – all types except *Herpesvirus simiae (Monkey B virus)* and *Marburg virus,* which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

CLASS 3

Alastrim, Smallpox, Monkey pox, and *Whitepox,* when used *in vitro*
Arboviruses – all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)
Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic chorimeningitis virus (LCM)
Psittacosis-Ornithosis-Trachoma group of agents
Rabies street virus, when used in inoculations of carnivores (See Class 2.)

Rickettsia – all species except *Vole rickettsia* when used for transmission or animal inoculation experiments

*Vesicular stomatitis virus**

Yellow fever virus – wild, when used *in vitro*

CLASS 4

Alastrim, Smallpox, Monkey pox, and *Whitepox,* when used for transmission or animal inoculation experiments

Hemorrhagic fever agents, including *Crimean hemorrhagic fever (Congo), Junin,* and *Machupo* viruses, and others as yet undefined

Herpesvirus simiae (Monkey B virus)

Lassa virus

Marburg virus

Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever,* and *Central European encephalitis viruses*

Venezuelan equine encephalitis virus, epidemic strains, when used for transmission or animal inoculation experiments

Yellow fever virus – wild, when used for transmission or animal inoculation experiments

IV. LEVEL OF COMPETENCE AND PHYSICAL CONTAINMENT RECOMMENDED FOR EACH CLASS

The following recommendations describe the level of competence and physical containment suggested for working with agents of each Class.

*USDA permit also required for import or interstate transport.

CLASS 1

Distribution to all users; no special competence or containment required.

CLASS 2

Distribution to laboratories whose staffs have levels of competency equal to or greater than one would expect in a college department of microbiology. Requests for agents in Class 2 are placed on institutional letterhead.

CLASS 3

Distribution to laboratories whose staffs have levels of competency equal to or greater than one would expect in a college department of microbiology and who have had special training in handling dangerous agents and are supervised by competent scientists. For aerosol studies, passage in animals, and infection of arthropod vectors, the laboratory should be located in a geographical area in which the chance of accidental establishment of the agent in a susceptible ecologic focus is minimal. Requests for agents in Class 3 are signed by the chairman of the department or the head of the laboratory or research institute where the work will be carried out. Conditions for containment include:

1. A controlled access facility: suite or room separated from the activities of individuals not engaged in handling Class 3 agents and from the general traffic pattern of the rest of the building or laboratory.
2. Negative air pressure is maintained at the site of work in a preparation cubicle or under a hood. Air is recirculated only after it has been adequately decontaminated through high efficiency filters.
3. Animal experiments, including cage sterilization, refuse handling, disposal of animals, etc., are conducted with a level of precaution equivalent to conditions required for laboratory experiments.
4. Personnel at risk are immunized against agents for which immune prophylaxis is available.

CLASS 4

Distribution to laboratories whose staffs have levels of competency equal to or greater than one would expect in a college department of microbiology and who have had special training in handling dangerous pathogens and are supervised by competent scientists. For aerosol studies, passage in animals and infection of arthropod vectors, the laboratory should be located in a geographic area in which the risk of accidental establishment of the agent in a susceptible ecologic focus is minimal. Requests for agents in Class 4 are signed by the director of the institute or laboratory where the work is to be carried out. Conditions for containment include all those required for Class 3 agents and the following:

1. Work areas are in a facility which is in effect a separate building, or they are separated from other work areas by effective airlocks.
2. If the work area is not in a separate building, the entire area used for Class 4 agents has a separate air exhaust and negative pressure with respect to other areas of the building. Exhaust air is decontaminated by filtration through high efficiency filters or by some other suitable process. Class 4 agents are manipulated only in safety cabinets equipped with absolute filters.
3. Access to work areas is restricted to individuals immunized or otherwise under specific control.
4. Protective clothing is worn, and it is decontaminated before being removed from the laboratory area.
5. When an agent is used in entomological experiments, the windows, walls, floor, ceiling, and airlock of the work area are insect-proof, and pure pyrethrum insecticide or a suitable insect killing device is available in the airlock.

V. GENERAL SAFETY

The best way to maintain laboratory safety is to practice correct and careful laboratory techniques, including effective decon-

tamination and sterilization procedures, at all times. The laboratory's isolation and containment requirements are to supplement, not to supplant, good laboratory practice and sound scientific judgment. However, in an adequately isolated and properly equipped laboratory with correctly directed airflow, a scientifically and technically competent investigator can confidently work even with the most hazardous agents, provided the safety cabinets are selected to meet the requirements of the work. Of the several available cabinet types, the investigator should select the one which meets requirements for the maximum risk he expects to encounter.

The Office of Biosafety of the Center for Disease Control, 1600 Clifton Road, N.E., Atlanta, Georgia 30333, is available for consultation on the handling of etiologic agents.

VI. FEDERAL REGULATIONS COVERING ETIOLOGIC AGENTS

Several Federal agencies have regulations which cover the importation, interstate shipment, and safe packaging of etiologic agents. Even though the requirements of the agencies differ somewhat, the same safety principles should apply to all shipments. The investigator who wishes to import etiologic agents from abroad or to forward imported agents to other laboratories should be aware of and observe the restrictions, thus avoiding delays, unpleasant situations and embarrassment.

The principal agencies concerned with the transportation of etiologic agents are the USDA and the PHS. The following should be consulted for current regulations and requirements:

1. For importation or interstate transportation of agents which are animal pathogens:

   Chief Staff Veterinarian
   Organisms and Vectors
   Veterinary Services, APHIS, USDA
   Federal Building
   Hyattsville, Maryland 20782

2. For importation and interstate movement of agents that cause human disease:**

Center for Disease Control
Attn: Office of Biosafety
Atlanta, Georgia 30333

**In the case of zoonotic agents, both the USDA and the PHS should be consulted.

APPENDIX 10

federal register

THURSDAY, SEPTEMBER 9, 1976



PART III:

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

■

RECOMBINANT DNA RESEARCH GUIDELINES

Draft Environmental Impact Statement

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

RECOMBINANT DNA RESEARCH GUIDELINES

Draft Environmental Impact Statement

On Wednesday, June 23, 1976, the Director of the National Institutes of Health, with the concurrence of the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health, issued Guidelines that will govern the conduct of NIH-supported research on recombinant DNA molecules.

The decision by the NIH Director to release the Guidelines was reached after extensive scientific and public airing of the issues. The issues were discussed at public meetings of the Recombinant DNA Molecule Program Advisory Committee (Recombinant Advisory Committee) and the Advisory Committee to the NIH Director. The Recombinant Advisory Committee debated three different versions of the Guidelines during this period, and made detailed recommendations to the NIH Director on how this line of research could proceed effectively with maximum protection of workers and the environment against possible hazards.

The Advisory Committee to the NIH Director, augmented with consultants representing law, ethics, consumer affairs, and the environment, was asked to advise on whether the proposed Guidelines balanced responsibility to protect the public with the potential benefits through the pursuit of new knowledge. The many points of view expressed at an open meeting of the Committee on February 9 and 10, 1976, and in subsequent correspondence, were taken into consideration in the Director's decision.

A number of public commentators urged NIH to consider preparing an environmental impact statement on recombinant DNA research activity. They evoked the possibility that organisms containing recombinant DNA molecules might escape and affect the environment in potentially harmful ways. It should be noted that the development of the guidelines was in large part tantamount to conducting an environmental impact assessment. For example, the objectives of recombinant DNA research were considered and the potential hazards and risks analyzed. Possible alternative approaches to the objectives were thoroughly explored, to maximize safety and minimize potential risks. And an elaborate review structure to ensure safety has been created.

The Guidelines are premised on physical and biological containment to prevent the release or propagation of DNA recombinants outside the laboratory. Deliberate release of organisms into the environment is prohibited. The stipulated physical and biological containment ensures that this research will proceed with a high degree of safety and precaution.

With a view to promoting public understanding of its issuance of the Guidelines, NIH conducted an environmental impact assessment and prepared the present draft environmental impact statement in accordance with the National Environmental Policy Act of 1969. Notice of the availability of this document appeared in the FEDERAL REGISTER of September 2.

In order to extend the opportunity for public comment and consideration, the present draft environmental impact statement is offered for general comment. Please address any comments on this draft statement to the Director, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014. All comments should be submitted by October 18, 1976.

Additional copies of this draft are available from Dr. Rudolf G. Wanner, Associate Director for Environmental Health and Safety, Building 12A, Room 4051, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014.

Dated: August 26, 1976.

DONALD S. FREDRICKSON,
Director,
National Institutes of Health.

DRAFT ENVIRONMENTAL IMPACT STATEMENT

GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

August 19, 1976

GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

National Institutes of Health, Public Health Service, DHEW, Bethesda, Maryland

(X) Draft ( ) Final Environmental Impact Statement.

Name of Action

(X) Administrative ( ) Legislative Action.

Additional Information

Additional information on the proposed action, including technical documents pertinent to this statement may be obtained from:

Dr. Donald S. Fredrickson, Director, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014, Telephone: (301) 496-2433.

A copy of the "Guidelines for Research Involving Recombinant DNA Molecules" is attached. (Appendix D)

COMMENTS

The Department, in issuing this draft, is requesting comments on the accuracy of the factual information (including the absence of relevant material) and projections contained therein. Comments shall be submitted by October 18, 1976, the Council on Environmental Quality weekly notice in the FEDERAL REGISTER. Address comments to Dr. Donald S. Fredrickson.

CONTENTS

I. Foreword.
II. Authority.
III. Objective of the NIH Action.
IV. Background.
A. Description of the recombinant DNA experimental process.
B. Events leading to the development of guidelines.
C. Description of issues raised by recombinant DNA research.
1. Possible hazardous situations.
2. Expected benefits of DNA recombinant research.
3. Long-range implications.
4. Possible deliberate misuse.
V. Description of the proposed action.
VI. Description of alternatives.
A. No action.
B. NIH prohibition of funding of all experiments with recombinant DNA.
C. Development of different guidelines.
D. No guidelines but NIH consideration of each proposed project on an individual basis before funding.
E. General Federal regulation of all such research.
VII. Environmental impact of the guidelines.
A. Impact of issuance of NIH guidelines:
1. Impact on the safety of laboratory personnel and on the spread of possibly hazardous agents by infected laboratory personnel.
2. Impact on the environmental spread of possibly hazardous agents.
3. Cost impact.
4. Secondary impacts.
B. Impact of experiments conducted under the guidelines.
1. Possible undesirable impacts.
2. Beneficial impacts of DNA recombinant research.

APPENDICES

A. Glossary.
B. Suggested references for additional reading.
C. Documents describing the implementation of the guidelines.
D. "Recombinant DNA Research" containing "Decision of the Director, National Institutes of Health to Release Guidelines for Research on Recombinant DNA Molecules" and "Guidelines for Research Involving Recombinant DNA Molecules" as published in the FEDERAL REGISTER, Part II, July 7, 1976.

FOREWORD

Recent developments in molecular genetics, particularly in the last 4 years, open avenues to science that were previously inaccessible. In the "recombinant DNA" experiments considered here, genes—deoxyribonucleic acid (DNA) molecules—from virtually any living organism can be transferred to cells of certain completely unrelated organisms. For example, the genes from one species of bacteria have been transferred to bacteria of another species. And genes from toads and from fruit flies have been introduced into the bacterium *Escherichia coli*.

If the recipient bacterium is then allowed to multiply, it will propagate these newly acquired genes as part of its own geoetic complement. It appears likely that any kind of gene from any kind of organism could be introduced into *E. coli* and certain other organisms.

This ability to join together genetic material from two different sources and to propagate these hybrid elements in bacterial and animal cells has resulted in a profound and qualitative change in the field of genetics. Now, for the first time, there is a methodology for crossing very large evolutionary boundaries, and for moving genes between organisms that are believed to have previously had little genetic contact.

The promise of recombinant DNA research for better understanding and improved treatment of human disease is great. There is also a possible risk that microorganisms with foreign genes might cause disease or alter the environment should they escape from the laboratory and infect human beings, animals, or plants. However, in the absence of further experimental data neither the benefits nor the risks can be precisely identified or assessed.

On June 23, 1976, the Director of the National Institutes of Health released Guidelines governing the conduct of NIH-supported research on recombinant DNA molecules (See Appendix D). Promulgation of these Guidelines followed 2 years of intensive discussion and debate within the scientific community and NIH itself, with public participation, concerning the possible hazards of such research and the best means for averting them, although the possible hazards remain speculative. The Guidelines prohibit certain kinds of recombinant DNA experiments and, for those experiments that are permitted, they specify safety precautions and conditions designed to protect the health of laboratory workers, the general public, and the environment should the putative hazards prove real.

The issuance of Guidelines establishing conditions and precautions with respect to such experiments is viewed by NIH as a Federal action that may significantly affect the quality of the human environment, and NIH Director Dr. Donald S. Fredrickson ordered the preparation of this statement pursuant to the National Environmental Policy Act.

Although NEPA assumes that such Federal actions will not be taken until the NEPA procedures are completed, the Director of NIH concluded that the public interest required immediate issuance of the Guidelines, rather than deferral for the months that would be required for completion of the NEPA process. This was because the escape of potentially hazardous organisms was more likely in the absence of NIH action. Further, prompt issuance of the Guidelines was believed necessary in order to promote their acceptance by scientists in the United States and abroad who do not come under the purview of NIH.

Issuance of and compliance with the Guidelines is, in itself, expected to decrease the chance of any detrimental environmental impact. However, since there has been little actual experience to date with recombinant DNA experiments, the indicated confidence in the Guidelines rests essentially upon the judgment of scientists. Their confidence is based on two premises. First, it is believed that the containment measures specified in the Guidelines make the escape of potentially harmful recombinant organisms into the environment highly improbable. Second, it is believed that, even if an experiment performed in accordance with the Guidelines does result in accidental release of recombinant organisms, adverse effects will either not occur or not be serious.

In the absence of an adequate base of data derived from either experiments or experience, it must be recognized that future events may not conform to these judgments. There is some statistical probability that recombinant organisms will find their way into the environment either from experiments under NIH auspices or from the activities of others. It is not difficult to construct scenarios in which injury could result. Although the possibility of significant environmental consequences is entirely speculative, the chance of an event that could cause severe injury, however low the probability, must be treated as an environmental impact.

The NIH Guidelines, in addition to ensuring the safety of NIH-supported researchers, the general public and the environment, are serving as a model for other laboratories throughout the world, thereby promoting environmental protection beyond that achievable through other actions available to the Federal Government. And the experiments themselves may be expected ultimately to lead to an increase of knowledge and the advancement of medicine and other sciences.

Although the action in question—that is, issuance of the Guidelines—has already been taken, the Director of NIH believes that the NEPA review will further enlighten the public and focus attention on the important issues involved, in the interest of gaining the understanding and views of the broadest possible segment of the American people. In issuing the Guidelines, the NIH Director pointed out that they will be subject to continuous review and modification in the light of changing circumstances. Constructive modification could result from information received during the NEPA process.

II. Authority

The Federal action discussed in this document is taken under the authority of Title III of the Public Health Service Act—General Powers and Duties of Public Health Service; Part A—Research and Investigation; sections 301 and 307 (42 U.S.C. 241 and 242l).

III. Objective of the NIH Action

The objective of the proposed action—release of the NIH Guidelines—is the protection of laboratory workers, the general public, and the environment from infection by possibly hazardous agents that may result from recombinant DNA research. The Guidelines are meant to ensure that experiments involving recombinant DNA molecules and which are supported by NIH, are carried out under conditions and safeguards that minimize the possibility of the harmful exposure of any human being or other component of the environment to these possibly hazardous agents.

It is NIH policy that all work supported by NIH, either in its own laboratories or through grants or contracts to various organizations, must be carried out according to the Guidelines. As part of this objective, the Guidelines describe procedures that will be used to ensure implementation. A further objective of establishing the Guidelines is to influence, to the extent possible, other Federal, non-Federal, and foreign organizations in their efforts to assure that recombinant DNA experiments will be carried out with minimal risk to laboratory workers, the general public, and the environment.

IV. Background

A. Description of the Recombinant DNA Experimental Process

All living things, from subcellular particles to higher organisms, require specific information for their reproduction and functions. The basic source of this information is deoxyribonucleic acid (DNA), which is the principal substance of the genes, the units of heredity (1). Each cell of an organism is composed of various organized structures, several of which contain DNA. Figure IV-1 illustrates a typical cell.

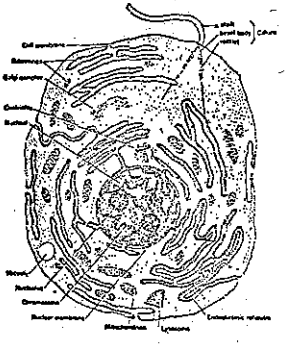

Figure IV-1

DNA plays two roles: (1) Provides information for the reproduction, growth, and functions of the cell, and (2) preserves and directs replication of this information and transfers it to the offspring. These two roles of DNA are common to animals, plants, single-cell organisms, and many viruses. The DNA of cells is mainly found in organized structures called chromosomes.

Intracellular DNA also occurs outside of the chromosomes as separately replicating molecules. Such DNA molecules include the plasmids, found in bacteria; the DNA of chloroplasts, common to green plants; and the DNA of mitochondria, the energy-producing units of the cells of complex organisms. These DNAs, while not strictly part of the inherent genetic make-up of a cell, help define the cell's functional capability. Another type of DNA commonly found in cells is the DNA of infecting viruses.

In the past 30 years the structure of the DNA molecule has been studied in-

tensively, and it can now be described in much detail. The molecule may be compared to a very long, but twisted step-ladder with thousands to millions of rungs (shown in Figure IV–2). The sides of the ladder are formed of sugar molecules (deoxyribose) attached end to end through phosphate groups. At right angles to each sugar molecule is one of four possible bases—adenine, guanine, thymine, and cytosine. The precise sequence of these bases, the rungs of the ladder, codes the information content. The "reading" of the code contained in the sequence of bases results in the formation of proteins which in turn permit the essential functions of the cell.

A gene is a portion of the DNA molecule which codes for the manufacture of a single protein. In higher organisms, much of the DNA may not serve as genes in this sense, but may regulate the activity of nearby genes. It is possible to break open cells and isolate DNA, free of other cellular constituents.

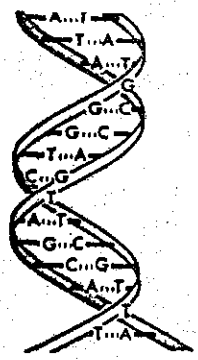

FIGURE IV–2

In recombinant DNA experiments, DNA is first isolated from two different cell types. Each DNA is then broken into segments. Each segment may contain one or more genes, or it may contain a portion of the DNA that lacks functional genes. The breaking is accomplished by means of bacterial enzymes (restriction endonucleases), which cut the DNA in such a way that the chemical structure at the ends of the segments permits interchangeable rejoining when the two different DNAs are mixed. In this way single DNA molecules containing portions of the two different DNAs are constructed. The DNA recombined in these experiments can be derived from widely divergent sources. The DNA from one of the sources serves as a carrier, or vector, for the insertion of the recombined DNA into a cell, or host. The vector may be DNA from a virus or a plasmid, usually derived from the same species as will serve as the host of the recombinant DNA. From a growth culture of the host cells, those containing the DNA fragment of particular interest are selected and allowed to multiply. The resulting population of identical cells is called a "clone." In some experiments the DNA will be extracted from the cells for study; in others, the properties of the cells themselves will be investigated.

In the experiments discussed in the Guidelines, the host cells are generally single-cell microorganisms such as bacteria, or animal or plant cells that were originally obtained from living tissue but are grown as single cells under special laboratory conditions.

The process of producing recombinant DNA molecules and introducing them into cells is illustrated in Figure IV–3.

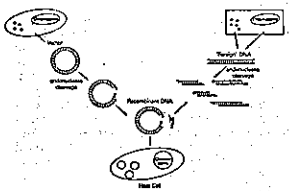

FIGURE IV–3

The cell represented at the upper left contains chromosomal DNA and several separately replicating DNA molecules. The non-chromosomal DNA molecules can be isolated from the cell and manipulated to serve as vectors (carriers) for DNA from a foreign cell. Most DNA molecules used as vectors are circular. They can be cleaved, as shown, by enzymes (restriction endonucleases) to yield linear molecules with rejoinable ends.

At the upper right is another cell, represented here as a rectangle. It serves as the source of the foreign DNA to be inserted in the vector. This DNA can also be cleaved by enzymes. The rectangular cell could be derived from any living species, and the foreign DNA might contain chromosomal or non-chromosomal DNA, or both.

In the next steps, the foreign DNA fragment is mixed and combined with the vector DNA, and the recombinant DNA is reinserted into a host cell. In most experiments this host cell will be of the same species as the source of the vector. The recipient cells are then placed under conditions where they grow and multiply by division. Each new cell will contain recombinant DNA.

B. EVENTS LEADING TO DEVELOPMENT OF GUIDELINES

On June 23, 1976, the Director, NIH, released "National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules" (see Appendix D). This action was approved by the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health. The Guidelines established carefully controlled conditions for the conduct of experiments involving the insertion of recombinatn genes into organisms, such as bacteria. The chronology leading to the present Guidelines and the decision to release them are outlined below.

It was some of the scientists engaged in recombinant DNA research who called for a moratorium on certain kinds of experiments in order to assess the risks and devise appropriate guidelines. The capability to perform DNA recombinations, and the potential hazards, had become apparent at the Gordon Research Conference on Nucleic Acids in July 1973. Those in attendance voted to send an open letter to Dr. Philip Handler, President of the National Academy of Sciences, and to Dr. John R. Hogness, President of the Institute of Medicine, NAS. The letter, appearing in "Science" (2), suggested that the Academy "establish a study committee to consider this problem and to recommend specific actions or guidelines, should that seem appropriate."

In response, NAS formed a committee, and its members published another letter in "Science" in July of 1974 (3). Under the title "Potential Biohazards of Recombinant DNA Molecules," the letter proposed:

First, and most important, that until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring * * * [certain] experiments * * *.

Second, plans to link fragments of animal DNAs to bacterial plasmid DNA or bacteriophage DNA should be carefully weighed * * *.

Third, the Director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (i) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (ii) developing procedures which will minimize the spread of such molecules within human and other populations; and (iii) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.

Fourth, an international meeting of involved scientists from all over the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

On October 7, 1974, the NIH Recombinant DNA Molecule Program Advisory Committee (hereafter "Recombinant Advisory Committee") was established to advise the Secretary of HEW, the Assistant Secretary for Health, and the Director of NIH" concerning a program for developing procedures which will minimize the spread of such molecules within human and other populations, and for devising guidelines to be followed by investigators working with potentially hazardous recombinants."

The international meeting proposed in the "Science" article (2) was held in February 1975 at the Asilomar Conference Center, Pacific Grove, California. It was sponsored by the National Academy of Sciences and supported by the National Institutes of Health and the National Science Foundation. One hundred and fifty people attended, including 52 foreign scientists from 15 countries, 16 representatives of the press, and 4 attorneys.

The conference reviewed progress in research on recombinant DNA molecules and discussed ways to deal with the potential biohazards of the work. Participants felt that experiments on con-

struction of recombinant DNA molecules should proceed: *Provided*, that appropriate containment is utilized. The conference made recommendations for matching levels of containment with levels of possible hazard for various types of experiments. Certain experiments were judged to pose such serious potential dangers that the conference recommended against their being conducted at the present time.

A report on the conference was submitted to the Assembly of Life Sciences, National Research Council, NAS, and approved by its Executive Committee on May 20, 1975. A summary statement of the report (4) was published in "Science, Nature," and the "Proceedings of the National Academy of Sciences." The report noted that "in many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazards. Until these are established, we urge individual scientists to use the proposals in this document as a guide."

The NIH Recombinant Advisory Committee held its first meeting in San Francisco immediately after the Asilomar conference. It proposed that NIH use the recommendations of the Asilomar conference as guidelines for research until the committee had an opportunity to elaborate more specific guidelines, and that NIH establish a newsletter for informal distribution of information. NIH accepted these recommendations.

At the second meeting, held on May 12–13, 1975, in Bethesda, Maryland, the committee received a report on biohazard-containment facilities in the United States and reviewed a proposed NIH contract program for the construction and testing of microorganisms that would have very limited ability to survive in natural environments and would thereby limit any possible hazards. A subcommittee chaired by Dr. David Hogness was appointed to draft guidelines for research involving recombinant DNA molecules, to be discussed at the next meeting.

The NIH committee, beginning with the draft guidelines prepared by the Hogness subcommittee, prepared proposed guidelines for research with recombinant DNA molecules at its third meeting, held on July 18–19, 1975, in Woods Hole, Massachusetts.

Following this meeting, many letters were received which were critical of the guidelines. The majority of critics felt that they were too lax, others that they were too strict. The committee reviewed all letters, and a new subcommittee, chaired by Dr. Elizabeth Kutter, was appointed to revise the guidelines.

A fourth committee meeting was held on December 4–5, 1975, in La Jolla, California. For this meeting a "variorum edition" had been prepared, comparing line-for-line the Hogness, Woods Hole, and Kutter guidelines. The committee reviewed these, voting item-by-item for their preference among the three variations and, in many cases, adding new material. The result was the "Proposed Guidelines for Research Involving Recombinant DNA Molecules," which were referred to the Director, NIH, for a final decision in December 1975.

The Director of the National Institutes of Health called a special meeting of the Advisory Committee to the Director to review these proposed guidelines. The meeting was held at NIH, Bethesda, on February 9–10, 1976. The Advisory Committee is charged to advise the Director, NIH, on matters relating to the broad setting—scientific, technological, and socioeconomic—in which the continuing development of the biomedical sciences, education for the health professions, and biomedical communications must take place, and to advise on their implications for NIH policy, program development, resource allocation, and administration. The members of the committee are knowledgeable in the fields of basic and clinical biomedical sciences, the social sciences, physical sciences, research, education, and communications. In addition to current members of the committee, the Director, NIH, invited a number of former committee members as well as other scientific and public representatives to participate in the special February session.

The purpose of the meeting was to seek the committee's advice on the guidelines proposed by the Recombinant Advisory Committee. The Advisory Committee to the Director was asked whether, in their judgment, the guidelines balanced scientific responsibility to the public with scientific freedom to pursue new knowledge.

Public responsibility weighs heavily in this genetic research area. The scientific community must have the public's confidence that the goals of this profoundly important research accord respect to important ethical, legal, and social values of our society. A key element in achieving and maintaining this public trust is for the scientific community to ensure an openness and candor in its proceedings. Representatives of the international press were invited to the Asilomar conference, and the proceedings received extensive coverage. The meetings of the Director's Advisory Committee and the Recombinant Advisory Committee have also reflected the intent of science to be an open community in considering the conduct of recombinant DNA experiments. Notification of all the meetings was published in the FEDERAL REGISTER and all the meetings were attended and reported by representatives of the press. At the Director's Advisory Committee meeting, there was ample opportunity for comment and an airing of the issues, not only by the committee members but by public witnesses as well. All major points of view were broadly represented.

The guidelines were reviewed in light of the comments and suggestions made by participants at that meeting, as well as the written comments received afterward. As part of that review the Recombinant Advisory Committee was asked to consider at its meeting of April 1–2, 1976, a number of selected issues raised by the commentators. Those issues and the response of the Recombinant Advisory Committee were taken into account in arriving at the final decision on the Guidelines.

The history of the events and discussions leading to the development of the Guidelines are described in greater detail in the "Decision of the Director, NIH," published as a preamble to the Guidelines in the FEDERAL REGISTER, Part II, July 7, 1976 (See Appendix D).

C. DESCRIPTION OF ISSUES RAISED BY RECOMBINANT DNA RESEARCH

1. *Possible hazardous situations.* The stable insertion of DNA derived from a different species into a cell or virus (and therefore the progeny thereof) may change certain properties of the host. The changes may be advantageous, detrimental, or neutral with regard to (a) the survival of the recipient species, (b) other forms of life that come in contact with the recipient and (c) aspects of the nonliving environment. Current knowledge does not permit accurate assessment of whether such changes will be advantageous, detrimental or neutral, and to what degree, when considering a particular recombinant DNA experiment. At present it is only possible to speculate on ways in which the presence of recombinant DNA in a cell or virus could bring about these effects. It should be emphasized that there is no known instance in which a hazardous agent has been created by recombinant DNA technology. The following discussion is speculative and consider ways in which hazardous agents might be produced.

a. *The effect of foreign DNA on the survival of recipient species (host cells or viruses).* The effect of foreign DNA on the survival of recipient species is important to the discussion of possible hazards of recombinant DNA experiments because although a recipient species may acquire a potential for harmful effects as a result of the foreign DNA, the possibility that the harmful effect will occur will depend on the survival of the recipient and its ability to multiply. If acquisition of foreign DNA increases the probability of survival and multiplication the possibility of harmful effects will increase. Similarly, if acquisition of foreign DNA decreases the probability of survival or multiplication, the possibility of harmful effects will decrease. It is important to recognize, in evaluating the potential for harmful effects, that significant infections of animals and plants by bacteria or viruses may require contact with either a large or small number of the infectious agent, depending on the agent.

There are various indications that bacteria and viruses containing inserted foreign DNA are less likely to survive and multiply than are the original organisms. Natural evolution results in the survival of well-balanced and efficient organisms. Essential functions are carefully controlled, and can be switched on and off as needed. It is unlikely that uncontrolled, nonessential properties such as might be introduced by foreign genes would result in any advantage to the survival and multiplication of an other-

wise well-balanced organisms. It is more likely that the new properties accompanying insertion of foreign genes will confer some relative disability to the recipient organisms. Therefore it is likely that bacterial cells containing inserted foreign DNA will multiply more slowly than the same cells without foreign DNA. Thus, in a natural competitive environment, bacteria containing recombinant DNA would generally be expected to disappear. The rate of disappearance will depend on the relative rate of growth compared to other, competing bacteria. The following calculation demonstrates this point.

Assume that a new organism constitutes 90 percent of a population, but grows 10 percent less rapidly than its natural counterpart. The new organism will drop from a concentration of 90 percent to a concentration of 0.0001 percent (1 part in 1,000,000) in 207 generations. If the generation time of the natural organism is one hour, this amounts to about 8½ days.

One example of a situation in which the capability of recipient bacterial host cells to survive may be significantly increased as the result of the presence of a foreign DNA is the case of resistance to antibiotics and drugs. It is well known that such resistance is often genetically determined and genes specifying resistance have been described. Furthermore it is well known that such genes may be transferred, by natural DNA recombination, from one species of microorganism to another. Such natural events are in fact responsible for the rapid and wide spread of resistance to clinically important drugs that has been observed during the last 20 years.

The ability of recipient bacterial host cells to survive and multiply might also be enhanced by acquisiton and expression of a foreign gene conferring the ability to metabolize particular nutrients. In an environmental niche containing the metabolite, such a recombinant might compete succesfully against organisms native to the niche. This could result in destruction of an environmental component—that is, the metabolite. Also, if the native organisms were performing beneficial functions, those functions could be lost upon the successful establishment of the recombinant in the niche.

b. *The effect of bacteria and viruses containing recombined DNA on other forms of life.* The analysis leading to the Guidelines centered on the possibility of deleterious effects, since the concern was the health and safety of living organisms, including humans, and the environment. Agents constructed by recombinant DNA technology could prove hazardous to other forms of life by becoming pathogenic (disease-producing) or toxigenic (toxin-producing), or by becoming more pathogenic or toxigenic than the original agent.

There are two basic mechanisms by which a recipient microorganism might be altered with regard to its pathogenicity or toxicity as a result of a resident recombinant:

(1) *The recombinant DNA may result in formation of a protein that has undesirable effects.* The case in which bacterial cells are used as carriers of foreign DNA is discussed first. A foreign protein, specified by the foreign DNA, might act after being liberated from the microorganism, or it could function within the microorganism and alter, secondarily, normal microbial cell function in such a way that the cell is rendered harmful to other living things. Either means depends on the expression of the foreign genes; that is, the information in the foreign genes must be used by the recipient bacterium to produce a foreign protein. Examples of protein that might prove harmful to other organisms are hormones, enzymes and toxins.

The weight of present evidence suggests that foreign DNA from bacteria of one species, when inserted into bacteria of another species, may be expressed in the recipient. For example, if the donor of the foreign DNA produces a toxic substance, then the recipient cell may produce such a substance if the gene for the toxic substance is present in the recombinant. The recipient may or may not be more hazardous than the original donor organism, depending on the relative ability of the two organisms to grow and infect an animal or plant species at risk.

The evidence available at present is insufficient to predict whether or not foreign genes derived from a complex organism (animals, plants, yeasts, and fungi) will be expressed in a bacterium in any particular instance. It may be that specific manipulations will be required to permit bacteria to express information of a foreign DNA efficiently. Faithful expression of a gene requires accurate functioning of the complex bacterial machinery involved in protein synthesis. At each step, specific signals originating in the foreign gene must be recognized by the bacterial machinery. Evolutionary divergence has resulted in different signals in bacteria and complex organisms.

Attempts to translate animal virus and animal cell genes into portein, using cell-free systems containing the protein-synthesizing machinery isolated from bacteria such as *E. coli* yield some protein-like products. The protein products characterized to date were not faithful products of the information in the genes.

In a few cases, intact bacteria containing recombined genes from complex organisms have been tested for evidence of expression of the inserted gene. By and large, accurate expression of the genes has not yet been demonstrated, although it may occur at a low frequency. In some instances, a new protein has been found, replacing one encoded by a bacterial gene. This result is expected if a bacterial gene is interrupted by insertion of the new DNA sequence within it, and does not necessarily indicate expression of the foreign gene. DNA fragments from yeast have been inserted into a strain of the bacterium *E. coli* which cannot manufacture the amino acid histidine (5). (Histidine is a component of most proteins and therefore is required for the growth of all organisms.) After insertion, some cells no longer required histidine; thus, the presence of the yeast DNA overcame the requirement for histidine. This is the first suggestion that a foreign gene from an organism more complex than bacteria may actually function in a bacterial cell. (Although yeast is a single-cell organism, it contains an organized nucleus like cells of higher organisms.) However, the detailed mechanism explaining this observation is unknown.

Analogous issues must be considered for the case in which animal viruses are the carriers of foreign DNA. Many viruses are simply described as DNA molecules enclosed and protected by coats of protein molecules. The protein coat protects the DNA from environmental effects, thus increasing the ability of the viral DNA to infect a cell. If viral DNAs are recombined with foreign DNAs in such a way that necessary viral genes remain intact, then the recombinant DNA may in turn be able to produce, and be packaged in, the coat of the virus. Inadvertent dispersal of such a viral particle outside of the laboratory might then result in entry of the recombinant DNA into cells of living organisms. The foreign genes may be expressed, resulting in the formation of a protein foreign to the infected cell, or the uncontrolled synthesis of a normal protein. The likelihood of expression of the foreign genes will probably depend on the degree of relatedness between its source and the infected organism as well as its location in the viral DNA used as vector. Currently, few if any relevant experimental data are available so that estimates of the probability of expression are, in these instances, impossible.

(2) *The recombined DNA may itself cause pathogenic or toxic effects.* Foreign DNA inserted in a bacterial gene, might so alter the microbial cell's properties that it becomes harmful to other organisms. This might happen, for example, through a change in the growth rate and competitive advantage of the recipient microbial cell, resulting in increased virulence of a mildly pathogenic bacteria. In general, one would expect the inserted DNA to result in a reduced growth rate and a selective disadvantage to the organism, as discussed in "a" above. Similar issues arise where animal viruses serve as carriers of foreign DNA.

It is also necessary to consider situations in which DNA molecules themselves may escape from the laboratory or from the experimental host cell and enter cells of living organisms with which they come in contact. Although free DNA molecules are themselves relatively fragile (and the probability that they would survive, in a significant form or for a significant time, in air, water, or any other medium, is considered remote), they can be protected in nature in a variety of ways and be released either into, or close to, a living cell.

When a cell or virus dies, or comes close to or invades the tissue of another living organism, the recombinant DNA may effectively enter a new cell. A hazardous situation similar to that described above might ensue if foreign proteins were manufactured in this "secondary" recipient. The recombinant DNA might survive as an independent cellular component, or it could recombine by natural

process with the DNA of the secondary recipient. Various possible deleterious consequences of such a recombination may be considered.

If the secondary recipient is another microorganism, the same considerations described in IV–C–1–a apply. If the secondary recipient is one of the cells of an animal or plant, different considerations apply. The latter include alterations of normal cellular control mechanisms, synthesis of a foreign protein (such as a hormone), and insertion of genes involved in cancer production (if, for example, the foreign DNA were derived from a cancer-producing virus).

It should be pointed out that the likelihood of causing inheritable changes in the offspring of complex organisms by such a mechanism is extremely low in animals because of the protection afforded germ-line cells (eggs and sperm) by their location. Thus, the possibility that recombined foreign DNA would reach germ line cells at a time in the life of such cells when secondary recombination can occur is extremely remote. With one-celled organisms, plants, or simple multicellular organisms, the probability of causing heritable change by secondary recombination may be higher.

What is the probability of secondary recombination between prokaryotes and eukaryotes in nature? It is generally held that recombination in nature is more likely if similar or identical sequences of bases (rungs in the DNA ladder) occur in the two recombining DNAs. The greater the degree of similar sequences, the more likely is recombination. In general, the more closely two species are related, the more likely it is that similar sequences will be found in their DNAs. Thus, DNA from primates has more DNA sequences in common with human DNA than does DNA from mice, or fish, or plants. Recombination may also occur between DNAs not sharing sequences but at lower frequencies.

It is possible that the capacity for interspecies recombination between distantly related species exists in nature. For example, bacteria in animal intestines are constantly exposed to fragments of animal DNA released from dead intestinal cells. Significant recombination requires the uptake of intact segments of animal DNA and their subsequent incorporation into the bacterial DNA. The frequency of such events is unknown.

There are very few available data permitting assessment of the reverse process—namely, the incorporation of bacterial DNA into the cells, or DNA, of more complex organisms. Although there are reports of experiments in which bacterial DNA was inserted into animal and plant species and production of the bacterial protein followed, the process is very inefficient and many investigators have been unable to repeat these experiments (6–8).

There are certain well-documented instances in which the DNAs of different living things become more or less permanently recombined in nature. These instances involve recombination between the DNAs of nonchromosomal genes, such as those of viruses or plasmids, or recombination between the DNAs of viruses or plasmids and chromosomal genes. The former instance, for example, is the mechanism behind the rapid spread of resistance to antibiotics among different bacterial species (9, 10). This spread accompanied the prevalent use of antibiotics in medicine and agriculture. Some viral DNAs recombine into and persist in chromosomal DNA of cells of receptive organisms (11, 12). Some viral DNAs acquire, in stable form, DNA sequences derived from their host cells (13, 14). There is also strong evidence for recombination of the DNA form of RNA tumor virus genes with chromosomal genes (15–17).

2. *Expected benefits of DNA recombinant research.* Benefits may be divided into two broad categories: An increased understanding of basic biological processes, and practical applications for medicine, agriculture, and industry.

At this time the practical applications are, of course, speculative. It is important to stress that the most significant results of this work, as with any truly innovative endeavor, are likely to arise in unexpected ways and will almost certainly not follow a predictable path.

a. *Increased understanding of basic biological processes.* There are many important fundamental biomedical questions that can be answered or approached by DNA recombinant research. In order to advance against diseases in inheritance, we need to understand the structure of genes and how they work. The DNA recombinant methodology provides a simple and inexpensive way to prepare large quantities of specific genetic information in pure form. This should permit elucidation of the organization and function of the genetic information in higher organisms. For example, current estimates of the fraction of this information that codes for proteins are simply educated guesses. There are almost no clues about the function of the portions of DNA that do not code for proteins, although these DNA sequences are suspected of being involved in the regulation of gene expression.

The existing state of ignorance is largely attributable to our previous inability to isolate discrete segments of the DNA in a form that permits detailed molecular analysis. Recombinant DNA methodology remove this barrier. Furthermore, ancillary techniques have been developed whereby pure DNA segments that contain particular sequences of interest can be identified and selected. Of particular interest is the isolation of pure DNA segments that contain the genes for the variable and constant portions of the immunoglobin proteins. The analyses of such segments obtained from both germline and somatic cells should be of inestimable value in determining the mechanism of immunologic diversity.

A major problem in understanding the mechanism by which certain viruses cause cancer is how and where the infecting or endogenous viral genomes are integrated into the cell's chromosome. This bears on the question of how the expression of the integrated viral genes affects cellular regulation, thus leading to the abnormal growth characteristics of cancer cells. With the recombinant DNA techniques for isolation and purification of specific genes, this research problem is reduced to manageable proportions. It is possible to isolate the desired DNA segment in pure form. Large quantities can be obtained for detailed study by simply extracting a culture of the bacteria carrying the viral DNA segment in a plasmid.

b. *Potential practical applications for medicine, agriculture and industry.* Certain of the potential applications will only be realized if the reproduction of the recombined foreign DNA in a recipient host cell is followed by expression of the genetic information contained in the DNA in the form of synthesis of proteins. Since the efficient translation of eukaryote genes in bacterial (prokaryote) hosts has yet to be proved, these potential applications are speculative at this time. Applications that depend on the expression of foreign prokaryotic genes in prokaryotic recipient cells are presently more certain.

(1) *Synthesis of medically important proteins and other substances.* It has been suggested that genes coding for medically important substances be attached to bacterial vectors, and that the bacteria then be used to produce large quantities of the desired material. A number of costly and/or rare substances would be prime candidates for such synthesis:

Human insulin (a future shortage of currently used animal insulin appears to be likely);

Human growth hormone (presently available only from human cadavers and in short supply);

Clotting factor VIII (for treatment of hemophilia).

Specific antibodies and antigens (for preventing and treating infectious, allergic, and autoimmune disease, and perhaps even cancer);

Certain enzymes, such as fibrinolysin and urokinase (promising agents in the treatment of embolism) and lysosomal enzymes.

(2) *Endowment of plants with new synthesis capabilities.* Whole plants may be generated from a single cell, and thus insertion of recombinant DNA into such cells might make it possible to endow plant species with the capability of—

Improved photosynthetic fixation of carbon dioxide;

Nitrogen fixation by presently inept species (thereby reducing the need for costly chemical fertilizers that cause pollution—e.g., eutrophication);

Producing a higher quality or quantity of food protein.

(3) *Some industrial applications.* A number of industrial processes utilize microorganisms containing enzymes (which are proteins) to produce important chemicals (e.g., steroid hormones or other drugs, vitamins) or foodstuffs (e.g., cheese). Such processes could be improved through innovations effected by DNA recombinant research. Completely new biosynthetic reactions may thereby become available, permitting the synthesis of large amounts of complex and

valuable compounds with ease and at low cost.

Some highly speculative applications relate to the area of energy production and neutralization of pollutants—e.g., as in oil spills. Genetic modification through DNA recombination might it possible to devise microorganisms tailor-made for such important purposes.

3. *Long-range implications.* The experimental situations treated in the Guidelines are those that appear feasible either currently or in the near future. The experiments primarily involve insertion of recombined DNA into bacteria or into single cells derived from more complex organisms and maintained under special laboratory conditions. It is only in the case of plants that the Guidelines cover experiments involving insertion of DNA into cells capable of developing into complex, multicellular organisms. The Guidelines and the discussions leading to their development have focused on problems of safety.

It is possible that techniques similar to or derived from current recombinant DNA methodology may, in the future, be applicable to the deliberate modification of complex animals, including humans. Such modification might have as its aim correction of an inherited defect in an individual, or alteration of heritable characteristics in the offspring of individuals of a given species. The latter type of alteration has been successfully achieved in agriculture for centuries, by classical breeding techniques. It may be that recombinant DNA methods, should they develop in appropriate ways, may offer new opportunities for specificity and accuracy in animal breeding.

The deliberate application of such methods for the correction of individual genetic defects or the alteration of heritable characteristics in man raises complex and difficult problems. In addition to philosophical, moral, and ethical questions of concern to individuals, serious societal issues are involved. Broad discussion of these problems in a variety of forums will be required to inform both private and public decision-making.

4. *Possible deliberate misuse.* In the event that recombinant DNA technology can yield hazardous agents, such agents might be considered for deliberate perpetration of harm to animals (including humans), plants or the environment. The possibilities include biological warfare or sabotage. Because it is not known whether recombinant DNA technology can yield such agents, discussion of these problems such as theft by saboteurs is hypothetical and difficult. With regard to biological warfare, a July 3, 1975 letter to Dr. David Baltimore from James L. Malone, General Counsel of the United States Arms Control and Disarmament Agency says, "you raise the question as to whether the Biological Weapons Convention prohibits production of recombinant DNA molecules for purposes of constructing biological weapons. In our opinion the answer is in the affirmative. The use of recombinant DNA molecules for such purposes clearly falls within the scope of the Convention's provisions."

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V. DESCRIPTION OF THE PROPOSED ACTION

The Director, National Institutes of Health, has issued Guidelines that will govern the conduct of NIH-supported research on recombinant DNA molecules. The Guidelines will apply to all NIH-supported research on such molecules—that is, molecules which are made by combining segments of DNA from different organisms in a cell free-system and which can be inserted into some living cell, there to replicate. The objective of the Guidelines is the protection of the laboratory worker, the general public, and the environment from infection by possibly hazardous agents that may result from this research. The complete text of the Guidelines is found in the FEDERAL REGISTER, Part II, for Wednesday, July 7, 1976. As an integral part of this Draft Environmental Impact Statement the Guidelines are found in Appendix D.

The mechanisms by which the NIH will implement the application of the Guidelines are outlined in the Guidelines themselves and are specified in greater detail in Appendix C. Noncompliance with the Guidelines will result in termination of funding of research grants and contracts.

The Guidelines describe (1) safeguards that protect the laboratory worker, the general public, and the environment, (2) the criteria for assessing the possible dangers from experiments involving recombinant DNA molecules, (3) the criteria for matching the assessed possible dangers of individual experiments with the appropriate safeguards, and (4) the roles and responsibilities of principal investigators, their institutions, and NIH for ensuring the implementation of the requirements specified in these Guidelines. The emphasis on protection of laboratory workers from infection reflects the fact that laboratory workers are the persons at the greatest risk of infection and that the most likely route of escape of possibly hazardous agents from the laboratory is the laboratory worker.

The physical safeguards have been grouped into four levels providing increasing capability for containment. The four levels approximate those recommended by the Center for Disease Control for the control of known infectious agents that have been determined to be of (1) no or minimal, (2) ordinary, (3) special, or (4) extreme hazard to man and other living things. These correspond to the terms Minimal, Low, Moderate, and High risk, respectively, as used in the NIH Guidelines. The safeguards include usual and special microbiological safety practices, primary physical barriers that isolate the experiment from the laboratory worker, and facility installations that either markedly reduce or eliminate the potential for accidental dissemination of recombinant DNA molecules to the environment. The four levels, designated P1 to P4, provide increasing protection against contact with or accidental release of microorganisms containing recombinant DNA molecules.

Additional safeguards are provided by the use of host cells and vectors with demonstrably limited ability to survive in other than specially designed laboratory environments. This concept is called "biological containment" in the Guidelines. In the case of bacterial host cells and vectors, this means that particular strains of cells and vectors with genetically determined and fastidious survival requirements must be used. For those experiments judged to be of potentially moderate or high risk, the properties of the bacterial strains to be used

must be certified by the NIH Recombinant Advisory Committee prior to initiation of experiments. In the case of a vector derived from an animal virus, the virus itself must be a low risk agent (CDC or National Cancer Institute), and a strain of the virus that is defective in infection must serve as the source of the vector DNA.

The selection of containment (safeguard) levels is dependent on the assessed possible dangers of the experiment. The Guidelines provide standards for evaluating the conceivable dangers of particular experiments involving recombinant DNA molecules. In the absence of evidence of any hazard actually occurring, these standards are based on relevant current knowledge. Permissible experiments are placed into four classes of increasing possible danger which correspond to the four levels of increasing containment capability (safeguards). Certain experiments, judged to have the potential for extreme hazard, should they prove dangerous, are prohibited.

The possibility for danger depends on—

(1) The biohazard associated with the DNA of the cell or microorganism that serves as the DNA source (e.g., genes for toxin production),

(2) The degree to which the DNA segment has been purified away from other genes and shown to be free of harmful characteristics,

(3) The biohazard associated with the vector that serves to transmit the source DNA to a recipient host cell,

(4) The ability of the vector to survive in natural environments or habitats,

(5) The kinds and number of different organisms that are susceptible to infection by the recipient or vector,

(6) The biohazard of the recipient host cell that serves to replicate the recombinant DNA molecule,

(7) The ability of the recipient cell to survive in natural environments or habitats,

(8) The ability of the recipient cell to transmit the recombinant DNA molecule to other cells capable of surviving in natural environments or habitats,

(9) The potential of the recipient cell to obtain the source DNA by natural means, and

(10) The evolutionary relatedness of the DNA source to humans.

The Guidelines prohibit a number of types of experiments, including those in which an organism contributing DNA is itself a biohazard of greater than low risk as determined by conventional methods of risk assessment (low risk corresponds to class 2 agents as defined by the Center for Disease Control). The host cells and vectors are required to be of no or minimal risk. The potential dangers are considered to increase as the organism providing the source DNA approaches humans phylogenetically. Thus, source DNA from primate cells is considered to have greater potential dangers than source DNA from lower eukaryotes. In general, greater possible dangers are assigned to recombinants than are present in the most hazardous component used to construct the DNA.

The risk-assessment standards are specified in detail for one prokaryote host-vector system employing a variant of *E. coli* called strain K12, which is, by itself, of no or minimal risk. Eukaryote host-vector systems using defective viral vectors are also described. The descriptions of these systems provide principles by which the potential dangers of recombinant DNA experiments with other host-vector systems can be assessed.

The Guidelines also establish an administrative framework for assigning the responsibility for ensuring safety in recombinant DNA research supported by NIH. This responsibility is shared among the principal investigators, their institutions, and NIH. The principal investigators have the primary responsibility for hazard assessment and for implementation of appropriate safeguards. The institutions are responsible for ensuring that the principal investigators have the capabilities for meeting the requirements stipulated in the Guidelines. NIH is responsible for securing an independent assessment of the potential dangers of this research and for ensuring that no research is supported unless it conforms to the requirements stipulated in the Guidelines.

The Guidelines require that the institutions establish biohazard committees to carry out the institutional responsibility, and stipulate the qualifications and expertise of the committee membership. NIH responsibilities are detailed in the Guidelines and are divided among (1) NIH Initial Review Groups, (2) the NIH Recombinant DNA Molecule Program Advisory Committee, and (3) the NIH staff.

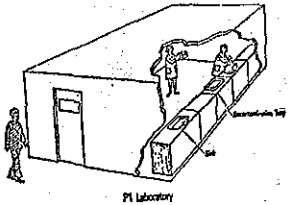
Physical containment requirements

The safeguards in the Guidelines require the use of procedures and physical containment systems to protect laboratory workers and the environment from exposure to potentially harmful organisms. The requirements include procedures and equipment in which work is to be done and special laboratory room and building features, as well as appropriate training of workers. The systems are grouped into four levels of containment—P1, P2, P3, and P4—each providing a level of containment greater than the one preceding it. The level of containment that must be provided by a laboratory in which an experiment is to be done is based on an assessment of the degree of hazard involved.

The following description of the physical containment levels is presented to outline these requirements. A complete description may be found in the Guidelines (Appendix B).

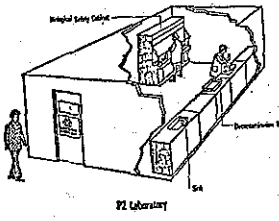
P1 Level (Minimal). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P1 level is shown in Figure V-1. Such a laboratory possesses no special engineering design features. Work in this laboratory is generally conducted on open bench tops. Special containment equipment is neither required nor generally available. The laboratory is not separated from the general traffic patterns of the building, and public access is permitted. Control of biohazards is provided by standard microbiological practices.

P2 Level (Low). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P2 level (see Figure V-2) is similar in construction and design to the P1 laboratory. The P2 laboratory must have access to an autoclave within the building, and it may have a biological safety cabinet. Work that does not produce a considerable aerosol is conducted on the open bench. However, when excessive aerosols may be produced, low-risk experiments must be conducted in special cabinets (biological safety cabinets) that provide physical barriers against possible release of organisms. Although this laboratory is not separated from the general traffic patterns of the building, access to it is limited when experiments requiring P2-level physical containment are being conducted.



P1 Laboratory

FIGURE V-1



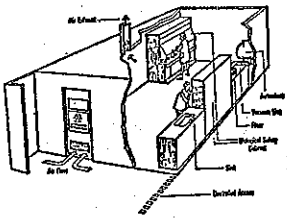
P2 Laboratory

FIGURE V-2

P3 Level (Moderate). As shown in Figure V-3, a laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P3 level has special engineering design features and physical containment equipment. The laboratory is separated from areas that are open to the general public. Separation is generally achieved by controlled access corridors and air locks, locker rooms, or other double-doored facilities not available for use by the general public. Access to the laboratory is controlled. Biological safety cabinets are available within the controlled laboratory area. An autoclave shall be available within the building and preferably within the controlled laboratory area. Environmental protection is provided by waste sterilization techniques. The surfaces of walls, floors,

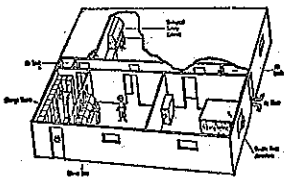
bench tops, and ceilings are easily cleanable to facilitate housekeeping and space decontamination. The laboratory ventilation system is balanced to provide for an inflow of supply air from the access corridor into the laboratory. No work in open vessels is conducted on the open bench; all such procedures are confined to biological safety cabinets.

P4 Level (High). As shown in Figure V–4, experiments involving recombinant DNA molecules requiring physical containment at the P4 level shall be confined to work areas in a maximum-security facility of the type designed to contain microorganisms that are extremely hazardous to man or may cause serious epidemic disease. The facility is either a separate building or a controlled interior area completely isolated from all other areas of a building. Access to the facility is under strict control. Class III biological safety cabinets are available.



P4 Laboratory

FIGURE V–3



P4 Laboratory

FIGURE V–4

A P4 facility has engineering features, shown in Figure V–5, designed to prevent the escape of microorganisms to the environment (1–4). The special features in a P4 facility include:

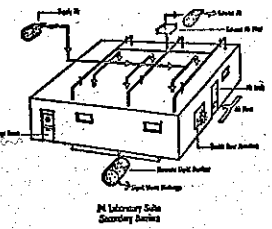
Monolithic walls, floors, and ceilings in which all penetrations such as for air ducts, electrical conduits, and utility pipes are sealed to ensure the physical isolation of the work area and to facilitate housekeeping and space decontamination.

Air locks through which supplies and materials can be brought safely into the facility.

Contiguous clothing change and shower rooms through which personnel enter into and exit from the facility.

Double-door autoclaves to sterilize and safely remove wastes and other materials from the facility.

A biowaste treatment system to sterilize liquid effluents if facility drains are installed.



P4 Laboratory Suite
Secondary Barriers

FIGURE V–5

A separate ventilation system that maintains negative air pressures and directional airflow within the facility.

A treatment system to decontaminate exhaust air before it is dispersed to the atmosphere. A central vacuum utility system is not encouraged; if one is installed, each branch line leading to a laboratory shall be protected by a high-efficiency particulate air filter.

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VI. Description of Alternatives

The following general classes of action have been considered as alternatives to, or in addition to, the proposed action. The impact of each is described briefly, and reference is made to other portions of this document which have a more complete discussion of the particular impact in question.

A. No Action

This alternative would perpetuate the situation existing prior to June 23, 1976. At that time the only restrictions on recombinant DNA research stemmed from voluntary compliance of the research community with the guidelines developed at the International Conference on Recombinant DNA Molecules, held at Asilomar, California, in February of 1975, which were published in scientific journals. The Asilomar guidelines differ in substance from the NIH Guidelines, and are considerably less stringent and less detailed in their requirements for containment of potentially hazardous organisms. For example, experiments that may be carried out with minimal containment according to the specific language of the Asilomar guidelines (e.g., the construction of an *E. coli* plasmid containing the noncancer-producing DNA segment of SV40) require P3 or P4 according to the NIH Guidelines. In addition, while the Asilomar guidelines recommend that certain experiments be deferred, the list of experiments to be deferred is expanded in the NIH Guidelines. Furthermore, disregard of the Asilomar guidelines carries no sanctions on investigators, and it could be expected that the currently high level of voluntary compliance would be eroded with time.

The "no action" alternative would greatly increase the probability that possibly hazardous organisms would be released into the environment. In addition, public concern would be increased in the absence of any Federal action. It is concluded that the "no action" alternative would not afford adequate protection of laboratory workers, the general public, and the environment from the possible hazards described in section IV–C–1.

The alternative of "no action" would essentially remove from the conduct of research the restrictions inherent in the NIH Guidelines. Experiments concerning basic biological processes, and the development of technology applicable to medical, agricultural, and industrial problems, would proceed at a faster rate. Moreover, the immediate cost of conducting research would be markedly decreased with the "no action" alternative, since the need for costly physical containment would be less.

B. NIH Prohibition of Funding of All Experiments With Recombinant DNA

NIH could refuse to fund any any recombinant DNA experiments. This would not necessarily result in the cessation of such research, since it may still be supported by non-NIH funds both in this country and abroad. Therefore a reduction of risks but not elimination of risks might be achieved by total NIH prohibition. Because the NIH funds a large proportion of the total biomedical research effort, a significant delay might be expected in the achievement of the goals and missions of programs designed to elucidate basic biological processes and, in turn, the mechanisms underlying various disease states. It is widely anticipated that a variety of research—impacting on health and other areas of human concern—will benefit from recombinant DNA technology (see Section IV–C–2).

American scientists have played a leading role in bringing the potential hazards of recombinant DNA research to the attention of scientists, governments, and international organizations. As a result, there is an effort to adopt safety procedures for the conduct of this research in many countries. Although nations differ in their perceptions of the need to adopt safety measures, and of what the exact measures should be, the NIH Guidelines are being used as a model. NIH prohibition of the work would undermine American leadership in the establishment of worldwide standards for safety.

Finally, prohibition would be likely to have important impacts on American science, both in research and in development of technology. The leadership of

the United States in biological research would be threatened. Further, historical precedents indicate that measures which interfere with free inquiry in one area of interest, often inhibit the vitality of other aspects of society.

C. DEVELOPMENT OF DIFFERENT GUIDELINES

Each of the stipulations in the NIH Guidelines was made after assessment of the possible hazards associated with particular experiments. The available data, however, were limited, and different conclusions could have been reached. Some issues addressed in the preparation of the Guidelines which could have led to different specifications are as follows:

1. *Levels of physical containment.* For certain experiments in which the potential risk is controversial, the physical containment level could have been higher or lower. Examples of controversial issues are the recommendations with respect to containment levels for recombinant experiments involving bacterial cells and DNA derived from cold-blooded animals, and for experiments involving the use of DNA from animal viruses.

2. *Establishment of a few national P3 facilities openly available to all investigators, with the requirement that all experiments requiring P3 containment be conducted therein.* In effect, this will be the situation with respect to P4 facilities under the Guidelines. There are several advantages to working in regional centers:

a. It would be less expensive to construct and staff a few such regional centers than many such facilities.

b. Training would be centralized.

c. P3 facilities would be more uniformly accessible to qualified investigators from a variety of institutions.

d. There would be greater assurance that the facilities meet the specified requirements.

e. Banks of cells containing recombinant DNA could be maintained, with a view to decreasing the number of times the actual recombination process would be performed (such banks can also be maintained in the absence of centralized P3 facilities).

f. The sites could be placed away from population centers.

The disadvantages of establishing regional centers include:

a. Long-range planning would be necessary.

b. Scheduling would be a problem.

c. The investigator's independence would be diminished.

d. Competition for access might favor established investigators or established ideas.

e. The nature of the process, which might require only brief access of P3 facilities in a given day but over a lengthy period of time.

f. Access problems might unnecessarily discourage valuable research.

3. *All permissible recombinant DNA experiments be conducted in P4 facilities.* This alternative implies no distinction among experiments. It does not recognize that certain recombinant DNA experiments are widely agreed to pose little, if any, possible hazard. It is equivalent to a total prohibition on much recombinant DNA research because of the limited number of P4 facilities that are available and the high cost of construction. Because of access problems, interesting and important research of low or moderate possible hazard would be discouraged.

4. *Experiments prohibited at this time.* Certain types of experiments are prohibited by the Guidelines. Their selection was a matter of judgment, and depended on the assessment of the seriousness of the possible hazard. Alternative assessments would result in either an expansion or a contraction of the list of prohibited experiments and consequent decrease or increase in the possible risks. Some of the controversial recommendations are—

a. *The prohibition of experiments involving more than 10 liters of culture fluid containing recombinant DNAs known to make harmful products without the express approval of the NIH Recombinant Advisory Committee.* Controversy over this recommendation relates to the fact that some investigators and laboratories contend that larger volumes of culture fluid can be safety contained by special procedures and facilities. The recommendation places responsibility for evaluating the containment on the NIH Recombinant Advisory Committee.

b. *Sanction of the use of the bacterium Escherichia coli as a recipient for recombinant DNA molecules.* This organism has been studied extensively and is well suited to recombinant DNA research. It has been argued, however, that *E. coli* should not be used at the present time. This is because many *E. coli* strains are intimately associated with humans and other living things, and because they readily exchange DNA (genes) with certain other bacteria in nature.

Theoretically, the most desirable bacterial recipient of recombinant DNA would be a species uniquely adapted to carefully controlled laboratory environments and unable to survive or transmit DNA to other organisms in any natural environment. This means that the bacteria should be unable to survive in normal ecological niches, either in the laboratory or neighboring areas. It should be unable to colonize or survive in or on other living things, or in soil or water. In addition, these properties should not be significantly altered by the insertion into the bacterium of the recombined DNA. The bacteria must also be able to be manipulated for successful execution of the proposed experiment.

No bacteria is known to meet all these requirements. The guidelines permit the use of various forms of a particular strain of *E. coli* called K12. (The forms are called EK1, EK2 and EK3 in the Guidelines where they are discussed in detail.) Some of these forms already exist, others need to be constructed. Although related to other *E. coli* strains that do not in any way meet the definition of the ideal organism, these permissible strains of *E. coli* partially fulfill many of the criteria in the definition of the ideal strain. At present, no other bacterial species is known to approximate the definition as closely as *E. coli* K–12 and its derivatives. In the future, other bacteria, closer to the ideal, may become known, or the properties of already known species may be shown to approach the ideal more closely than *E. coli* strain K12 and its derivatives, as defined in the Guidelines.

c. *Sanction of the use of Simian Virus 40 (SV 40) as a carrier of a foreign DNA fragment.* It has been argued that SV40 should not be permitted, since it is known to cause cancer in laboratory animals. There is little evidence that SV40 results in disease in humans. However, SV40 infects humans, and demonstrable antibodies to SV40 indicate that infection has occurred in some members of the general population. Some of the infection may have resulted from the inadvertent inoculation of millions of individuals during the initial mass program of immunization against polio virus before SV40 was identified as a contaminant in the vaccine. The antibodies may have been formed against SV40-like viruses known to exist naturally in humans (1). It is possible that a recombined DNA carried by SV40 could infect humans and significantly affect their health (2). The Guidelines restrict the use of SV40 DNA to DNA from strains of the virus that are defective in the infection process. In addition, stringent physical containment is required.

d. *Sanction of experiments involving the transfer of uncharacterized mixtures of DNA segments derived from warm-blooded animals into bacteria.* Such experiments are believed to present a greater possible risk than others because they involve a conglomeration of undefined genes that might include DNA capable of causing disease.

e. *Sanction of the use of oncogenic viruses.* It has been argued that the introduction into *E. coli* of the whole DNA or any purified segment of the DNA of any virus oncogenic in any species should not be permitted.

D. *No guidelines but NIH consideration of each proposed project on an individual basis before funding.* With this alternative, individual investigators requesting NIH funds for projects involving recombinant DNA research would bring plans for proposed experiments to an NIH committee that would, without the use of formal guidelines, recommend suitable containment measures. Depending on the criteria used by the committee, this might result in lower or higher containment levels than are currently imposed by the Guidelines. The advantages of such a procedure would include constant reevaluation of potential hazards and containment measures, and up-to-date information for investigators. The disadvantages include the enormous time and resources required for review, given the size of the biological research enterprise in the United States, the problem of finding knowledgeable individuals to serve on such a committee—essentially a full-time occupation—the opportunity for arbitrary decisions, and the bypassing of local input in assessment of hazards.

It should be pointed out that under the present NIH Guidelines, local institutional biohazards committees must consider proposed research projects on an individuals basis and may impose more

stringent safeguards than those required by the Guidelines. The judgments of the investigator and his local committee will be reevaluated by the NIH Study Section reviewing the scientific merit of the proposal.

E. GENERAL FEDERAL REGULATION OF ALL SUCH RESEARCH

The NIH Guidelines control only recombinant DNA research supported by NIH. Nevertheless, NIH has assumed a real responsibility to work toward the promulgation of safety measures for all such research. Nationally, NIH has conducted and is continuing to conduct meetings with representatives of other Federal agencies and of private industry. In the case of the Federal Government, consideration is being given to the imposition of the Guidelines either by individual agency adoption or through an Executive Order. Non-Federal groups have indicated that they will voluntarily comply with reasonable guidelines designed to be applicable to their specific needs.

From the international standpoint, the NIH has been in communication with relevant national bodies, the World Health Organization, the European Molecular Biology Organization, and the International Council of Scientific Unions, among others, to encourage the widest possible application of the Guidelines.

A variety of administrative mechanisms could be employed to regulate recombinant DNA research. Relevant agencies are the Center for Disease Control (CDC), including the National Institute for Occupational Safety and Health (NIOSH), or the Occupational Safety and Health Administration, Department of Labor (OSHA). For example NIH could petition OSHA to enforce and monitor such research through its standard procedures. If OSHA concurred, the adopted guidelines could be extended to all facilities under OSHA's responsibility.

Legislation could be passed to impose procedures and specify containment for recombinant DNA experiments. Specific guidelines, as well as appropriate enforcement mechanisms and penalties, could be established as statute. The advantages of this approach would include uniformity in coverage and process. The disadvantages include the need for establishment of a new administrative mechanism and consequent costs, the long time generally required for enactment of legislation, and the relative inflexibility of law. Flexibility is desirable because presently recommended containment procedures will surely require timely revision as knowledge and experience are accumulated.

A body like the National Commission on the Protection of Human Subjects of Biomedical and Behavioral Research could be legislatively established. It should be noted that a bill (S. 2515) currently under consideration in the Congress would assign responsibility for consideration of recombinant DNA experiments to a permanent President's Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. A real concern would be the inability of a group with such a broad mandate to deal effectively with the highly specialized subject of recombinant DNA research.

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VII. ENVIRONMENTAL IMPACT OF THE GUIDELINES

A. IMPACT OF ISSUANCE OF NIH GUIDELINES

The primary impact of issuance of the Guidelines is to provide a mechanism for the protection of the laboratory worker, the general public, and the environment from the possible hazards that might result from recombinant DNA molecule research. These hazards are purely speculative at present; the speculations may prove to be wrong. Nevertheless the Guidelines take cognizance of the possibility of dangers to the laboratory worker, other persons, and the environment posed by the emergency research technology involving recombinant DNA molecules, and call for a number of measures aimed at reducing or eliminating human and environmental exposure to materials containing recombinant DNA molecules, in case they should prove hazardous. The Guidelines govern only work supported by the NIH, including NIH supported research at various institutions (grants and contracts) and research carried out within NIH intramural laboratories.

With regard to the anticipated but speculative benefits of recombinant DNA research, adherence to the Guidelines may postpone their realization. Certain experiments are prohibited; many permissible experiments will be delayed pending availability of suitable containment facilities and certification of appropriate hosts and vectors.

1. *Impact on the safety of laboratory personnel and on the spread of possibly hazardous agents by infected laboratory personnel.* The NIH Guidelines are directly concerned with reducing and eliminating exposures of laboratory personnel and all other persons to host cells and microorganisms containing recombinant DNA molecules. Because laboratory personnel would be the chief source of infection of other people, protection of personnel is of primary importance. Lack of knowledge about the real risks of such molecules makes it impossible to determine either the nature of the hazards or the extent to which laboratory personnel are endangered by exposures to the materials. Nevertheless present understanding of biology permits a ranking of the possible risks that may be associated with a given experiment.

Four levels of possible risk have been established: minimal, low, moderate, and high. Protection of personnel from minimal risk materials is provided by ordinary microbiological techniques. Since these procedures are generally performed on the open bench, exposures may occur. The avoidance of harmful effects depends more on the exceedingly low potential of these materials to cause a harmful infection than of the elimination of potential exposures. Potential harmful effects would require exposure to large numbers of organisms, e.g., due to accidental ingestion by poor pipetting techniques or self-inoculation by needle and syringe). Such exposures should be prevented by adherence to practices recommended for this risk level.

The safety of personnel handling materials of minimal risk in the prescribed manner is supported by the absence of any documented laboratory-acquired bacterial or viral infections involving known human etiologic agents that are customarily handled in the same fashion—i.e., CDC class 1 agents (see *Glossary*).

The protection of personnel from potential dangers associated with low- and moderate-risk materials is provided by a greater reliance on physical barriers separating the laboratory personnel from the experimental process as well as on safe microbiological practices. Accidental exposure by ingestion would be prevented by the adherence to the required use of mechanical pipetting for low- and moderate-risk materials. Potential exposure to low-risk materials through aerosols is reduced by the requirement that all processes that produce significant aerosols are to be confined to biological safety cabinets. Potential exposure to moderate-risk materials through aerosols is further reduced by the requirement to contain all processes that produce any aerosol. The use of Class I and Class II biological safety cabinets that comply with the standards specified in the Guidelines can reduce the potential exposure by a factor of 10,000 (1). Potential exposures of laboratory personnel not involved in these experiments are further controlled by the specified laboratory access procedures. These measures do not provide absolute protection from exposures, and the required primary barriers can be compromised by lack of attention to technique, poor placement of equipment, and human error. Experience demonstrates that the use of these measures reduces but does not prevent the potential for laboratory-acquired infections with relatively infectious agents such as class 2 and class 3 agents.

The nature of the harmful effects from exposures to low- and moderate-risk recombinant DNA materials cannot be determined. However, the ability for these materials to cause disease or injury, should they be hazardous can be estimated by comparison of their infectivity with that of known class 2 and class 3 agents. The requirement that recipient bacterial cells be class 1 agents (no or minimal risk) and that animal virus vectors be similarly low risk agents (in the absence of recombined DNA) reduces the likelihood that they will have the infectious properties of class 2 or 3 agents upon insertion of foreign DNA.

Recombinant DNA experiments assessed to have high-risk potential require special precautions designed to prevent exposures, as specified in the Guidelines. All such experimental procedures are required to be surrounded by absolute primary barriers that are gas-tight. These are barriers that physically isolate the experimental process from the laboratory worker. Research is conducted within these barriers through attached gloves. Materials are not removed from the barriers until they have been sterilized or put into hermetically sealed containers, which are then surface sterilized.

Experience with class 3 and 4 human etiologic agents demonstrates that the absolute primary barriers can be operated without exposure of the operators under standardized procedures, employing stable, well trained and well-disciplined personnel (2). This conclusion is based on those data in reference 2 that refer to the experience of recent years; the earlier experience is less relevant because of important recent developments in the design and availability of containment equipment. The procedures for combining segments of DNA and inserting them into recipient cells can be standardized, and the Guidelines require that research personnel be well trained and proficient in the necessary operational practices. Inspection and certification of all high-risk research facilities by NIH personnel provide additional assurances that these requirements will be met.

Thus, potentially harmful effects from research with high risk recombinant DNA molecules should be extremely unlikely given strict adherence to the NIH Guidelines.

Insofar as research sponsored by NIH is concerned, potentially harmful effects from experiments judged to present the possibility of very severe hazard should be prevented completely since those experiments are prohibited.

2. *Impact on the environmental spread of possibly hazardous agents.* The NIH guidelines are directly concerned with preventing the release of cells and microorganisms containing recombinant DNA molecules, or the release of recombinant DNA molecules themselves, into the environment, thus preventing potential exposures of humans, other animals and plant communities.

The Guidelines require decontamination of all liquid and solid wastes generated by low-, moderate-, or high-risk experiments. As the potential risk of these materials increases (low → high), further measures are required to increase the certainty of containment. The Guidelines recommend the decontamination of no- or minimal-risk materials before their disposal to the environment. This is standard microbiological practice.

The Guidelines prohibit the release of contaminated air under ordinary conditions. Procedures involving low- and moderate-risk materials that may produce aerosols are confined to primary barriers. Contaminants in the exhaust air from these barriers are removed by filtration.

The potential for accidental release of recombinant DNA materials into the atmosphere, however, increases with decreasing containment requirements (moderate → minimal). Harmful secondary effects from such accidental release of minimal-, low-, or moderate-risk materials are exceedingly remote. An analysis of 36 reported laboratory-acquired micro-epidemics in the period 1925–1975 involving over 1,000 infections with class 2, class 3, and class 4 human etiologic agents demonstrated no infections among persons who were never in the laboratory building or who were not associated in some way with the laboratory (2). Almost all of these outbreaks occurred in the absence of genuine efforts to control contaminated air, liquid wastes, refuse, and laundry.

Any potential release of high-risk materials to the environment should be prevented by adherence to the NIH Guidelines. All high-risk materials are required to be isolated in physically contained, absolute primary barriers. All effluents from these barriers are sterilized. The barriers themselves are located in maximum-security facilities, which are provided with additional barriers to prevent any accidental release. Air locks, negative air pressure, clothes-change rooms, filtration and incineration of all air exhausted from the facility, and the secondary sterilization of all liquid and solid wastes, provide additional protection to the environment.

The NIH Guidelines also define requirements for protecting the environment from potential dangers that may be associated with the shipment of recombinant DNA materials. Federal packaging standards appropriate for the shipment of class 4 human etiologic agents are required for the shipment of all recombinant materials.

3. *Cost impact.* The direct cost impact of the NIH guidelines is the cost of complying with their provisions. The costs will vary according to the level of potential risk of the research. There are no special facility requirements for work with minimal- and low-risk recombinant DNA materials (P1 and P2). There are equipment requirements for work involving low-risk recombinant DNA materials that will involve little cost impact. Low-risk research requires a biological safety cabinet for procedures that may produce significant aerosols and an autoclave for sterilizing waste materials. These items of equipment, however, are generally available within the existing facilities where such research is being conducted. The cost impact of the NIH guidelines on minimal- and low-risk research is therefore not significant.

Special equipment and facility requirements are specified for moderate-risk recombinant DNA research (P3). All work at this level of potential risk is to be conducted within biological safety cabinets (Class I or II). This requirement will necessitate the acquisition of many additional cabinets, the number being dependent on the scope of the research effort. It is estimated that one cabinet will be required for every three persons involved in the research. The cost of each cabinet is approximately $5,000.

Directional air flow, single-pass ventilation, and provisions for *ensuring* restricted access are facility requirements specified for moderate risk (P3) recombinant DNA research. While many new facilities (those constructed in the last decade) have been constructed with this capability, few older facilities can provide this capability without extensive renovation. Creating adequate access control by construction of architectural barriers (e.g., air locks, double-door alcove, etc.) is not expensive. However, th cost of renovation of air-handling systems to provide for single-pass, directional air flow may prevent some institutions from conducting moderate-risk research. It has been estimated that installation of air-handling systems that comply with the NIH Guidelines would cost approximately $200 per square foot of space serviced by the system.

The NIH Guidelines require that high-risk (P4) research involving recombinant DNA materials be conducted only in class III biological safety cabinets (glove boxes) that are installed in maximum security facilities. Fewer than 30 facilities within the United States have the potential for meeting the requirements specified in the Guidelines for such facilities. A smaller number may actually be available for this research. It is estimated that approximately $750,000 would be required to construct and equip a maximum-security facility having two 10-foot by 20-foot laboratory modules with class III cabinetry. This great cost is due to sophisticated mechanical support systems (e.g., negative pressure, exhaust air filtration, air waste treatment plant) and architectural barriers (e.g., clothes-change rooms, air locks, waste-staging areas, and monolithic walls, floors, and ceilings). The cost of class III cabinetry installed is approximately $3000 per linear foot. In addition, the cabinetry line and the facility each require a double-door autoclave, costing a minimum of $15,000 and $65,000 respectively.

4. *Secondary impacts.* There are three secondary impacts which further provide for environmental protection—i.e., reduce the potential risk to the environment from recombinant DNA research:

a. *Limited maximum-security containment capability.* The small number of facilities available to support high-risk research greatly restricts the number of such experiments that can be conducted. The reduction in the number of experiments minimizes the probability of accidental exposure of laboratory workers and subsequent secondary environmental impacts.

b. *Safety awareness.* The safe performance of biomedical research is dependent on an awareness of the risks and the safeguards required to control the risks. Issuance of the NIH Guidelines should strengthen safety performance in general by providing safety information and increasing the awareness of the laboratory worker to the potential hazards associated with biomedical research.

c. *Early recognition of potential hazards.* The Guidelines require that the principal investigator notify NIH of any serious or extended illness or accident that may result in serious exposure to man or to the environment. This monitoring procedure will provide an early warning of possible unforeseen hazard. For example, if a laboratory infection from exposure to a recombinant DNA molecule is confirmed, indicating a real hazard, an increase in safeguards or cessation of experiments can be required to minimize the hazard to other investigators conducing similar studies. This upgrading will also reduce any potential for environmental effects.

B. IMPACT OF EXPERIMENTS CONDUCTED UNDER THE GUIDELINES

1. *Possible undesirable impact—a. Dispersion of potentially hazardous agents.* The hypothetical mechanisms by which insertion of foreign genes into cells or viruses might result in the formation of hazardous agents are described in Section IV–C. There is, as stated before, no known instance in which a hazardous agent has been created by recombinant DNA technology. Current knowledge permits no more than speculation that such agents may be produced and an equally speculative assessment of the nature and extent of hazards that may follow upon a particulr recombinant DNA experiment. This is the underlying reason that the thrust of the Guidelines is to minimize contact of organisms containing recombinant DNA with other organisms or the environment. Therefore the following analysis of possible undesirable impacts due to dispersion of potentially hazardous agents emphasizes the likelihood of significant dispersion rather than the nature of the hazard itself. The analysis given does not apply in detail to all the possible situations, but can serve as a model for analyzing different situations.

In order that any potential hazard be realized, it is necessary that each of a number of sequential events occur. Each event in the sequence is possible only if the earlier events have occurred. The organism must—

(a) Contain foreign genes,
(b) Escape from the experimental situation,
(c) Survive after escape,
(d) Become established in an environment permitting its growth and multiplication,
(e) Contact other living organisms in a significant manner, including contact by a sufficient number of organisms to ensure survival and growth and to cause infection. (Note that the environment in (d) may be a living organism itself).

In those cases where the detrimental effect results from the formation of a harmful protein, the organism containing the recombinant DNA must—

(f) Contain a gene for a potentially harmful protein,
(g) Be able to express the foreign gene—that is, synthesize the foreign protein,
(h) Synthesize the protein in sufficient quantity to be deleterious to the infected organism.

In those cases where the foreign DNA itself may be the cause of undesirable effects, another set of events must be considered. In the case where the foreign DNA increases the pathogenicity of the initial host cell or virus, the inserted DNA must—

(i) Impart a selective advantage for growth to the carrier of the recombinant DNA as compared with the original cell or virus,
(j) Alter the metabolism of the carrier so that it becomes disease producing.

In the case where the foreign DNA causes undesirable effects by virtue of its transfer out of the original recipient and reinsertion into cells of another species, the DNA must—

(k) Leave the original recipient without being destroyed,
(l) Survive transfer to another cell,
(m) Become associated with the other cell in a stable manner, either as an independent element or by natural recombination.

For example, in a hypothetical experiment classified as low-risk and carried out according to the requirements of the Guidelines, events (a) through (h) might be required to yield a hazardous situation. Available data might permit assignment of probabilities of : 1 for (a); of 10^{-2} (1 in 100) for (b); of 10^{-4} (1 in 10,000) for (c); and of 10^{-6} (1 in a million) for (d). Lack of any pertinent knowledge concerning events (e) through (h) would make assignment of probabilities impossible. Even assuming a probability of one for each event (e) through (h), the overall probability of a deleterious effect on a member of a species at risk in this hypothetical situation would then be the product of all probabilities (a) through (h), namely 10^{-12} (one in a trillion). This probability then needs to be compared with the number of organisms grown for the experiment. Typically, bacteria are grown in liquid mixtures to a concentration of between 10^8 and 10^9 organisms per ml. The probability will also need to be corrected for the length of time over which the experiment is to be conducted. In reality, it may frequently be difficult to assess the relevant probabilities.

It is currently impossible to assign specific probabilities for many experiments, although crude estimates can often be made from current knowledge of laboratory-acquired infections, from prototype experiments set up to measure bacterial or viral escape (4), and from knowledge concerning the stability of organisms and DNA. NIH is currently supporting research designed to improve the ability to evaluate certain of these probabilities.

b. Other considerations. The foregoing descriptions of the kinds of possibly hazardous situations that might arise from organisms obtained through recombinant DNA experiments must be considered in the light of certain more general issues.

(1) *Monitoring for release of organisms containing recombined DNA.* Control of the spread of any agent outside of an experimental situation to laboratory workers or the outside environment is greatly assisted by adequate means for monitoring the agent in question. A pertinent example is the monitoring for spillage and spread of radioisotopes. The presence of radioisotopes is readily measured, and the exposure of laboratory personnel or the environment to radiation can be quantified. The situation is fundamentally different in the case of organisms or viruses containing recombined DNA. No simple general procedure exists for identifying an organism released from the laboratory against the large background level of related and unrelated organisms occurring naturally.

It is possible, however, to devise special pertinent procedures for detection of some of the agents used in recombinant DNA experiments. For example, development of bacterial strains, phages, or plasmids carrying readily detectable genetic traits would enable the monitoring of laboratory personnel, people working in the area, and their families for the presence of those agents. This would be analogous to the examination of drinking water, lakes, etc., for fecal contamination with enteric organisms. Detection in such instances could be at levels as low as 10^{-7} (1 part in 10,000,000). The adequacy of such screening is not presently known.

Given the nature of the series of events that might characterize a hazardous situation, the time factors involved in those events become relevant. Certain possible types of organisms containing recombinant DNA might, if they escaped and if they were hazardous, be immediately perceived as such—e.g., production of toxic foreign proteins. We might therefore be aware of the potential problem soon after dispersal of the organism, and reasonable means for minimizing further dispersal could be undertaken. In other instances—e.g., a cancer-producing DNA fragment—evidence of harmful effects might not be apparent for many years. The connection between the causative organisms and the observed harmful effects could be difficult to establish. Further, dispersal of the hazardous agent might then be so widespread as to make control difficult or impossible.

(2) *Natural occurrence of DNA recombination between unrelated organisms.* Concern over the potential for hazard in organisms containing recombined DNA develops from the central idea that such recombinants will be unique types of organisms, not normally arising in nature, and that their properties will therefore be unknown and unpredictable. Natural environments provide many opportunities for recombination of DNA between unrelated species, as for example, in the intestines of animals. Whether, or at what frequency, such recombinations may occur is not known at present, but it is probably low given the very low extent of shared base sequences that can be detected in DNAs derived from distantly related organisms. It would appear that naturally occurring interspecies recombinants, if they occur in nature, may have been selected against in evolution. However tests for shared base sequences are of limited sensitivity.

(3) *Relative irreversibility of spread of organisms.* Should organisms containing recombined DNA be dispersed into the environment, they might, depending on their fitness relative to naturally occurring organisms, find a suitable ecological niche for their own reproduction, and a potentially dangerous organism could then multiply and possibly spread. Subsequent cessation of experiments would not stop the diffusion of the hazardous agent. While means to eradicate the organism might be found, as in the case of smallpox, it is also possible that such means will not be available, or that they will be available too late to prevent or stop untoward events.

As described earlier, the likelihood is that newly constructed organisms will be less fit than those occurring naturally and therefore will disappear over time.

2. *Beneficial impacts of recombinant DNA research.* Section IV–C–2 describes the various anticipated benefits of recombinant DNA research. As with the possible hazards, many of the proposed benefits are speculative. Assessment of the likelihood that they will be realized will depend on information acquired from future experimentation. For example, assessment of the category of anticipated benefits that depends on the synthesis of eukaryote proteins in prokaryote cells (see IV–C–1–b) awaits additional data on the expression of the foreign genes. Should these benefits be realized, it may be expected that the cost of manufacturing certain clinically important proteins can be markedly decreased. Other clinically important proteins that are either in short supply (e.g. human growth hormone) or unobtainable by existing techniques may be made readily available. Innovative approaches to immunization against infectious diseases can also be expected.

Some of the indicted benefits appear certain. These are the benefits to be derived from an increased understanding of both basic biological processes and the mechanisms underlying a variety of disease states.

Application of the restrictions imposed by the Guidelines will retard progress toward the realization of the possible benefits. In addition to the prohibitions on certain experiments, there are many permissible experiments which will need to be postponed until the requirements in the Guidelines can be met. The acquisition and installation of P3 facilities requires adequate funds, extensive planning and installation. P4 facilities are limited in number. Experiments that require hosts and vectors with demonstrably limited ability to survive in natural environments must await development of appropriate hosts and vectors, their testing, and finally their certification by the NIH Recombinant Advisory Committee. Time will also be required for the various review processes that are required.

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APPENDIX A

GLOSSARY

1. Aerosol: A colloid of liquid or solid particles suspended in a gas, usually air.

2. Antibody: A protein which is formed in the body as a result of the inoculation of an antigen.

3. Antigen: A substance which when injected into an animal causes the formation of antibodies.

4. Autoclave: An apparatus for effecting sterilization by steam under pressure. It is fitted with a gauge that automatically regulates the pressure, and therefore the degree of heat to which the contents are subjected.

5. Bacteriophage: A virus that infects only bacteria.

6. Bld: Bureaus, Institutes, and Divisions of NIH.

7. Biohazard: A contraction of the words biological hazard; infectious agents presenting a risk or potential risk to the well-being of man, or other animals, either directly through infection or indirectly through disruption of the environment.

8. Biohazardous Agent: Any microbial unit capable or potentially capable of presenting a biohazard.

9. Biohazard Area: Any area (a complete operating complex, a single facility, a single room within a facility, etc.) in which work has been, or is being performed with biohazardous agents or materials.

10. Biohazard Control: Any set of equipment and procedures utilized to prevent or minimize the exposure of man and his environment to biohazardous agents or materials.

11. Biohazardous Material: Any substance which contains or potentially contains biohazardous agents.

12. Biowaste: Liquid wastes from biological research procedures.

13. CDC: Center for Disease Control, United States Public Health Service, Atlanta, Georgia.

14. CDC Classification of etiologic agents on the basis of hazard: A system for evaluating the hazards associated with various etiologic agents, and definition of minimal safety conditions for their management in microbiological investigations. The basis for Agent Classification is as follows:

Class 1: Agents or no or minimal hazard under ordinary conditions of handling.

Class 2: Agents of ordinary potential hazard. This class includes agents which may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration but which are contained by ordinary laboratory techniques.

Class 3: Agents involving special hazard or agents derived from outside the United States which require a federal permit for importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment.

Class 4: Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This class includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

Class 5: Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.

NOTE: Federally licensed vaccines containing live bacteria or viruses are not subject to these classifications. These classifications are applicable, however, to cultures of the strains used for vaccine production, or further passages of the vaccine strains.

15. Class I biological safety cabinet: A ventilated cabinet for personnel protection only, having an open front with inward flow of air away from the operator. The cabinet exhaust air is filtered through a high efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet can be used for work with low- to moderate-hazard risk agents where no product protection is required.

16. Class II biological safety cabinet: An open-front cabinet for personnel and product protection with mass recirculated airflow with HEPA filtered exhaust and HEPA filtered recirculated air. This cabinet can be used for work with low- to moderate-hazard risk agents. It is not suitable for use with explosive and flammable substances, toxic agents, or radioactive materials.

17. Class III biological safety cabinet: A gas-tight cabinet providing total isolation for personnel and product protection with a HEPA-filtered air supply and a HEPA-filtered exhaust. The cabinet is fitted with gloves and is maintained under negative air pressure. This cabinet provides the highest containment reliability and should be utilized for all activities involving high-hazard risk agents.

18. Clone: A population of cells derived, by asexual reproduction, from a single cell. Every cell in the population is presumed to be genetically identical. In recombinant DNA research, every cell in a clone contains the same recombinant DNA species.

19. Coding sequence: The orderly array of codons which are subunits of a gene.

20. Chromosome: One or more small rod-shaped body(s) in the nucleus of a cell that contains genetic information for that cell. A collection of genes.

21. Deoxyribonucleic acid, or DNA: A complex substance of which genes are composed.

22. Effluent: A liquid or gas flowing from a process.

23. Endogenous: Developing or originating within the organism, or arising from causes within the organism.

24. Escherichia coli: A bacterium commonly found in the intestinal tract of animals.

25. Etiologic agent: A viable microorganism or its toxin which causes, or may cause, human disease.

26. Eukaryotic cell: A cell that contains a nucleus with a nuclear membrane surrounding multiple chromosomes; also contains extranuclear organelles.

27. Gene: The smallest portion of a chromosome that contains the hereditary information for the production of a protein.

28. Genetic engineering: Directed intervention with the content and/or organization of an organism's genetic complement.

29. Genome: The complete set of hereditary information in a cell as the chromosomes in

a eukaryote or the single chromosome in a prokaryote.

30. HEPA filter: (High Efficiency Particulate Air Filter) A disposable, extended medium, dry type filter with a particle removal efficiency of no less than 99.97% for 0.3um particles.

31. Infectious: Capable of invading a susceptible host, replicating, and causing an altered host reaction commonly referred to as a disease.

32. Laminar air flow: Air flow in which the entire body of air within a designated space moves with uniform velocity in one direction along parallel flow lines.

33. Laboratory acquired infection: Any infection resulting from exposure to biohazardous materials in a laboratory environment. Exposure may be the result of a specific accident or inadequate biohazard control procedure or equipment.

34. Messenger ribonucleic acid (mRNA): A complex molecule that transmits the information from the gene to a template on which a protein is formed.

35. Mitochondrion: A DNA-containing structure present in all aerobic eukaryotic cells. The mitochrondria produce energy for the cell and divide by fission after cell division has occurred.

36. Nucleotide: A basic unit of the polymeric structure of DNA. Each unit contains a sugar (deoxyribose), phosphoric acid, and one of the following organic substances: adenine, guanine, thymine or cytosine.

37. Oncogenesis: The process of tumor formation.

38. Organelle: An independent structural body existing within cells, generally related to a particular cellular function, and containing a special group of genes within an extrachromosomal DNA molecule (e.g., mitochrondria and chloroplasts).

39. Pathogenic: Producing or capable of producing disease.

40. Phenotype: The visible traits of an organism as determined by the genome or genotype.

41. Plasmid: A genetic element outside of the chromosome that is capable of replicating independently of the chromosome.

42. Polymer: A large molecule composed of simpler repeating units. DNA is a polymer composed of nucleotides, while starch and cellulose are polymers composed of sugars.

43. Prokaryotic organism or Prokaryote: Cells of bacteria or blue-green algae which are characterized as being rather small, having a single chromosome that is not enclosed by a nuclear membrane, and lacking organelles.

44. Restriction endonuclease: An enzyme capable of breaking DNA at specific sites. The action of the enzyme is unique in that "sticky" ends are formed which can join with other fragments of DNA to form a recombinant DNA molecule. In nature, these bacterial enzymes restrict invasion of foreign DNA.

45. Reverse transcriptase: An enzyme found in certain viruses which reverses the normal synthesis of RNA from DNA. DNA is formed for the replication of viral RNA.

46. R plasmid: A plasmid that carries genetic information for resistance to antibiotics and/or other antibacterial drugs.

47. Shotgun experiment: An experiment in which all the DNA fragments cleaved by a restriction endonuclease are inserted into a vector DNA, which is then put into a cell. This is in contrast to other recombinant DNA experiments where only selected fragments of DNA are inserted into a vector DNA.

48. Sterilize: Any act which results in the absence of all life on or in an object.

49. Vector: A carrier of a recombinant DNA molecule; usually a plasmid or bacteriophage.

50. Viable: Literally, "capable of life." Generally refers to the ability of microbial cells to grow and multiply as evidenced by, for example, formation of colonies on an agar culture medium. Frequently organisms may be viable under one set of culture conditions and not under another set, making it extremely important to define precisely the conditions used for determining viability.

APPENDIX B

SUGGESTED REFERENCES FOR ADDITIONAL READING

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Appendix C

DOCUMENTS DESCRIBING THE IMPLEMENTATION OF THE GUIDELINES

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE, PUBLIC HEALTH SERVICE, NATIONAL INSTITUTES OF HEALTH

June 18, 1976.

To: Director, NIH, Through: Director, NIOMS, NIH.

From: Executive Secretary, Recombinant DNA Molecule Program Advisory Committee.

Subject: Operation of the Office of Recombinant DNA Activities (ORDA).

The proposed structure and responsibilities of an Office of Recombinant DNA Activities (ORDA) were described in Dr. Kirchstein's memorandum to you of April 28, 1976. The purpose of this document is to present our views as to how such an office can function effectively at the NIH. Consequently, the following relationships and activities are discussed:

1. *Office of the Director, NIH (OD, NIH).* The Office of Recombinant DNA Activities (ORDA) will be responsible for keeping the Office of the Director, NIH, (OD, NIH) and

institutions and investigators regarding NIH policies and procedures. Dissemination of major changes in the Guidelines should also be handled as above.

The NIAID will have responsibility for publication of the "Nucleic Acid Recombinant Scientific Memoranda" (NARSM). Major announcements in NARSM, such as distribution of Guidelines, statements of NIH policies and procedures, announcements of certified host-vector systems, training courses, workshops, conferences, etc. will be coordinated with ORDA.

III. *Division of research grants.* ORDA will work with the Associate Director for Scientific Review, DRG on procedures for the review of grant applications involving recombinant DNA technology. On request, ORDA will brief executive secretaries and study sections on NIH policies and procedures.

Involvement of DRG in the processing of grant applications is discussed in Appendix A.

IV. *Institutes and divisions.* Institutes and Divisions will be required to report to ORDA on all activities involving recombinant DNA technology, including: intramural research projects, extramural grants and contracts, workshops, training courses, conferences, etc. BIDs will provide ORDA with copies of all incoming and outgoing correspondence dealing with recombinant DNA activities. Awarding components must consult with ORDA prior issuing Requests for Proposals (RFPs) and *Requests for Applications (RFAs) likely* to result in projects utilizing recombinant DNA technology. Procedures for obtaining information on extramural research, and for the processing of applications are proposed in Appendix A. Procedures for monitoring intramural research are proposed in Appendix B.

ORDA will be a source of information for the Institutes and Divisions, and their initial review groups, regarding current NIH policies and procedures. ORDA may establish an inter-BID Recombinant DNA Coordinating Committee, to facilitate interchange of information. Because of the procedures proposed, NIH awarding components may wish to consider naming specific staff for liaison with ORDA.

V. *Special NIH relationships.* ORDA will maintain close working relationships with the Director, Office of Research Safety, NCI, the Associate Director for Environmental Health and Safety, DRS and the NIH Biohazards Committee, and will coordinate their activities on matters relating to recombinant DNA technology. The Office of Research Safety, NCI will continue to have primary responsibility for matters relating to physical containment of recombinant DNA materials, and will continue to maintain a register of high containment facilities in this country and abroad. However, plans for site inspections of P4 physical containment facilities currently or envisaged to be engaged in recombinant DNA research, and of other facilities as deemed necessary, will be coordinated through ORDA, and copies of site visit reports will be filed with ORDA.

VI. *Federal relationships.* ORDA will develop interrelationships with other federal agencies concerned with recombinant DNA technology, including but not limited to the following: National Academy of Sciences, National Science Foundation, Energy Research and Development Administration, Department of Agriculture, Environmental Protection Agency, National Aeronautics and Space Administration, and the Occupational Safety and Health Administration.

Parenthetically, it should be mentioned that representatives of the National Academy of Sciences and National Science Foundation are already attending meetings of the Recombinant Advisory Committee on a regular basis, and the National Aeronautics and Space Administration and the Energy Research and Development Administration have sent a representative to several of the meetings.

VII. *Non-Federal and international relationships.* ORDA will attempt to develop interrelationships with private foundations, professional societies, scientific journals and industry, and to coordinate NIH policies with international bodies concerned with recombinant DNA technology. The Executive Secretary of the Recombinant Advisory Committee already has a working relationship with the European Molecular Biology Organization.

VIII. *Institutional Biohazards Committees.* ORDA will receive directly from each institution involved in recombinant DNA activities the roster of its institutional biohazards committee. The minimum information should include the names, addresses, occupations and qualifications of the chairman and members of the committee. Institutions will be notified by the appropriate instrument (NIH Guide, NIH Manual, etc.) of the necessity for filing this information with ORDA.

As stipulated in the Guidelines, ORDA will assist in the formation of area biohazards committees composed of members of a given institution and/or other organizations beyond its own staff. An area committee will be necessary when expertise from outside a given institution is necessary for the biohazards committee to fulfill its functions.

ORDA will review the composition of institutional biohazards committees for compliance with the recommendations stated in the Guidelines, and will maintain updated lists of such complying committees. Serious questions about the suitability of a committee will be brought to the attention of the Executive Committee.

IX. *Information on accidents, containment and innovations.* As stipulated by the Guidelines, investigators are required to report to ORDA and to their institutional biohazards committees any serious or extended illness of a worker, or any accidents of the type described in the Guidelines. The Guidelines also require that investigators report to ORDA and their biohazards committee any problems pertaining to operation and implementation of biological and physical containment safety practices and procedures, or equipment or facility failure.

ORDA will receive from investigators information on purported EK2 and EK3 systems, and information bearing on the Guidelines, such as technical information relating to hazards and new safety procedures or innovations.

ORDA will receive and file these reports and, as appropriate bring them to the attention of the Deputy Director for Science, NIH, the Office of Research Safety, NCI, and the Recombinant Advisory Committee or subcommittees thereof. ORDA may, after review, recommend appropriate action to the Deputy Director for Science, NIH.

X. *Recombinant DNA molecule program advisory committee.* ORDA will have the management responsibilities for the Recombinant Advisory Committee, will serve as its staff, and will provide the Executive Secretary. Staff functions will include the gathering, analysis and dissemination of information, the presentation of issues, etc.

XI. *Transition and implementation.* Proposals for initial gathering of information on NIH activities in this area and implementation of the Guidelines are discussed in this Appendix C.

The relationships, responsibilities and procedures proposed in this memorandum and its appendices are wide-ranging and complex. They are, however, an attempt to describe how NIH might administer the Guidelines governing this controversial technology responsibly and effectively. I look forward to

hearing your comments and those of your staff on these proposals.

WILLIAM J. GARTLAND, JR., Ph. D.

APPENDIX A TO APPENDIX C

PROCESSING, REVIEW, AND MANAGEMENT OF EXTRAMURAL PROJECTS INVOLVING RECOMBINANT DNA TECHNOLOGY

I. *General.* The purpose of this appendix is to discuss procedures for the processing, review and management of NIH supported projects which involve the use of recombinant DNA technology. The term "application", as used here, refers to all contract proposals and grant applications for research projects, program projects, centers, training, fellowships, research career development, etc., as defined in NIH Manual Issuance 4101: "Activity Codes, Organizational Codes and Definitions used in Extramural Programs." The procedures would apply to applications reviewed by DRG study sections and Institute and Division initial review groups.

II. *Capture of information.* One of the primary functions of the Office of Recombinant DNA Activities (ORDA) is to maintain a central register of NIH supported projects which involve recombinant DNA technology so that the NIH will know where these projects are located and will have the capability of rapidly communicating with project directors should the need arise.

In order to maintain an updated central register of projects it will be necessary for the NIH to modify application forms to indicate on the face sheet whether or not recombinant DNA technology is involved in the project. This could be modeled after the statement currently in use regarding research involving human subjects. The information would be captured, as is the case for human experimentation, and permit sorting by different parameters such as awarding component, geographic location of projects, etc.

III. *Receipt of applications.* Through the use of appropriate instrument (NIH Guide, NIH Manual, etc.), the NIH will inform applicants of the necessity for assessing the physical and biological containment required for the proposed experiments as stipulated in the NIH guidelines. This assessment must be incorporated into the application.

All applications requesting support for projects involving recombinant DNA technology will be required to have on file two documents: A Memorandum of Understanding and Agreement (MUA) and a Certification Statement (Certification) from the institutional biohazards committee. The NIH must and will require that a properly executed MUA and Certification accompany all applications proposing to use recombinant DNA technology. This will eliminate the need for tracking these documents after the application is accepted for review. The originals of these documents will be placed in the official files of the NIH awarding components with copies on file in ORDA.

IV. *Review of applications.* The executive secretaries of initial review groups are responsible for identifying all applications involving recombinant DNA technology, and for placing on the first page of every summary statement the following special note:

RECOMBINANT DNA MOLECULES—POTENTIAL BIOHAZARD

The executive secretaries are responsible for ensuring that the initial review groups make an independent assessment of the biological and physical containment levels required for the proposed experiments, and for stating in the text of the summary statement the initial review group's determination as to whether the containment levels proposed by the investigator meet the levels stipulated in the NIH guidelines. If the proposed containment levels are inadequate, the initial review group should discuss, in as much detail as possible, the inadequacies of the proposed containment and under what circumstances, if at all, the application should be eligible for funding. Initial review groups should be encouraged to disapprove applications if the proposed containment levels are so inadequate as to be irresponsible. It will be the responsibility of the awarding unit to inform applicants directly as to the fact that this was the reason for disapproval.

Executive secretaries and members of relevant initial review groups will receive a copy of the Guidelines to permit them to make these judgments. Problems relating to assessment of biological and physical containment levels proposed by investigators versus those required by the Guidelines will be referred to ORDA.

As in the case of human experimentation, national advisory councils and boards and final review bodies are expected to carefully scrutinize proposals, involving recombinant DNA technology, and make appropriate recommendations.

V. *Award of new and competing renewal projects.* Prior to the award of any project involving recombinant DNA technology, the NIH awarding component will forward to ORDA one copy of each of the following documents: The application, summary statement, MUA, Certification, any comments of the final review body, and a request for clearance to award. In those cases in which the initial or final review group, or staff of an awarding BID finds that a project requires a higher level of containment than that originally proposed by the applicant or the institution, a properly executed revised MUA and Certification Statement will be required prior to a request for approval to award. ORDA will review the documents and indicate concurrence or non-concurrence with the request for clearance to award. In the latter case, ORDA will prepare a memorandum outlining the reasons for disapproval but emphasizing that the action is independent of scientific merit or other reasons. Such a memorandum should be forwarded to the applicant through the awarding unit. In those cases in which ORDA is not able to reach a decision, or in which the awarding component or applicant disputes the decision, the decision will be submitted to the Executive Committee, and, if necessary, to the Recombinant Advisory Committee, for review. The final decision will rest with the Deputy Director for Science, NIH.

BIDs will forward to ORDA a copy of all award statements involving these applications.

ORDA will retain in its files the documents cited above. In the event that the volume of filed documents becomes excessive, ORDA will retain a copy of the face sheet of a funded application rather than the entire application. In those cases in which the proposed project involving recombinant DNA technology is but one component of a multiproject application, ORDA will retain in its files the face sheet of the application and the section(s) describing the project(s) involving recombinant DNA technology.

It must be emphasized that the primary responsibility for ensuring that applications have been properly reviewed, and that required documents are properly executed lies with NIH staff involved with the initial review groups and program areas. The procedures proposed above are intended to serve as a final review prior to award. ORDA will be available to NIH staff for advice and consultation, but it can not be expected to make decisions for review units and awarding components.

VI. *Award of non-competing renewals and incrementally-funded contracts.* Each non-competing renewal of a grant and subsequent budget period of an incrementally-funded contract utilizing recombinant DNA technology must be accompanied by an updated Certification Statement from the institutional biohazards committee. Prior to any award of this type the program official in the awarding component has the responsibility for reviewing the application for conformity with the Guidelines, for determining whether the proposed protocols do or do not require a higher level of containment than was required in the application as reviewed by the initial review group, and for ensuring that the required documents are properly executed. The program official will then forward to ORDA one copy of the application and the certification statement, along with a request for clearance to award. The latter will include a statement to the effect that the program official has reviewed the application for conformity with the Guidelines, and that the proposed containment levels are adequate. Thereafter, the procedures described in V will be followed, and BIDs will forward to ORDA a copy of all award statements involving these projects.

If the investigator proposes to significantly alter an approved protocol at the time of the non-competing renewal or subsequent budget period of an incrementally-funded contract, then the procedures described in VII must be followed. It is the responsibility of the program official to ensure that all the information and properly executed documents required in VII are present in the application prior to forwarding the request to ORDA.

VII. *Changes in awarded projects.* Since in many cases the NIH supports projects for project periods longer than one year, a number of situations will arise in funded projects. One situation arises when an investigator makes a decision to utilize recombinant DNA technology after the project has been reviewed and awarded. Another situation arises when an investigator decides to clone DNA segments other than those originally reviewed, and for which higher levels of containment may be required. In these cases, and in all cases in which an investigator wishes to significantly alter an approved protocol, the investigator must first apply to the NIH awarding component for permission before proceeding. This requirement should be stated in the appropriate NIH instrument (NIH Manual, NIH Guide, etc.) The investigator will be required to submit to the awarding component a proposed protocol, an assessment of the levels of physical and biological containment required by the Guidelines, an MUA, and a Certification Statement from the institutional biohazards committee. The program official in the awarding component has the responsibility for reviewing the request in light of the Guidelines, for ensuring that the required documents are properly executed, and for forwarding to ORDA a copy of all the documents along with a recommendation. The latter should include the program official's independent assessment of the levels of physical and biological containment required by the NIH Guidelines, and a recommendation as to how to proceed. ORDA will review the request, and, when appropriate, refer the request to the initial review group, the Recombinant Advisory Committee, or ad hoc consultants.

VIII. *Requests for lowering of containment levels.* Under "characterized clones of DNA recombinants derived from shotgun experiments," the Guidelines state:

• • • before containment conditions lower than the ones used to clone the DNA can be adopted, the investigator must obtain approval from the granting agency. Such ap-

proval would be contingent upon data concerning: (a) The absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or which code for toxic substances), (b) the relation between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable), and (c) maintenance of the biological properties of the vector.

This stipulation for NIH approval may be one of the most difficult sections of the Guidelines to implement. This is because of the technical nature of the data to be evaluated, and because of the volume of requests which can be anticipated. Therefore, the following proposed procedures are especially viewed as a feasibility trial.

An investigator who wishes to use lower levels of containment for characterized clones derived from shotgun experiments must state, in writing, the justification for the request to the program official of the NIH awarding component. Such justification will provide data on (a), (b) and (c) as stated above. The program official will retain the original request in the awarding component's file, and forward a copy to ORDA which will submit the request to the Recombinant Advisory Committee or to a subcommittee thereof for evaluation, or, if a precedent has been established, will make a decision independently. The decision will be forwarded to the program official who may appeal. The final decision rests with the Deputy Director for Science, NIH.

IX. *Large-scale experiments.* The Guidelines state that:

* * * at this time large-scale experiments (e.g., more than 10 liters of culture) with recombinant DNAs known to make harmful products are not to be carried out * * *. However, specific experiments in this category may be exempted from this rule if special biological containment precautions and equipment designed for large-scale operations are used, and provided that these experiments are expressly approved by the Recombinant DNA Molecule Program Advisory Committee.

An investigator who wishes to conduct such experiments must submit a request, along with a properly executed MUA and Certification Statement from the institutional biohazards committee, to the program official of the NIH awarding component. The program official will retain the original request in the awarding component's file, and forward copies to ORDA. ORDA will bring the request to the attention of the Recombinant Advisory Committee or subcommittees thereof, by mail, telephone, or presentation at the next meeting or, if a precedent has been established, will make a decision independently.

APPENDIX B TO APPENDIX C

NIH INTRAMURAL RESEARCH

Because NIH intramural research projects are reviewed in a very different fashion than extramural projects, different procedures are applicable than those proposed in Appendix A.

At present, the Chief of the Laboratory in which an investigator plans to utilize recombinant DNA technology requests approval through the Scientific Director of the relevant BID to the Deputy Director for Science, NIH with copies to the Associate Director for Environmental Health and Safety, DRS. The request for approval is in the form of a draft Memorandum of Understanding and Agreement (MUA) which describes the type of experiment, nature of host-vector system, assessment of potential risk, proposed safety measures, proposed training of personnel, etc. The Deputy Director for Science then requests the NIH Biohazards Committee to review the research plan and procedures proposed in the draft MUA. The recommendations of the NIH Biohazards Committee are forwarded to the Deputy Director for Science, NIH. Recommendations of the NIH Biohazards Committee must be included in a final MUA, and the Associate Director for Environmental Health and Safety, DRS must certify that the safety measures included in the final MUA are available. The research cannot proceed until the final MUA is fully approved. The original copy of the MUA is sent to the Associate Director for Environmental Health and Safety, DRS with copies to the requesting investigator, the Laboratory Chief, the Scientific Director and the Executive Secretary of the NIH Biohazards Committee.

It is proposed here that a copy of the final MUA be forwarded to ORDA for review. If ORDA does not concure with the recommendations of the NIH Biohazards Committee, it may request the Deputy Director for Science, NIH to bring the matter to the attention of the Executive Committee or the Recombinant Advisory Committee for resolution.

ORDA will assist the NIH Biohazards Committee with problems relating to assessment of biological and physical containment levels proposed by investigators versus those required by the Guidelines, with requests for the use of lower containment levels for characterized clones derived from shotgun experiments, and with requests for permission to do large-scale experiments with recombinants known to make harmful products. ORDA will also assist the NIH Biohazards Committee in periodic review and revisions of MUAs. If ORDA does not concur with the decisions of the NIH Biohazards Committee, it may request the Deputy Director for Science, NIH to bring the matter to the attention of the Executive Committee or the Recombinant Advisory Committee.

APPENDIX C TO APPENDIX C

TRANSITION AND IMPLEMENTATION

The procedures proposed in Appendices A and B should be implemented as soon as possible. However, clearly there will be an interim period after the Guidelines are issued and before all the procedures are functioning. It is the purpose of this Appendix to propose how the Office of Recombinant DNA Activities (ORDA) might initiate coordination and gathering of information during this period.

I. *Intramural research.* ORDA will brief the Scientific Directors of the BIDs who will be expected to assure ORDA and the Deputy Director for Science, NIH of present and future compliance by intramural research scientists with the Guidelines.

ORDA will request the Deputy Director for Science, NIH to provide a copy of the final MUA on all intramural projects, utilizing recombinant DNA technology, which are already in progress. After review of the MUAs, ORDA will report any concerns to the Deputy Director for Science, NIH.

II. *Extramural programs.* ORDA will brief the Executive Committee for Extramural Affairs on NIH policies and procedures.

BIDs will be required to report to ORDA all presents or planned workshops, training courses, conferences, etc., relating to recombinant DNA technology. BIDs must also report all present or planned RPFs and RFAs likely to result in projects utilizing recombinant DNA technology. After review of this information, ORDA will report any concerns to the Deputy Director for Science, NIH and/or the Executive Committee.

With regard to active grants and contracts, BIDs will be required to submit to ORDA a copy of the application, summary statement and award statement for each currently funded project involving recombinant DNA technology. NIH awarding components will be responsible for ensuring that this reporting is as complete as possible.

BIDs will send a letter to investigators identified in the paragraph above to determine whether active research projects are in compliance with the Guidelines. Responses to this query will be retained in BID official files, and a copy will be forwarded to ORDA for review. If ORDA is satisfied that a project is in compliance with the Guidelines, no further action is required. If the investigator reports that the project is not in full compliance with the guidelines, those aspects of the project which are not in compliance will have to be terminated. However, investigators will have the opportunity to petition the Recombinant Advisory Committee to permit continued use of characterized clones already in existence and constructed under Asilomar guidelines. Presumably, the use of these clones will be permitted to continue until the Recombinant Advisory Committee or a subcommittee thereof, has rendered its opinion.

The above procedures assume that all investigators are already at least in compliance with Asilomar guidelines. If projects are identified which appear not to be in compliance with Asilomar guidelines, they will be brought to the immediate attention of the Deputy Director for Science, NIH and the Recombinant Advisory Committee.

APPENDIX D

RECOMBINANT DNA RESEARCH

Guidelines
as published in the
FEDERAL REGISTER, Part II,
July 7, 1976

On Wednesday, June 23, 1976, the Director, National Institutes of Health, with the concurrence of the Secretary of Health, Education, and Welfare, and the Assistant Secretary for Health, issued guidelines that will govern the conduct of NIH-supported research on recombinant DNA molecules. The NIH is also undertaking an environmental impact assessment of these guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969.

The NIH Guidelines establish carefully controlled conditions for the conduct of experiments involving the production of such molecules and their insertion into organisms such as bacteria. These Guidelines replace the recommendations contained in the 1975 "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules." The latter would have permitted research under less strict conditions than the NIH Guidelines.

The chronology leading to the present Guidelines is described in detail in the NIH Director's decision document that follows. In summary, scientists engaged in this research called, in 1974, for a moratorium on certain kinds of experiments until an international meeting could be convened to consider the potential hazards of recombinant DNA molecules. They also called upon the NIH to establish a committee to provide advice on recombinant DNA technology.

The international meeting was held at the Asilomar Conference Center, Pacific Grove, California, in February 1975. The consensus of this meeting was that certain experiments should not be done at the present time, but that most of the work on construction of recombinant DNA molecules should proceed with appropriate physical and biological barriers. The Asilomar Conference report also

made interim assignments of the potential risks associated with different types of experiments. The NIH then assumed responsibility for translating the broadly based Asilomar recommendations into detailed guidelines for research.

The decision by the NIH Director on these Guidelines was reached after extensive scientific and public airing of the issues during the sixteen months which have elapsed since the Asilomar Conference. The issues were discussed at public meetings of the Recombinant DNA Molecule Program Advisory Committee (Recombinant Advisory Committee) and the Advisory Committee to the NIH Director. The Recombinant Advisory Committee extensively debated three different versions of the Guidelines during this period.

The Advisory Committee to the NIH Director, augmented with consultants representing law, ethics, consumer affairs and the environment, was asked to advise as to whether the proposed Guidelines balanced responsibility to protect the public with the potential benefits through the pursuit of new knowledge. The many different points of view expressed at this meeting were taken into consideration in the decision.

The NIH recognizes a special obligation to disseminate information on these guidelines as widely as possible. Accordingly, the Guidelines will be sent to all of the approximately 25,000 NIH grantees and contractors. Major professional societies which represent scientists working in this area will also be asked to endorse the Guidelines. The Guidelines will be sent to medical and scientific journals and editors of these journals will be asked to request that investigators include a description of the physical and biological containment procedures used in any recombinant research they report on. International health and scientific organizations will also receive copies of the guidelines for their review.

Filing of an environmental impact statement will provide opportunity for the scientific community, Federal, State and local agencies and the general public to address the potential benefits and hazards of this research area. In order for there to be further opportunity for public comment and consideration, these guidelines are being offered for general comment in the FEDERAL REGISTER. It must be clearly understood by the reader that the material that follows is not proposed rulemaking in the technical sense, but is a document on which early public comment and participation is invited.

Please address any comments on these draft policies and procedures to the Director, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014. All comments should be received by November 1, 1976.

Additional copies of this notice are available from the Acting Director, Office of Recombinant DNA Activities, National Institute of General Medical Sciences, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20014.

DONALD S. FREDRICKSON, M.D.,
Director,
National Institutes of Health.

JUNE 25, 1976.

DECISION OF THE DIRECTOR, NATIONAL INSTITUTES OF HEALTH TO RELEASE GUIDELINES FOR RESEARCH ON RECOMBINANT DNA MOLECULES

JUNE 23, 1976.

CONTENTS

Introduction
I. General Policy Considerations
A. Science Policy
B. Implementation Within the NIH
C. Implementation Beyond the Purview of NIH
D. Environmental Policy
II. Methods of Containment (See Guidelines II)
III. Prohibited Experiments (See Guidelines III, A)
IV. Permissible Experiments: *E. Coli* K-12 Host-Vector Systems (See Guidelines III, B, 1)
V. Classification of Experiments Using the *E. Coli* K-12 Containment Systems (See Guidelines III, B, 2)
VI. Classification of Experiments Using Containment Systems Other than *E. Coli* K-12 (See Guidelines III, B, 4)
VII. Roles and Responsibilities (See Guidelines IV)

INTRODUCTION

Today, with the concurrence of the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health, I am releasing guidelines that will govern the conduct of NIH-supported research on recombinant DNA molecules (molecules resulting from the recombination in cell-free systems or segments of deoxyribonucleic acid, the material that determines the hereditary characteristics of all known cells). These guidelines establish carefully controlled conditions for the conduct of experiments involving the insertion of such recombinant genes into organisms, such as bacteria. The chronology leading to the present guidelines and the decision to release them are outlined in this introduction.

In addition to developing these guidelines, NIH has undertaken an environmental impact assessment of these guidelines for recombinant DNA research in accordance with the National Environmental Policy Act of 1969 (NEPA). The guidelines are being released prior to completion of this assessment. They will replace the current Asilomar guidelines, discussed below, which in many instances allow research to proceed under less strict conditions. Because the NIH guidelines will afford a greater degree of scrutiny and protection, they are being released today, and will be effective while the environmental impact assessment is under way.

Recombinant DNA research brings to the fore certain problems in assessing the potential impact of basic science on society as a whole, including the manner of providing public participation in those assessments. The field of research involved is a rapidly moving one, at the leading edge of biological science. The experiments are extremely technical and complex. Molecular biologists active in this research have means of keeping informed, but even they may fail to keep abreast of the newest developments. It is not surprising that scientists in other fields and the general public have difficulty in understanding advances in recombinant DNA research. Yet public awareness and understanding of this line of investigation is vital.

It was the scientists engaged in recombinant DNA research who called for a moratorium on certain kinds of experiments in order to assess the risks and devise appropriate guidelines. The capability to perform DNA recombinations, and the potential hazards, had become apparent at the Gordon Research Conference on Nucleic Acids in July 1973. Those in attendance voted to send an open letter to Dr. Philip Handler, President of the National Academy of Sciences, and to Dr. John R. Hogness, President of the Institute of Medicine, NAS. The letter, appearing in "Science 181," 1114, (1973), suggested "that the Academies [*sic*] establish a study committee to consider this problem and to recommend specific actions or guidelines, should that seem appropriate."

In response, NAS formed a committee, and its members published another letter in "Science 185," 303, (1974). Entitled "Potential Biohazards of Recombinant DNA Molecules," the letter proposed:

First, and most important, that until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring * * * [certain] experiments * * *.

Second, plans to link fragments of animal DNAs to bacterial plasmid DNA or bacteriophage DNA should be carefully weighed * * *.

Third, the Director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (i) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (ii) developing procedures which will minimize the spread of such molecules within human and other populations; and (iii) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.

Fourth, an international meeting of involved scientists from all over the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

On October 7, 1974, the NIH Recombinant DNA Molecule Program Advisory Committee (hereafter "Recombinant Advisory Committee") was established to advise the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH, "concerning a program for developing procedures which will minimize the spread of such molecules within human and other populations, and for devising guidelines to be followed by investigators working with potentially hazardous recombinants."

The international meeting proposed in the "Science" article (*185*, 303, 1974) was held in February 1975 at the Asilomar Conference Center, Pacific Grove, California. It was sponsored by the National Academy of Sciences and supported by the National Institutes of Health and the National Science Foundation. One hundred and fifty people attended, including 52 foreign scientists from 15 countries, 16 representatives of the press, and 4 attorneys.

The conference reviewed progress in research on recombinant DNA molecules and discussed ways to deal with the potential biohazards of the work. Participants felt that experiments on construction of recombinant DNA molecules should proceed, provided that appropriate biological and physical containment is utilized. The conference made recommendations for matching levels of containment with levels of possible hazard for various types of experiments. Certain experiments were judged to pose such serious potential dangers that the conference recommended against their being conducted at the present time.

A report on the conference was submitted to the Assembly of Life Sciences, National Research Council, NAS, and approved by its Executive Committee on May 20, 1975. A summary statement of the report was published in "Science 188," 991 (1975), "Nature 225," 442, (1975), and the "Proceedings of the National Academy of Sciences 72," 1981, (1975). The report noted that "in many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard. Until these are

established, we urge individual scientists to use the proposals in this document as a guide."

The NIH Recombinant Advisory Committee held its first meeting in San Francisco immediately after the Asilomar conference. It proposed that NIH use the recommendations of the Asilomar conference as guidelines for research until the committee had an opportunity to elaborate more specific guidelines, and that NIH establish a newsletter for informal distribution of information. NIH accepted these recommendations.

At the second meeting, held on May 12–13, 1975, in Bethesda, Maryland, the committee received a report on biohazard-containment facilities in the United States and reviewed a proposed NIH contract program for the construction and testing of microorganisms that would have very limited ability to survive in natural environments and would thereby limit the potential hazards. A subcommittee chaired by Dr. David Hogness was appointed to draft guidelines for research involving recombinant DNA molecules, to be discussed at the next meeting.

The NIH committee, beginning with the draft guidelines prepared by the Hogness subcommittee, prepared proposed guidelines for research with recombinant DNA molecules at its third meeting, held on July 18–19, 1975, in Woods Hole, Massachusetts.

Following this meeting, many letters were received which were critical of the guidelines. The majority of critics felt that they were too lax, others that they were too strict. All letters were reviewed by the committee, and a new subcommittee, chaired by Dr. Elizabeth Kutter, was appointed to revise the guidelines.

A fourth committee meeting was held on December 4–5, 1975, in La Jolla, California. For this meeting a "variorum edition" had been prepared, comparing line-for-line the Hogness, Woods Hole, and Kutter guidelines. The committee reviewed these, voting item-by-item for their preference among the three variations and, in many cases, adding new material. The result was the "Proposed Guidelines for Research Involving Recombinant DNA Molecules," which were referred to the Director, NIH, for a final decision in December 1975.

As Director of the National Institutes of Health, I called a special meeting of the Advisory Committee to the Director to review these proposed guidelines. The meeting was held at NIH, Bethesda, on February 9–10, 1976. The Advisory Committee is charged to advise the Director, NIH, on matters relating to the broad setting—scientific, technological, and socioeconomic—in which the continuing development of the biomedical sciences, education for the health professions, and biomedical communications must take place, and to advise on their implications for NIH policy, program development, resource allocation, and administration. The members of the committee are knowledgeable in the fields of basic and clinical biomedical sciences, the social sciences, physical sciences, research, education, and communications. In addition to current members of the committee, I invited a number of former committee members as well as other scientific and public representatives to participate in the special February session.

The purpose of the meeting was to seek the committee's advice on the guidelines proposed by the Recombinant Advisory Committee. The Advisory Committee to the Director was asked to determine whether, in their judgment, the guidelines balanced scientific responsibility to the public with scientific freedom to pursue new knowledge.

Public responsibility weighs heavily in this genetic research area. The scientific community must have the public's confidence that the goals of this profoundly important research accord respect to important ethical, legal, and social values of our society. A key element in achieving and maintaining this public trust is for the scientific community to ensure an openness and candor in its proceedings. The meetings of the Director's Advisory Committee, the Asilomar group, and the Recombinant Advisory Committee have reflected the intent of science to be an open community in considering the conduct of recombinant DNA experiments. At the Director's Advisory Committee meeting, there was ample opportunity for comment and an airing of the issues, not only by the committee members but by public witnesses as well. All major points of view were broadly represented.

I have been reviewing the guidelines in light of the comments and suggestions made by participants at that meeting, as well as the written comments received afterward. As part of that review I asked the Recombinant Advisory Committee to consider at their meeting of April 1–2, 1976, a number of selected issues raised by the commentators. I have taken those issues and the response of the Recombinant Advisory Committee into account in arriving at my decision on the guidelines. An analysis of the issues and the basis for my decision follow:

I. General Policy Considerations

A word of explanation might be interjected at this point as to the nature of the studies in question. Within the past decade, enzymes capable of breaking DNA strands at specific sites and of coupling the broken fragments in new combinations were discovered, thus making possible the insertion of foreign genes into viruses or certain cell particles (plasmids). These, in turn, can be used as vectors to introduce the foreign genes into bacteria or into cells of plants or animals in test tubes. Thus transplanted, the genes may impart their hereditary properties to new hosts. These cells can be isolated and cloned—that is, bred into a genetically homogeneous culture. In general, there are two potential uses for the clones so produced: as a tool for studying the transferred genes, and as a new useful agent, say for the production of a scarce hormone.

Recombinant DNA research offers great promise, particularly for improving the understanding and possibly the treatment of various diseases. There is also a potential risk—that microorganisms with transplanted genes may prove hazardous to man or other forms of life. Thus special provisions are necessary for their containment.

All commentators acknowledged the exemplary responsibility of the scientific community in dealing publicly with the potential risks in DNA recombinant research and in calling for a self-imposed moratorium on certain experiments in order to assess potential hazards and devise appropriate guidelines. Most commentators agreed that the process leading to the formulation of the proposed guidelines was a most responsible and responsive one. Suggestions by the commentators on broad policy considerations are presented below. They relate to the science policy aspects of the guidelines, the implementation of the guidelines for NIH grantees and contractors, and the scope and impact of the guidelines nationally and internationally.

A. Science Policy Considerations

Commentators were divided on how best to steer a course between stifling research through excessive regulation and allowing it to continue with sufficient controls. Several emphasized that the public must have assurance that the controls afford adequate protection against potential hazards. In the views of these commentators, the burden is on the scientific community to show that the danger is minimal and that the benefits are substantial and far outweigh the risks.

Opinion differed on whether the proposed guidelines were an appropriate response to the potential benefits and hazards. Several found the guidelines to so exaggerate safety procedures that inquiry would be unnecessarily retarded, while others found the guidelines weighted toward promoting research. The issue was how to strike a reasonable balance—in fact, a proper policy "bias"—between concerns to "go slow" and those to progress rapidly.

There was strong disagreement about the nature and level of the possible hazards of recombinant DNA research. Several commentators believed that the hazards posed were unique. In their view, the occurrence of an accident or the escape of a vector could initiate an irreversible process, with a potential for creating problems many times greater than those arising from the multitude of genetic recombinations that occur spontaneously in nature. These commentators stress the moral obligation on the part of the scientific community to do no harm.

Other commentators, however, found the guidelines to be adequate to the hazards posed. In their view, the guidelines struck an appropriate balance so that research could proceed cautiously. Still other commentators found the guidelines too onerous and restrictive in light of the potential benefits of this research for medicine, agriculture, and industry. Some felt that the guidelines are perhaps more stringent than necessary given the available evidence on the likelihood of hazards, but supported them as a compromise that would best serve the scientific community and the public at large. Many commentators urged that the guidelines be adopted as soon as possible to afford more specific direction to this research area.

I understand and appreciate the concerns of those who urge that this research proceed because of the benefits and of those who urge caution because of potential hazards. The guidelines issued today allow the research to go forward in a manner responsive and appropriate to hazards that may be realized in the future.

The object of these guidelines is to ensure that experimental DNA recombination will have no ill effects on those engaged in the work, on the general public, or on the environment. The essence of their construction is subdivision of potential experiments by class, decision as to which experiments should be permitted at present, and assignment to these of certain procedures for containment of recombinant organisms.

Containment is defined as physical and biological. Physical containment involves the isolation of the research by procedures which have evolved over many years of experience in laboratories studying infectious microorganisms. P1 containment—the first physical containment level—is that used in most routine bacteriology laboratories. P2 and P3 afford increasing isolation of the research from the environment. P4 represents the most extreme measures used for containing virulent pathogens, and permits no escape of contaminated air, wastes, or untreated materials. "Biological" containment is the use of vectors or hosts that are crippled by mutation so that the recombinant DNA is incapable of surviving under natural conditions.

The experiments now permitted under the guidelines involve no known additional haz-

ard to the workers or the environment beyond the relatively low risk known to be associated with the source materials. The additional hazards are speculative and therefore not quantifiable. In a real sense they are considerably less certain than are the benefits now clearly derivable from the projected research.

For example, the ability to produce, through "molecular cloning," relatively large *amounts of pure DNA from the chromosomes* of any living organism will have a profound effect in many areas of biology. No other procedure, not even chemical synthesis, can provide pure material corresponding to particular genes. DNA "probes," prepared from the clones will yield precise evidence on the presence or absence, the organization, and the expression of genes in health and disease.

Potential medical advances were outlined by scientists active in this research area who *were present at the meeting of the Director's* Advisory Committee. Of enormous importance, for example, is the opportunity to explore the malfunctioning of cells in complicated diseases. Our ability to understand a variety of hereditary defects may be significantly enhanced, with amelioration of their expression a real possibility. There is the potential to elucidate mechanisms in certain cancers, particularly those that might be caused by viruses.

Instead of mere propagation of foreign DNA, the expression of the genes of one organism by the cell machinery of another may alter the new host and open opportunities for manipulating the biological properties of cells. In certain prokaryotes (organisms with a poorly developed nucleus, like bacteria), this exchange of genetic information occurs in nature. Such exchange explains, for instance, an important mechanism for the changing and spreading of resistance to antibiotics in bacteria. Beneficial effects of this mechanism might be the production of medically important compounds for the treatment and control of disease. Examples frequently cited are the production of insulin, growth hormone, specific antibodies, and clotting factors absent in victims of hemophilia.

Aside from the potential medical benefits, a whole host of other applications in science and technology have been envisioned. Examples are the *large-scale production of* enzymes for industrial use and the development of bacteria that could ingest and destroy oil spills in the sea. Potential benefits in agriculture include the enhancement of nitrogen fixation in certain plants, permitting increased food production.

While the projected research offers the possibility of many benefits, it must proceed only with assurance that potential hazards can be controlled or prevented. Some commentators are concerned that nature may maintain a barrier to the exchange of DNA between prokaryotes and eukaryotes (higher organisms, with a well-formed nucleus)—a barrier that can now be crossed by experimentalists. They further argue that expression of the foreign DNA may alter the host in unpredictable and undesirable ways. Conceivable harm could result if the altered host has a competitive advantage that would foster its survival in some niche within the ecosystem. Other commentators believe that the endless experiments in recombination of DNA which nature has conducted since the beginning of life on the earth, and which have accounted in part for the evolution of species, have most likely involved exchange of DNA between widely disparate species. They argue that prokaryotes such as bacteria in the intestines of man do exchange DNA with this eukaryotic host and that the failure of the altered prokaryotes to be detected attests to a sharply limited capacity of such recombinants to survive. Thus nature, this argument runs, has already tested the probabilities of harmful recombination and any survivors of such are already in the ecosystem. The fact is that we do not know which of the above-stated propositions is correct.

The international scientific community, as exemplified by the Asilomar conference and the deliberations attendant upon preparation of the present guidelines, has indicated a desire to proceed with research in a conservative manner. And most of the considerable public commentary on the subject, while urging caution, has also favored proceeding. Three European groups have independently arrived at the opinion that *recombinant DNA research* should proceed with caution. These are the Working Party on Experimental Manipulation of the Genetic Composition of Micro-Organisms, whose "Ashby Report" was presented to Parliament in the United Kingdom by the Secretary of State for Education and Science in January 1975; the Advisory Committee on Medical Research of the World Health Organization, which issued a press release in July 1976; and the European Molecular Biology Organization *Standing Committee on Recombinant* DNA, meeting in February 1976.

There is no means for a flat proscription of such research throughout the world community of science. There is also no need to attempt it. It is likely that the evaluation engendered in the preparation and application of these guidelines will lead to beneficial review of some of the containment practices in other work that is not technically defined as recombinant DNA research.

Recombinant DNA research with which these guidelines are concerned involves microorganisms such as bacteria or viruses or cells of higher organisms growing in tissue culture. It is extremely important for the public to be aware that his research is not directed to altering of genes in humans although some of the techniques developed in this research may have relevance if this is attempted in the future.

NIH recognizes its responsibility to conduct and support research designed to determine the extent to which certain potentially harmful effects from recombinant DNA molecules may occur. Among these are experiments, to be conducted under maximum containment, that explore the capability of foreign genes to alter the character of host or vector, rendering it harmful, as through the production of toxic products.

Given the general desire that no rare and unexpected event arising from this research shall cause irreversible damage, it is obvious that merely to establish conservative rules of conduct for one group of scientists is not enough. The precautions must be uniformly and unanimously observed. Second, there must be full and timely exchange of experiences so that guidelines can be altered on the basis of new knowledge. The guidelines must also be implemented in a manner that protects all concerned—the scientific workers most likely to encounter unexpected hazards and all forms of life within our biosphere. The responsibility of the scientists involved is an inescapable and extreme as is their opportunity to beneficially enrich our understanding.

B. IMPLEMENTATION CONSIDERATIONS WITHIN THE NIH

All the commentators had suggestions concerning the structure and function of decision making as it relates to the principal investigator, the local biohazards committee, the peer review group, and the NIH Recombinant Advisory Committee. These comments and my response on the section of the guidelines relating to roles and responsibilities of investigators, their institutions, and *the National Institutes of Health* are presented below.

Of considerable concern to all commentators was the process by which NIH would proceed to implement the guidelines. The scientific community generally urged that there be no Federal regulations, while some of the public commentators recommended the regulatory process.

Many who opposed changing the proposed guidelines into Federal regulations expressed concern for flexibility and administrative efficiency, which could best be achieved, in their view, through voluntary compliance. Other commentators, however, believed it imperative to proceed toward regulation. In their view, the guidelines could be implemented for purposes of NIH funding and would govern the conduct of experiments until regulations were in effect. Another commentator who thought regulation would be harmful rather than helpful suggested that if there were to be regulations, they should be along lines similar to those that govern the sale, distribution, use, and disposal of radioisotopes.

The question of how best to proceed now that the guidelines have been released deserves careful attention. *I* share the concern of those who feel that the guidelines must remain flexible. It is especially important that there be opportunity to change them quickly, based on new information relating to scientific evidence, potential risks, or safety aspects of the research program.

The suggestions for regulation need further attention at this time. The process for regulation not only involves the Director of NIH, but also the Assistant Secretary for Health and the Secretary of Health, Education, and Welfare. These guidelines are being promulgated now in order to afford additional protection to all concerned. Consideration of their conversion to regulations can proceed with continuing review of their content and present and future implications. Meanwhile, the NIH shall continue to provide the opportunity for public comment and participation at least equivalent to that provided if steps towards regulations were to proceed immediately. The guidelines *will be published* in the FEDERAL REGISTER forthwith to allow for further public comment.

C. IMPLEMENTATION CONSIDERATIONS BEYOND THE PURVIEW OF NIH

Special concern has been expressed by many commentators regarding the application of the guidelines to research outside NIH by investigators other than its grantees or contractors. It has been urged that the *guidelines be made applicable to recombinant* DNA research conducted or supported by other agencies in HEW and by NSF, ERDA, DoD, and other governmental departments. Most commentators believe that these or similar guidelines should also govern research in the private sector, including industry, voluntary organizations, and foundations. Many feel that experiments conducted in colleges, universities, and even in high schools require some form of monitoring. And *finally,* all agree that *in view of the potential* hazards of recombinant DNA research to the biosphere, some form of international understanding on guidelines for the research is essential.

The committee, in the proposed guidelines, has suggested as one means of control that a description of the physical and biological containment procedures practiced in a research project be included in the publication of research results. In the scientific com*munity this can be a powerful force for con*formity, and we will undertake to present the recommendation to all appropriate journals. We are also prepared to take steps to disseminate the guidelines widely, and to arrange for a continual flow of information outward concerning the activities of the Re-

combinant Advisory Committee and the Advisory Committee to the Director, NIH, in the evolution of the guidelines and their implementation.

In response to these suggestions, I have already held a meeting with relevant HEW agencies and with representatives from other departments of the Federal Government. The purpose of the meeting was to exchange information on recombinant DNA research and to discuss the NIH guidelines. It served as an important beginning to address a common concern of these public institutions. A number of the representatives indicated that various departments might very well adopt the guideline for research conducted both in-house and supported outside. Following up, I have begun preliminary discussions with the Assistant Secretary for Health and the Secretary of HEW, to determine possible methods to ensure adoption of the guidelines by all Federal agencies. Encouraged by these efforts, we held a meeting on June 2 with representatives of industry to provide them with full information about the guidelines and to help determine the present and future interests of industrial laboratories in this type of research. The meeting provided one of the first opportunities for industry representatives to convene for a discussion of this research area, and an industry committee under the auspices of the Pharmaceutical Manufacturers Association will be formed to review the guidelines for potential application to the drug industry. Further meetings will be scheduled with other groups that have an active interest in recombinant DNA research.

It is my hope that the guidelines will be voluntarily adopted and honored by all who support or conduct such research throughout the United States, and that at least very similar guidelines will obtain throughout the rest of the world. NIH places the highest priority on efforts to inform and to work with international organizations, such as the World Health Organization and the International Council of Scientific Unions, with a review to achieving a consensus on safety standards in this most important research area.

There has been considerable international cooperation and activity in the past, and I expect it to continue in the future. The aforementioned Ashby Report, presented to Parliament in January 1975, describes the advances in knowledge and possible benefits to society of the experiments involving recombinant DNA molecules, and attempts to assess the hazards in these techniques. The Asilomar meeting also had a number of international representatives, as mentioned previously. The European Molecular Biology Organization (EMBO) has been involved in considering guidelines for recombinant DNA research. They have closely followed the activities of NIH, and will thus be encouraged, I believe, to monitor their research with augmented cooperation and coordination. For example, EMBO recently announced plans for a voluntary registry of recombinant DNA research in Europe. Following this EMBO initiative, NIH shall similarly maintain a voluntary registry of investigators and institutions engaged in such research in the United States. Plans for establishing this registry are under way.

D. ENVIRONMENTAL POLICY CONSIDERATIONS

A number of commentators urged NIH to consider preparing an environmental impact statement on recombinant DNA research activity. They evoked the possibility that organisms containing recombinant DNA molecules might escape and affect the environment in potentially harmful ways.

I am in full agreement that the potentially harmful effects of this research on the environment should be assessed. As discussed throughout this paper, the guidelines are premised on physical and biological containment to prevent the release or propagation of DNA recombinants outside the laboratory. Deliberate release of organisms into the environment is prohibited. In my view, the stipulated physical and biological containment ensures that this research will proceed with a high degree of safety and precaution. But I recognize the legitimate concern of those urging that an environmental impact assessment be done. In view of this concern and ensuing public debate, I have reviewed the appropriateness of such an assessment and have directed that one be undertaken.

The purpose of this assessment will be to review the environmental effects, if any, of research that may be conducted under the guidelines. The assessment will provide further opportunity for all concerned to address the potential benefits and hazards of this most important research activity. I expect a draft of the environmental impact statement should be completed by September 1 for comment by the scientific community, Federal and State agencies, and the general public.

It should be noted that the development of the guidelines was in large part tantamount to conducting an environmental impact assessment. For example, the objectives of recombinant DNA research, and alternate approaches to reach those objectives, have been considered. The potential hazards and risks have been analyzed. Alternative approaches have been thoroughly considered, to maximize safety and minimize potential risk. And an elaborate review structure has been created to achieve these safety objectives. From a public policy viewpoint, however, the environmental impact assessment will be yet another review that will provide further opportunity for the public to participate and comment on the conduct of this research.

II. METHODS OF CONTAINMENT

Comments on the containment provisions of the proposed guidelines were directed to the definitions of both physical and biological containment and to the safety and effectiveness of the prescribed levels. Several commentators found the concept of physical containment imprecise and too subject to the possibility for human error. Others questioned the concept of biological containment in terms of its safety and purported effectiveness in averting potential hazards. The commentators were divided on which method of containment would provide the most effective and safe system to avoid hazards. Several suggested that each of the physical containment levels be more fully explained.

W. Emmett Barkley, Ph.D., Director of the Office of Research Safety, National Cancer Institute, was asked to review the section on physical containment in light of these comments. Dr. Barkley convened a special committee of safety and health experts, who met to consider not only this section of the guidelines but also the section of the roles and responsibilities of researchers and their institutions. The committee thoroughly reviewed the section on physical containment and recommended a number of changes. The Recombinant Advisory Committee, meeting on April 1-2, 1976, reviewed the recommendations of the Barkley group. These are incorporated, with editorial revisions, in the final version of the guidelines.

The present section on physical containment is directly responsive to those commentators who asked for greater detail and explanation. Although different in detail, the four levels of containment approximate those given by the Center for Disease Control for human etiologic agents and by the National Cancer Institute for oncogenic viruses. For each of the proposed levels, optional items have been excluded, and only those items deemed absolutely necessary for safety are presented. Necessary facilities, practices, and equipment are specified. To give further guidance to investigators and their institutions, a supplement to the guidelines explains more fully safety practices appropriate to recombinant DNA research. And a new section has been added to ensure that shipment of recombinant DNA materials conforms, where appropriate, to the standards, prescribed by the U.S. Public Health Service, the Department of Transportation, and the Civil Aeronautics Board.

The section on physical containment is carefully designed to offer a constructive approach to meeting potential hazards for recombinant experiments at all levels of presumed risk. Certain commentators had suggested that the first level of physical containment (P1) be merged with the second level (P2). This suggestion, however, would tend to apply overly stringent standards for some experiments and might result in a lowering of standards necessary at the second level. I believe the level of control must be consistent with a reasonable estimate of the hazard; and the section on physical containment does provide this consistency. Accordingly, the first and second levels of physical containment remain as separate sections in the guidelines.

Because of the nature and operation of facilities required for experiments to be done at the fourth level of containment (P4), a provision has been included that the NIH shall review such facilities prior to funding them for recombinant DNA studies. The situation merits the special attention of experts who have maximum familiarity with the structure, operation, and potential problems of P4 installations. Several commentators advocated that NIH arrange for sharing of P4 facilities, both in the NIH intramural program and in institutions supported through NIH awards. In response to these suggestions, we are currently reviewing our facilities, including those at the Frederick Cancer Research Center (Fort Detrick), to determine how such a program can best be devised. It is most important that P4 facilities be made available to investigators. It should be noted that incidents of infection by even the most highly infectious and dangerous organisms are extremely infrequent at P4 facilities, and therefore the potential for hazard in certain complex experiments in recombinant DNA research is considerably reduced.

III. PROHIBITED EXPERIMENTS

1. Practically all commentators supported the present prohibition of certain experiments. There were suggestions for a clearer definition of the prohibition of certain experiments where increased antibiotic resistance may result. And it was urged by some that the prohibition be broadened to include experiments that result in resistance to any antibiotic, irrespective of its use in medicine or agriculture. Consideration of such a suggestion must take into account that antibiotic resistance occurs naturally among bacteria, and that resistance is a valuable marker in the study of microbial genetics in general and recombinants in particular.

In view of these concerns, however, the Recombinant Advisory Committee was asked to reconsider carefully the prohibition and related sections concerning antibiotic resistance. The committee noted that the prohibition relating to drug resistance was intended to ban those experiments that could compromise drug use in controlling disease agents in veterinary as well as human medicine and this is now clearly stated.

In the draft guidelines there were two statements concerning resistance to drugs which related to experiments with *E. coli*. The statements appeared to allow experi-

ments that would extend the range of resistance of this bacterium to therapeutically useful drugs and disinfectants, and thus seemed to be in conflict with the general prohibition on such research. There are numerous reports in the scientific literature indicating that E. coli can acquire resistance to all antibiotics known to not against it. Since E. coli acquires resistance naturally, the prohibition directed against increasing resistance does not apply. The ambiguous statements have been deleted from the present guidelines. On the other hand, new language has been inserted in the section dealing with other prokaryote species to set containment levels for permitted experiments.[1]

2. The Recombinant Advisory Committee was also asked to clarify whether the prohibition of use of DNA derived from pathogenic organisms (those classified as 3, 4, and 5 by the Center for Disease Control, USPHS) also included the DNA from any host infected with these organisms. The committee explained that this prohibition did extend to experiments with cells known to be so infected. To avoid misunderstanding, the prohibition as now worded includes such cells. In addition, the prohibitions have been extended to include moderate-risk oncogenic viruses, as defined by the National Cancer Institute, and cells known to be infected with them.

3. Two other issues relating to the section on prohibited experiments were raised by Roy Curtiss III, Ph.D., Professor, Department of Microbiology, University of Alabama School of Medicine, Birmingham, who is a member of the Recombinant Advisory Committee. Dr. Curtiss noted that for the class of experiments prohibited on the basis of production of highly toxic substances, only substances from microorganisms were cited as examples. He suggested that other examples be included, such as venoms from insects and snakes. The committee approved the suggestion and I concur.

In the proposed guidelines, release of organisms containing recombinant DNA molecules into the environment was prohibited unless a series of controlled tests had been done to leave no reasonable doubt of safety. Dr. Curtiss felt that the guidelines should provide greater specificity for testing and should include some form of review prior to release of the organism. I have decided that the guidelines should, for the present, prohibit any deliberate release of organisms containing recombinant DNA into the environment. With the present limited state of knowledge, it seems highly unlikely that there will be in the near future, any recombinant organism that is universally accepted as being beneficial to introduce into the environment. When the scientific evidence becomes available that the potential benefits of recombinant organisms, particularly for agriculture, are about to be realized, then the guidelines can be altered to meet the need for release. It is most important that the potential environmental impact of the release be considered.

IV. Permissible Experiments: E. coli K-12 Host-Vector Systems

The continued use of E. coli as a host has drawn considerable comment, including some suggestions that its use be prohibited presently or within a specified time limit. It should be stressed that the use of E. coli as detailed in the guidelines is limited to E. coli K-12, a strain that has been carried in the laboratory for decades, and does not involve the use of any strain of E. coli that is freshly isolated from a natural source. E. coli K-12 does not usually colonize the normal bowel, even when given in large doses, and exhibits little if any multiplication while passing through the alimentary canal. For years it has been the subject of more intense investigation than any other single organism, and knowledge of its genetic markup and recombinant behavior exceeds greatly that pertaining to any other organism. I believe that because of this experience, E. coli K-12 will provide a host-vector system that is safer than other candidate microorganisms.

NIH recognizes the importance of supporting the development of alternative host-vector systems (such as B. subtilis, which has no ecological niche in man) and will encourage such development. It should be noted, however, that for each new host-vector system, the same questions of risk from altered properties attendant upon the presence of recombinant genes will apply as apply to E. coli. NIH does not believe it wise to set a time limit on replacement of E. coli systems by other organisms.

There were specific suggestions concerning the three levels of biological containment prescribed for use of E. coli K-12 vectors. Some commentators requested a more detailed explanation of the adequacy of protection for laboratory personnel with the first level of containment (EK1).[2] Sections of the guidelines dealing with physical containment and roles and responsibilities now specify the need for safety practices and accident plans.

For the second level of containment (EK2), it is required that a cloned DNA fragment be contained in a host-vector system that has no greater than a 10^{-8} probability of survival in a laboratory culture or natural environment. It was suggested that the selection of this level of biological containment and the appropriate tests for vertification be more fully explained in the guidelines. The committee, in responding to a request for further examination of this point, reviewed at considerable length the testing for an EK2 system and recommended certain modifications. We have accepted the committee's new language that better explains testing of survival of a genetic marker carried on the vector, preferably on an inserted DNA fragment.

Possible tests to determine the level of biological containment afforded by these altered host-vector systems are outlined in this section. Because this is such a new area of scientific research and development, however, it is inappropriate to standardize such testing at the present time. Standards will gradually be set as more experience with EK2 host-vector systems is acquired. The committee, for example, during its April 1976 meetings gave its first approval to an EK2 host-vector system. What is necessary is that new and more effective tests be devised by investigators, and this effort is very likely to occur under the present guidelines. For example, one task recognized by the committee is to clarify how survival of the organism and the cloned DNA should be defined in terms of temperature, medium, and other variables.

It is also very important to note here that the stringent requirements set by the committee for EK2 biological containment jeopardize considerably the capacity of such crippled organisms to survive and replicate even under permissive laboratory conditions. More experience will be required to determine whether EK2 containment will permit some lines of important research to be followed.

Several commentators suggested that methods and procedures to confirm an EK system at the third level of containment (EK3) be more fully explained. The Recombinant Advisory Committee was asked to consider this suggestion. After considerable discussion the committee declined to define the procedures more fully at this time, because development of an EK3 system is still far enough in the future not to warrant specific testing procedures. Further, it is not clear what tests are best suited. The language, therefore, remains general. The committee, however, is aware of the concerns for a more completely defined system of testing, and has considered the possibility of organizing a symposium for purposes of designating tests. In my view, more fully developed protocols for testing EK3 systems are warranted, and it is necessary that guidelines here be more fully developed before the committee proceeds to certify such a system. In this regard the NIH is prepared through the National Institute of Allergy and Infectious Diseases to support contracts to accomplish this task. We will seek the advice and assistance of the committee to define the scope of necessary work.

These guidelines also include a statement that for the time being no EK2 or EK3 host-vector system will be considered bona fide until the Recombinant Advisory Committee has certified it. I share the concern of the commentators that new host-vector systems require the highest quality of scientific review and scrutiny. At this early stage of development, it is most important that the committee provide that scrutiny. Further, I believe that until more experience has been gained, the committee should encourage and the NIH support research that will independently confirm and augment the data on which certification of EK2 host-vector systems are based.

V. Classification of Experiments Using the E. Coli K-12 Containment Systems

The guidelines assign different levels of containment for experiments in which DNA from different sources is to be introduced into an *E. coli* K-12 host-vector system. The variation is based on both facts and assumptions. There are some prokaryotes (bacteria) which constantly exchange DNA with *E. coli*. Here it is assumed that experimental conditions beyond those obtained in careful, routine microbiology laboratories are superfluous, because any exchange experiments have undoubtedly been performed already in nature.

In every instance of artificial recombination, consideration must be given to the possibility that foreign DNA may be translated into protein (expressed), and also to the possibility that normally repressed genes of the host may be expressed and thus change, undesirably, the characteristics of the cell. It is assumed that the more similar the DNAs of donor and host, the greater the probability of expression of foreign DNA, or of possible derepression of host genes. In those cases where the donor exchanges DNA with *E. coli* in nature, it is unlikely that recom-

[1] Specifically, experiments that would extend realstance to therapeutically useful drugs must use P3 physical containment plus a host-vector comparable to EK1, or P2 containment plus a host-vector comparable to EK2.

[2] The EK1 system presently consists of a battery of different vectors and of E. coli K-12 mutants, all of which afford a considerable degree of biological containment. The diversity of vectors and of host mutants in this battery has parmitted a wide range of important scientific questions to be attacked. For example, the availability of different vectors with cleavage sites for different restriction endonucleases have increased the kind of DNA segments that can be cloned. By contrast, the first EK2 host-vector systems are only now being considered by the Recombinant Advisory Committee. While NIH is supporting the development of more EK2 host-vector systems, it is not expected that a battery equivalent to that available for the EK1 system will be certified by the Recombinant Advisory Committee in the near future.

bination experiments will create new genetic combinations. When prokaryote donors not known to exchange DNA with *E. coli* in nature are used, however, there is a greater potential for new genetic combinations to be formed and be expressed. Therefore, it is required that experiments involving prokaryotic DNA from a donor that is not known to exchange DNA with *E. coli* in nature be carried out at a higher level of containment. Recombination using prokaryotic DNA from an organism known to be highly pathogenic is prohibited.

There are only limited data available concerning the expression of DNA from higher forms of life (eukaryotes) in *E. coli* (or any other prokaryote). Therefore, the containment prescriptions for experiments inserting eukaryotic DNA into prokaryotes are based on risks having quite uncertain probabilities.

On the assumption that a prokaryote host might translate eukaryotic DNA, it is further presumed that the product of that foreign gene would be most harmful to man if it were an enzyme, hormone, or other protein that was similar (homologous) to proteins already produced by or active in man. An example is a bacterium that could produce insulin. Such a "rogue" bacterium could be of benefit if contained, a nuisance or possibly dangerous if capable of surviving in nature. This is one reason that the higher the phylogenetic order of the eukaryote, the higher the recommended containment, at least until the efficiency of expression of DNA from higher eukaryotes in prokaryotes can be determined.

There is a second, more concrete reason for scaling containment upward as the eukaryote host becomes similar to man. This is the concern that viruses capable of propagating in human tissue, and possibly causing diseases, can contaminate DNA, replicate in prokaryote hosts and infect the experimentalist. Such risks are greatest when total DNA from donor tissue is used in "shotgun" recombinant experiments; it diminishes to much lower levels when pure cloned DNA is used.

The commentators were clearly divided on the classification of containment criteria for different kinds of recombinant DNAs. Many commentators considered the guidelines too stringent and rigid. Others viewed the guidelines in certain instances as too permissive. And still others endorsed the guidelines as sensible and reasonable, affording the public an enormous degree of protection from the speculative risks. Several suggestions were made for the specific classes of experiments, and they follow:

1. Comment on the use of DNA from animals and plants in recombinant experiments varied widely. Some commentators suggested banning the use of DNA from primates, other mammals, and birds. Others suggested that higher levels of the containment be used for all such experiments. Still others believed that the guidelines were too strict for experiments of this class. I have carefully reviewed the issues raised by the commentators and the response of the committee to certain queries concerning use of animal and plant DNA in these experiments.

In my view, the classification for the use of DNA from primates, other mammals, and birds is appropriate to the potential hazards that might be posed. The physical and biological containment levels are very strict. For example, biological containment levels are at EK2 or EK3, and will effectively preclude experimentation until useful EK2 and EK3 systems are available. EK2 systems are still in the initial stages of development, and the first system was only certified at the most recent meeting of the Recombinant Advisory Committee. An EK3 host-vector system has yet to be tested, and its certification is far enough in the future to place a moratorium on those experiments requiring biological containment at an EK3 level. The physical containment levels of P3 or P4 themselves afford a very high degree of protection. I am satisfied that the guidelines demonstrate the caution and prudence that must govern the conduct of experiments in this category.

The guidelines allow reduced containment levels for primate DNA when it is derived from embryonic tissue or germ-line cells. This is based on evidence that embryonic material is less likely to contain viruses than is tissue from the adult. Obviously, the embryonic tissue must be free of adult tissue, and the present guidelines so indicate.

I have also carefully considered the special concerns arising from the use of DNA from cold-blooded vertebrates and other cold-blooded animals, because several commentators questioned the basis of lower physical and biological containment levels for DNA from these species. The Recombinant Advisory Committee has debated this extensively, and they were asked to do so once again in April.[a] The committee has now recommended high containment levels (P3+EK2) when the DNA is from a cold-blooded vertebrate known to produce a potent toxin. That recommendation is included in the present guidelines. Where no toxin is involved the committee supported lower containment levels. The guidelines specify P2+EK2 levels for such work. There was considerable discussion concerning the advisability of recommending lower containment (P2+EK1) when the DNA is isolated from embryonic tissue or germ-line cells from cold-blooded vertebrates. Those supporting lower containment levels argued that the justification for P2+EK2 was the possibility that cold-blooded vertebrates may carry viruses and that the distinction between adult and germ cell tissue is real. Others argued that, contrary to the situation with primate DNA, viruses are not a central problem with cold-blooded vertebrates and therefore no distinction should be made on the basis of tissue origin. Finally, the committee recommended, on a divided vote (8 to 4), to adopt P2+EK1 when the cold-blooded vertebrate DNA is isolated from embryonic tissue or germ-line cells. Upon reviewing these considerations, I have decided to retain the containment levels for embryonic or germ-line DNA from cold-blooded vertebrates as recommended by the committee.

In April the committee also reviewed, at our request, the classification of experiments where DNA is derived from other cold-blooded animals or lower eukaryotes. Several commentators, for example, had been concerned about the fact that insects are known to carry agents pathogenic to man. In the committee review, it was noted that viruses carried by insects and known to transmit disease to man are RNA rather than DNA viruses and do not reproduce via DNA copied from RNA. In order, however, to make the intent clearer, the guidelines have been rewritten for experiments of this class. New language is inserted to ensure that strict containment levels are employed when the DNA comes from known pathogens or species known to carry them. Further, to reduce the potential hazards, we have also included in the guidelines the requirement that any insect must be grown under laboratory conditions for at least 10 generations prior to its use as a DNA source.

2. As alluded to above, certain commentators expressed concern that when *E. coli* becomes the host of recombinant DNA from prokaryotes with which DNA is not usually exchanged, there is hazard of altered host characteristics resulting from translation of the DNA into functioning proteins. The committee was asked to review the guidelines and take into account this potential hazard. They agreed that the containment levels should be increased for this category of experiment, from P2+EK1 to either P2+EK2 or P3+EK1. That recommendation is included in the present guidelines.

Comments were made concerning that class of experiments in which the recombinant DNA, regardless of source, has been cloned. A clone is a population of cells derived from a single cell and therefore all the cells are presumed to be genetically identical. As outlined in the proposed guidelines, clones could be used at lower containment levels if they had been rigorously characterized and shown to be free of harmful genes. Several commentators inquired how the characterization was to be performed and the freedom from harmful genes demonstrated. Although the committee acknowledges that these terms are unavoidably vague, they do not appropriate scientific methods to make relevant determinations. Again, this is a rapidly changing area and more clarity and precision can be expected with experience. Reduced containment requirements for this class of experiment are warranted because of the purified nature of clones. Further, the granting agency must approve the clone before containment conditions can be reduced, thus providing an additional element of review.

4. Another comment was related to the use of DNA from organelles (intracellular elements that contain special groups of genes for particular cell functions). Concern was expressed about the potential contamination of purified organelle DNA with DNA from viruses because of the similarity of their structures. The committee agrees, and the guidelines now specify a requirement, that the organelles be isolated prior to extracting DNA, as a further means of reducing the hazard of viral contamination.

5. Some commentators were troubled about the lowering of containment for that class of experiments involving recombinations with cell DNA segments purified by chemical or physical methods. They asked that procedures for determining the state of purification be more fully detailed and that the Recombinant Advisory Committee certify the purity. There are, however, appropriate techniques, such as gel electrophoresis, with which a purity of 99 percent by mass can be achieved and ascertained. There is no way for the committee to certify these results beyond repeating the experiments themselves. These techniques are well documented and described in the literature. I do not believe it is necessary or feasible for the committee to review each procedure for purification of DNA.

6. Comments were made concerning the use of DNA derived from animal viruses. It

[a] A committee member, David S. Hogness, Ph. D., Professor, Department of Biochemistry, Stanford University, California, submitted a statement in support of lower containment levels based on current scientific evidence. That evidence is based on certain differences between cold- and warm-blooded vertebrates. One of the criteria used for the evaluation of the relative risk that might be encountered with different levels of shotgun experiment is the degree of sequence homology between the DNA of the given species and that of humans. This criterion is used to estimate the likelihood that segments of DNA from the given species might be integrated into the human genome by recombination; the greater the homology, the greater the likelihood of integration. Studies of sequence homologies indicate that there is a considerable degree of homology between human DNA and DNA from other primates, much less homology between primates and other mammals, and even lower but detectable homology between birds and primates. By contrast, no significant homologies between cold-blooded vertebrates and primates have been detected.

was urged that containment levels for this class of experiment be increased. On the basis of my review, I find the containment conditions appropriate to the potential hazard posed. As defined in the guidelines, experiments are to be done at very strict levels of containment and these can be lowered only when the cloned DNA recombinants have been shown to be free of possibly harmful genes by suitable biochemical and biological tests. This also pertains to DNA that is copied from RNA viruses. In no instance are the guidelines more lenient, and in most instances they are more stringent than conditions obtaining in many laboratories where such viruses are studied in non-DNA-recombinant experiments.

VI. CLASSIFICATION OF EXPERIMENTS USING CONTAINMENT SYSTEMS OTHER THAN E. COLI K-12

1. No issue with regard to these guidelines raised more comment than the use of animal viruses as vectors. Of special concern to many commentators was the use of the simian (monkey) virus 40 (hereafter "SV40"). Some suggested a complete ban on the use of this virus; others urged its retention as a vector. SV40 is not known to produce any disease in man, although it can be grown in human cells and on very rare occasions has been isolated from humans. Many humans have received SV40 virus inadvertently in vaccines prepared from virus grown in monkey kidney-cell cultures. An intensive search has been made and is continuing for evidence that SV40 might cause cancer or be otherwise pathogenic for man. At present, it is my view that the extensive knowledge we have of SV40 virus provides us with sufficient sophistication to ensure its safe handling under the conditions developed for its use in the guidelines.

I believe work with SV40 should continue under the most careful conditions, but I do recognize and appreciate the concerns expressed over its possible harmful effects in humans. In light of these concerns, I asked the Recombinant Advisory Committee to review this section of the guidelines. The committee reconsidered the containment conditions for this class of experiments and judged them appropriate to meet the potential hazards.[4]

This class of experiments will proceed under the most careful and stringent conditions. Work with SV40 virus will be done at the maximum level of physical containment (P4). The extraordinary precautions required in a P4 facility lessen the likelihood of a potential hazard from this work. Only defective SV40 virus will be used as vector; that is, the SV40 virus particles that carry the foreign DNA cannot multiply by themselves. When a number of strict conditions are met, this work will be permitted to go on at the third level of containment (P3), which in itself requires care and precision. It should be noted that SV40 virus and its DNA can be efficiently disinfected by Clorox and autoclaving. These are customary procedures for disinfecting glassware and other items used in SV40 animal-cell work.

Some commentators suggested that the containment criteria for experiments using polyoma virus as the vector be strengthened. There is no evidence that polyoma infects humans or replicates to any significant extent in human cells. It holds promise as a vector, as is more fully documented in an appendix to these guidelines.

[4] One member dissented from this position. During the discussion, additional language was recommended (and adopted) to ensure that the defective SV40-virus/helper-virus system, with its inserted non-SV40 DNA segment, does not replicate in human cells with significantly more efficiency than does SV40.

2. Several commentators found the guidelines inadequate regarding experiments with plant host-vector systems. Because NIH shared these concerns, a group of extensive experience with plants was appointed to review this section. The group met concurrently with the Recombinant Advisory Committee in April 1976 and made several modifications. The suggested revisions were acceptable to the full committee, and we have included them in the guidelines.

The modifications are responsive to the stated concerns of the commenators. A description of greenhouse facilities is given, and physical containment conditions have been modified to take into account operations with whole plants. On the whole the respective portions of the guidelines relating to plants are more fully explained and the intent is clarified.

I have also accepted the recommendation of the subcommittee to lower the biological containment level from EK2 to EK1 for experiments in which the DNA from plants is used in conjunction with the *E. coli* K-12 host-vector system, thereby setting containment in this instance at the same level required for experiments with lower-eukaryote DNA.

VII. ROLES AND RESPONSIBILITIES

1. Most commentators had suggestions for the section on the roles and responsibilities of investigators, their local institutions, and NIH. Commentators generally urged openness, candor, and public participation in the process, emphasizing shared responsibility and accountability from the local to the national level. We reviewed that section of the guidelines in light of these comments and have asked the Recombinant Advisory Committee to review certain issues.

It is clear that much of the success of the guidelines will lie in the wisdom with which they are implemented. Because of the importance of this section, especially in terms of safety programs and plans, we have carefully weighed the comments and suggestions made in this regard. NIH has a special responsibility to take a leading role in ensuring that safety programs are part of all recombinant DNA research. Dr. Barkley and a specially convened committee were asked to provide greater detail for safety, accident, and training plans for this section of the guidelines. Based on their recommendations, the section has been extensively rewritten to clarify the respective responsibilities of the principal investigator, the institution (including the institutional biohazards committee), the NIH initial review group (study section), the NIH Recombinant DNA Molecule Program Advisory Committee, and NIH staff.

This section has a definitive administrative framework for assuring that safety is an essential and integrated component of research involving recombinant DNA molecules. The guidelines require investigators to institute, monitor, and evaluate containment and safety practices and procedures. Before research is done, the investigator must have safety and accident plans in place and training exercises for the staff well under way.

Some commentators suggested that the investigator be required to obtain informed consent of laboratory personnel prior to their participation. Rather than rely explicitly on an informed consent document, the guidelines now make the investigator responsible for advising his program and support staff as to the nature and assessment of the real and potential biohazards. He must explain and provide for any advised or requested precautionary medical policies, vaccinations, or serum collections. Further, an appendix to the guidelines includes detailed explanations for dealing with accidents, as well as instructions for the training of staff in safety and accident procedures.

In response to suggestions for epidemiological monitoring, the guidelines now require the principal investigator to report certain categories of accidents, in writing, to appropriate officials. NIH is investigating procedures for long-term surveillance of workers engaged in recombinant DNA research.

2. A number of comments on the role and responsibilities of the institutional biohazards committee were received. Comments were directed to the structure of the committee, the scope of its responsibility, and the methods for operation. Comments on structure included suggestions that the committee have a broadly based representation, especially in terms of health and safety expertise. Some others suggested NIH require certain classes of representation. In response to these suggestions, the guidelines now recommend membership from a diversity of disciplines relevant to recombinant DNA molecule technology, biological safety, and engineering.

For broader representation beyond the immediate scientific expertise, the guidelines now recommend that local committees should possess, or have available, the competence necessary to determine the acceptability of their findings in terms of applicable laws, regulations, standards of practice, community attitudes, and health and environmental considerations. The names of and relevant background information on the committee members will be reported to NIH.

In response to suggestions that decisions of the committee be made publicly available, the guidelines now recommend that minutes of the meetings should be kept and made available for public inspection.

Commentators generally approved of the responsibility given to the institutional biohazards committee to serve as a source of advice and reference to the investigator on scientific and safety questions. It was further suggested that the committee's responsibility be broadened in the development, monitoring, and evaluation of safety standards and procedures. In response to these suggestions, the guidelines now indicate that the institutional biohazards committee has the responsibility to certify, and recertify annually, to NIH that the facilities, procedures, practices, training, and expertise of involved personnel have been reviewed and approved. The Recombinant Advisory Committee suggested that examination might be unnecessary for P1 facilities, but we believe that all facilities should be reviewed to emphasize the importance of safety programs.

Some commentators suggested that the guidelines should stipulate that the local committees be required to determine the containment conditions to be imposed for a given project (which the draft guidelines specifically noted was not their responsibility). The Recombinant Advisory Committee took exception to this suggestion. They urged NIH not to include these conditions as local requirements, arguing among other things that review by the NIH study sections would provide the necessary scrutiny at the national level and assure uniformity of standards in application of the guidelines. I do not believe that NIH should require the local institution to have its biohazards committee assess what containment conditions are required for a given project. On the other hand, the guidelines should not prohibit the local institution from having its biohazards committee perform this function. Accordingly, I have deleted the prohibition that appeared in the proposed guidelines.

Another suggestion was that the local committee ensure that research is carried out in accordance with standards and procedures under the Occupational Safety and Health

Act (OSHA). This is an area of importance to the local institutions under Federal and State law, but need not be included as a requirement in the guidelines. NIH will maintain liaison with the Occupational Safety and Health Administration (Department of Labor) to ensure maximum Federal cooperation in this venture.

I would also encourage all institutions, as suggested by several commentators, to review their insurance compensation programs to determine whether their laboratory personnel, in the research area, are covered for injuries.

3. The commentators approved of having the NIH study sections responsible for making an independent evaluation of the classification of the proposed research under the guidelines, along with the customary judgment of the scientific merit of each grant application. This additional element of review will ensure careful attention to potential hazards in the research activity. The study sections will also scrutinize the proposed safeguards. Biological safety expertise shall be available to the study section for consultation and guidance in this regard.

4. Several commentators made suggestions concerning the structure, function, and scope of responsibility of the NIH Recombinant DNA Molecule Program Advisory Committee.

Comments on possible structural mechanisms for decision making included suggestions that there be a scientific and technical committee and a general advisory public policy committee. It was also suggested that the scientific committee include scientists who are not actively engaged in recombinant research, and, that the public policy committee have a broad scientific and public representation.

I have carefully reviewed these comments and suggestions. In response, the following structure has been devised. The Recombinant Advisory Committee shall serve as the scientific and technical committee. Its membership shall continue to include scientists who represent disciplines actively engaged in recombinant DNA research. In my view, it is most important that this committee have the necessary expertise to assure that the guidelines are of the highest scientific quality. The committee has provided this expertise in the past, and it must continue to do so. The committee shall also include members from other scientific disciplines.

It should be noted that the present committee recommended on its own initiative that a nonscientist be appointed. Emmette S. Redford, Ph. D., LL. D., Ashbel Smith Professor of Government and Public Affairs at the Lyndon B. Johnson School of Public Affairs, University of Texas at Austin, serves in that capacity. An ethicist has also been nominated for appointment.

The Advisory Committee to the Director, NIH, shall serve to provide the broader public policy perspectives. This committee, at its meeting on February 9-10, 1976, reviewed the proposed guidelines with the participation of public witnesses, and shall continue to provide such review for future activities of the Recombinant Advisory Committee.

In response to suggestions, the responsibilities of the Recombinant Advisory Committee have been expanded. In addition to reviewing the guidelines for possible modification as scientific evidence warrants, the committee will certify EK2 and EK3 systems. In response to requests by the investigator, local committee, or study section, the committee will also provide evaluation and review in order to advise on levels of required containment, on lowering of requirements when cloned recombinants are to be used, and on questions concerning potential biohazard and adequacy of containment provisions.

Commentators also asked that the committee review ongoing research initiated prior to the implementation of the guidelines. Now that the guidelines are being released, NIH-funded investigators in this field will be asked to give assurance, within a given period, that they will comply. Any investigators who constructed clones under the Asilomar guidelines will be asked to petition NIH for special consideration of their case, if the new guidelines require higher containment than did the Asilomar guidelines. Here the advice of the Recombinant Advisory Committee will be sought.

There were also suggestions that the committee certify chemical purification of recombinant DNA, but as I indicated earlier, these procedures are too well known to require NIH monitoring.

5. In light of comments received, NIH will provide review, through appropriate NIH offices, of data from institutional biohazards committees (including accident reports) and will ensure dissemination of these findings as appropriate. Dr. William Gartland will head the newly created NIH Office of Recombinant DNA Activities for these purposes. In addition, NIH will provide for rapid dissemination of information through its Nucleic Acid Recombinant Scientific Memoranda (NARSM), distributed by the National Institute for Allergy and Infectious Diseases. NIH will also provide an appropriate mechanism for approving and certifying clones before containment conditions can be lowered.

With these extended modifications, the section of the guidelines dealing with roles and responsibilities now sets forth a more fully developed review structure involving the principal investigator, local biohazards committees, and the Recombinant Advisory Committee, as well as peer review committees. Guidelines now provide extensive opportunity for advice, from the local to the national level. Several levels of review and scrutiny are provided, ensuring the highest standards for scientific merit and conditions for safety.

The Recombinant Advisory Committee in conjunction with the Director's Advisory Committee shall continue to serve as an ongoing forum for examining progress in the technology and safety of recombinant DNA research. Their responsibility, and that of the NIH Director, is to ensure that the guidelines, through modification when called for, reflect the soundest scientific and safety evidence as it accrues in this area. Their task, in a sense, is just beginning.

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NATIONAL INSTITUTES OF HEALTH

GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

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CONTENTS

I.

Introduction.

II.

Containment.
A. Standard practices and training.
B. Physical containment levels.
P1 Level (Minimal).
P2 Level (Low).
P3 Level (Moderate).
P4 Level (High).
C. Shipment.
D. Biological containment levels.

III.

Experimental Guidelines.
A. Experiments that are not to be performed.
B. Containment guidelines for permissible experiments.
1. Biological containment criteria using *E. Coli* K-12 host-vectors.
EK1 host-vectors.
EK2 host-vectors.
EK3 host-vectors.
2. Classification of experiments using the *E. Coli* K-12 containment systems.
<a> Shotgun experiments.
(i) Eukaryotic DNA recombinants.
(ii) Prokaryotic DNA recombinants.
(iii) Characterized clones of DNA recombinants derived from shotgun experiments.
<b> Purified cellular DNAs other than plasmids, bacteriophages, and other viruses.
<c> Plasmids, bacteriophages, and other viruses.
(i) Animal viruses.
(ii) Plant viruses.
(iii) Eukaryotic organelle DNAs.
(iv) Prokaryotic plasmid and phage DNAs.
3. Experiments with other prokaryotic host-vectors.
4. Experiments with eukaryotic host-vectors.
<a> Animal host-vector systems.
<b> Plant host-vector systems.
<c> Fungal or similar lower eukaryotic systems.

IV.

Roles and Responsibilities.
A. Principal Investigator.
B. Institution.
C. NIH Initial Review Group (Study Sections).
D. NIH Recombinant DNA Molecule Program Advisory Committee.
E. NIH Staff.

V.

Footnotes.

VI.

References.

VII.

Members of the Recombinant DNA Molecule Program Advisory Committee.

APPENDICES

A. Statement on the use of *Bacillus subtilis* in recombinant molecule technology.
B. Polyoma and SV40 Virus.
C. Summary of Workshop on the Design & Testing of Safer Prokaryotic Vehicles & Bacterial Hosts for Research on Re-Combinant DNA Molecules.
D. Supplementary Information on Physical Containment (Including Detailed Contents).

I. INTRODUCTION

The purpose of these guidelines is to recommend safeguards for research on recombinant DNA molecules to the National Institutes of Health and to other institutions that support such research. In this context we define recombinant DNAs as molecules that consist of different segments of DNA which have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of the host's genome.

This is the first attempt to provide a detailed set of guidelines for use by study sections as well as practicing scientists for evaluating research on recombinant DNA molecules. We cannot hope to anticipate all possible lines of imaginative research that are possible with this powerful new methodology. Nevertheless, a considerable volume of written and verbal contributions from scientists in a variety of disciplines has been received. In many instances the views presented to us were contradictory. At present, the hazards may be guessed at, speculated about, or voted upon, but they cannot be

known absolutely in the absence of firm experimental data—and, unfortunately, the needed data were, more often than not, unavailable. Our problem then has been to construct guidelines that allow the promise of the methodology to be realized while advocating the considerable caution that is demanded by what we and others view as potential hazards.

In designing these guidelines we have adopted the following principles, which are consistent with the general conclusions that were formulated at the International Conference Center, Pacific Grove, California, in February 1975 (3): (i) There are certain experiments for which the assessed potential hazard is so serious that they are not to be attempted to the present time. (ii) The remainder can be undertaken at the present time provided that the experiment is justifiable on the basis that new knowledge or benefits to humankind will accrue that cannot readily be obtained by use of conventional methodology and that appropriate safeguards are incorporated into the design and execution of the experiment. In addition to an insistence on the practice of good microbiological techniques, these safeguards consist of providing both physical and biological barriers to the dissemination of the potentially hazardous agents. (iii) The level of containment provided by these barriers is to match the estimated potential hazard for each of the different classes of recombinants. For projects in a given class, this level is to be highest at initiation and modified subsequently only if there is a substantiated change in the assessed risk or in the applied methodology. (iv) The guidelines will be subjected to periodic review (at least annually) and modified to reflect improvements in our knowledge of the potential biohazards and of the available safeguards.

In constructing these guidelines it has been necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account existing and anticipated special procedures and information that will allow particular experiments to be carried out under different conditions than indicated here without sacrifice of safety. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that study sections give consideration to such procedures which may allow change in the containment levels recommended here.

It is recommended that all publications dealing with recombinant DNA work include a description of the physical and biological containment procedures practiced, to aid and forewarn others who might consider repeating the work.

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs (4–17). The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which either limit the infectivity of a vector or vehicle (plasmid, bacteriophage or virus) to specific hosts, or its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying different combinations of the physical and biological barriers to a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the guidelines for research on the different kinds of recombinant DNAs (Section III).

A. *Standard practices and training.* The first principle of containment is a strict adherence to good microbiological practices (4–13). Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. This should include at least training in aseptic techniques and instruction in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which an effective vaccine is available, all workers should be immunized. Serological monitoring, where appropriate, should be provided.

B. *Physical containment levels.* A variety of combinations (levels) of special practices, equipment, and laboratory installations that provide additional physical barriers can be formed. For example, 31 combinations are listed in "Laboratory Safety at the Center for Disease Control" (4); four levels are associated with the "Classification of Etiologic Agents on the Basis of Hazard" (5), four levels were recommended in the "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (3); and the National Cancer Institute uses three levels for research on oncogenic viruses (16). We emphasize that these are an aid to, and not a substitute for, good technique. Personnel must be competent in the effective use of all equipment needed for the required containment level as described below. We define only four levels of physical containment here, both because the accuracy with which one can presently assess the biohazards that may result from recombinant DNAs does not warrant a more detailed classification, and because additional flexibility can be obtained by combination of the physical with the biological barriers. Though different in detail, these four levels (P1<P2<P3<P4) approximate those given for human etiologic agents by the Center for Disease Control (i.e., classes 1 through 4; ref. 5), in the Asilomar summary statement (i.e., minimal, low moderate, and high; ref. 3), and by the National Cancer Institute for oncogenic virtues (i.e., low, moderate, and high; ref. 6), as is indicated by the P-number or adjective in the following headings. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of hazardous organisms.

We anticipate, and indeed already know of, procedures (14) which enhance physical containment capability in novel ways. For example, minaturization of screening, handling, and analytical procedures provides substantial containment of a given host-vector system. Thus, such procedures should reduce the need for the standard types of physical containment, and such innovations will be considered by the Recombinant DNA Molecule Program Advisory Committee.

The special practices, equipment and facility installations indicated for each level of physical containment are required for the safety of laboratory workers, other persons, and for the protection of the environment. Optional items have been excluded; only those items deemed absolutely necessary for safety are presented. Thus, the listed requirements present basic safety criteria for each level of physical containment. Other microbiological practices and laboratory techniques which promote safety are to be encouraged. Additional information giving further guidance on physical containment is provided in a supplement to the guidelines (Appendix D).

P1 Level (Minimal). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P1 level is a laboratory that possesses no special engineering design features. It is a laboratory commonly used for microorganisms of no or minimal biohazard under ordinary conditions of handling. Work in this laboratory is generally conducted on open bench tops. Special containment equipment is neither required nor generally available in this laboratory. The laboratory is not separated from the general traffic patterns of the building. Public access is permitted.

The control of biohazards at the P1 level is provided by standard microbiological practices of which the following are examples: (i) Laboratory doors should be kept closed while experiments are in progress. (ii) Work surfaces should be decontaminated daily and following spills of recombinant DNA materials. (iii) Liquid wastes containing recombinant DNA materials should be decontaminated before disposal. (iv) Solid wastes contaminated with recombinant DNA materials should be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. (v) Although pipetting by mouth is permitted, it is preferable that mechanical pipetting devices be used. When pipetting by mouth, cotton-plugged pipettes shall be employed. (vi) Eating, drinking, smoking, and storage of food in the working area should be discouraged. (vii) Facilities to wash hands should be available. (viii) An insect and rodent control program should be provided. (ix) The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

P2 Level (Low). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P2 level is similar in construction and design to the P1 laboratory. The P2 laboratory must have access to an autoclave within the building; it may have a Biological Safety Cabinet.[1] Work which does not produce a considerable aerosol is conducted on the open bench. Although this laboratory is not separated from the general traffic patterns of the building, access to the laboratory is limited when experiments requiring P2 level physical containment are being conducted. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

The P2 laboratory is commonly used for experiments involving microorganisms of low biohazard such as those which have been classified by the Center for Disease Control as Class 2 agents (5).

See footnotes on p. 38459.

The following practices shall apply to all experiments requiring P2 level physical containment: (i) Laboratory doors shall be kept closed while experiments are in progress. (ii) Only persons who have been advised of the potential biohazard shall enter the laboratory. (iii) Children under 12 years of age shall not enter the laboratory. (iv) Work surfaces shall be decontaminated daily and immediately following spills of recombinant DNA materials. (v) Liquid wastes of recombinant DNA materials shall be decontaminated before disposal. (vi) Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. Packaged materials shall be disposed of by incineration or sterilized before disposal by other methods. Contaminated materials that are to be processed and reused (i.e., glassware) shall be decontaminated before removal from the laboratory. (vii) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used. (viii) Eating, drinking, smoking, and storage of food are not permitted in the working area. (ix) Facilities to wash hands shall be available within the laboratory. Persons handling recombinant DNA materials should be encouraged to wash their hands frequently and when they leave the laboratory. (x) An insect and rodent control program shall be provided. (xi) The use of laboratory gowns, coats, or uniforms is required. Such clothing shall not be worn to the lunch room or outside the building. (xii) Animals not related to the experiment shall not be permitted in the laboratory. (xiii) Biological Safety Cabinets [1] and/or other physical containment equipment shall be used to minimize the hazard of aerosolization of recombinant DNA materials from operations or devices that produce a considerable aerosol (e.g., blender, lyophilizer, sonicator, shaking machine, etc.). (xiv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

P3 Level (Moderate). A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P3 level has special engineering design features and physical containment equipment. The laboratory is separated from areas which are open to the general public. Separation is generally achieved by controlled access corridors, air locks, locker rooms or other double-doored facilities which are not available for use by the general public. Access to the laboratory is controlled. Biological Safety Cabinets [1] are available within the controlled laboratory area. An autoclave shall be available within the building and preferably within the controlled laboratory area. The surfaces of walls, floors, bench tops, and ceilings are easily cleanable to facilitate housekeeping and space decontamination.

Directional air flow is provided within the controlled laboratory area. The ventilation system is balanced to provide for an inflow of supply air from the access corridor into the laboratory. The general exhaust air from the laboratory is discharged outdoors and so dispersed to the atmosphere as to prevent reentry into the building. No recirculation of the exhaust air shall be permitted without appropriate treatment.

No work in open vessels involving hosts or vectors containing recombinant DNA molecules requiring P3 physical containment is conducted on the open bench. All such procedures are confined to Biological Safety Cabinets.[2]

The following practices shall apply to all experiments requiring P3 level physical containment: (i) The universal biohazard sign is required on all laboratory access doors. Only persons whose entry into the laboratory is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the potential biohazards before entry and they shall comply with posted entry and exit procedures. Children under 12 years of age shall not enter the laboratory. (ii) Laboratory doors shall be kept closed while experiments are in progress. (iii) Biological Safety Cabinets [1] and other physical containment equipment shall be used for all procedures that produce aerosols of recombinant DNA materials (e.g., pipetting, plating, flaming, transfer operations, grinding, blending, drying, sonicating, shaking, etc.). (iv) The work surfaces of Biological Safety Cabinets [1] and other equipment shall be decontaminated following the completion of the experimental activity contained within them. (v) Liquid wastes containing recombinant DNA materials shall be decontaminated before disposal. Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. Packaged material shall be sterilized before disposal. Contaminated materials that are to be processed and reused (i.e., glassware) shall be sterilized in the controlled laboratory area or placed in a durable leak-proof container before removal from the controlled laboratory area. This container shall be sterilized before the materials are processed, (vi) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used, (vii) Eating, drinking, smoking, and storage of food are not permitted in the laboratory. (ix) Facilities to wash hands shall be available within the laboratory. Persons shall wash hands after experiments involving recombinant DNA materials and before leaving the laboratory. (x) An insect and rodent control program shall be provided. (xi) Laboratory clothing that protects street clothing (i.e., long sleeve solid-front or wrap-around gowns, no-button or slipover jackets, etc.) shall be worn in the laboratory. FRONT-BUTTON LABORATORY COATS ARE UNSUITABLE. Gloves shall be worn when handling recombinant DNA materials. Provision for laboratory shoes is recommended. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry. (xii) Raincoats, overcoats, topcoats, coats, hats, caps, and such street outerwear shall not be kept in the laboratory. (xiii) Animals and plants not related to the experiment shall not be permitted in the laboratory. (xiv) Vacuum lines shall be protected by filters and liquid traps. (xv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xvi) If experiments of lesser biohazard potential are to be conducted in the same laboratory concurrently with experiments requiring P3 level physical containment they shall be conducted only in accordance with all P3 level requirements. (xvii) Experiments requiring P3 level physical containment can be conducted in laboratories where the directional air flow and general exhaust air conditions described above cannot be achieved, provided that this work is conducted in accordance with all other requirements listed and is contained in a Biological Safety Cabinet [1] with attached glove ports and gloves. All materials before removal from the Biological Safety Cabinet [1] shall be sterilized or transferred to a non-breakable, sealed container, which is then removed from the cabinet through a chemical decontamination tank, autoclave, ultraviolet air lock, or after the entire cabinet has been decontaminated.

P4 Level High. Experiments involving recombinant DNA molecules requiring physical containment at the P4 level shall be confined to work areas in a facility of the type designed to contain microorganisms that are extremely hazardous to man or may cause serious epidemic disease. The facility is either a separate building or it is a controlled area, within a building, which is completely isolated from all other areas of the building. Access to the facility is under strict control. A specific facility operations manual is available. Class III Biological Safety Cabinets [1] are available within work areas of the facility.

A P4 facility has engineering features which are designed to prevent the escape of microorganisms to the environment (14, 15, 16, 17). These features include: (i) Monolithic walls, floors, and ceilings in which all penetrations such as for air ducts, electrical conduits, and utility pipes are sealed to assure the physical isolation of the work area and to facilitate housekeeping and space decontamination; (ii) air locks through which supplies and materials can be brought safely into the facility; (iii) contiguous clothing change and shower rooms through which personnel enter into and exit from the facility; (iv) double-door autoclaves to sterilize and safely remove wastes and other materials from the facility; (v) a biowaste treatment system to sterilize liquid effluents if facility drains are installed; (vi) a separate ventilation system which maintains negative air pressures and directional air flow within the facility; and (vii) a treatment system to decontaminate exhaust air before it is dispersed to the atmosphere. A central vacuum utility system is not encouraged; if one is installed, each branch line leading to a laboratory shall be protected by a high efficiency particulate air filter.

The following practices shall apply to all experiments requiring P4 level physical containment: (i) The universal biohazard sign is required on all facility access doors and all interior doors to individual laboratory rooms where experiments are conducted. Only persons whose entry into the facility or individual laboratory rooms is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the potential biohazards and instructed as to the appropriate safeguards to ensure their safety before entry. Such persons shall comply with the instructions and all other posted entry and exit procedures. Under no condition shall children under 16 years of age be allowed entry. (ii) Personnel shall enter into and exit from the facility only through the clothing change and shower rooms. Personnel shall shower at each exit from the facility. The air locks shall not be used for personnel entry or exit except for emergencies. (iii) Street clothing shall be removed in the outer facility side of the clothing change area and kept there. Complete laboratory clothing including undergarments, pants and shirts or jumpsuits, shoes, head cover, and gloves shall be provided and used by all persons who enter into the facility shall be placed in an entry air be stored in lockers provided for this purpose or discarded into collection hampers before personnel enter into the shower area. (iv) Supplies and materials to be taken into the facility shall be placed in an entry air lock. After the outer door (opening to the corridor outside of facility) has been secured, personnel occupying the facility shall retrieve the supplies and materials by opening the interior air lock door. This door shall be secured after supplies and materials are brought into the facility. (v) Doors to laboratory rooms within the facility shall be kept closed while experiments are in progress. (vi) Experimental procedures requiring P4 level physical containment shall be confined to Class III Biological Safety Cabinets.[2] All materials, before removal from these cabinets, shall be sterilized or transferred to a non-breakable sealed container, which is then removed from the system through a chemical decontaminated tank, autoclave, or after the

See footnotes on p. 38459.

entire system has been decontaminated. (vii) No materials shall be removed from the facility unless they have been sterilized or decontaminated in a manner to prevent the release of agents requiring P4 physical containment. All wastes and other materials and equipment not damaged by high temperature or steam shall be sterilized in the double-door autoclave. Biological materials to be removed from the facility shall be transferred to a non-breakable sealed container which is then removed from the facility through a chemical decontamination tank or a chamber designed for gas sterilization. Other materials which may be damaged by temperature or steam shall be sterilized by gaseous or vapor methods in an air lock or chamber designed for this purpose. (viii) Eating, drinking, smoking, and storage of food are not permitted in the facility. Foot-operated water fountains located in the facility corridors are permitted. Separate potable water piping shall be provided for these water fountains. (ix) Facilities to wash hands shall be available within the facility. Persons shall wash hands after experiments. (x) An insect and rodent control program shall be provided. (xi) Animals and plants not related to the experiment shall not be permitted in the facility. (xii) If a central vacuum system is provided, each vacuum outlet shall be protected by a filter and liquid trap in addition to the branch line HEPA filter mentioned above. (xiii) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xiv) If experiments of lesser biohazard potential are to be conducted in the facility concurrently with experiments requiring P4 level containment, they shall be confined in Class I or Class II Biological Safety Cabinets[3] or isolated by other physical containment equipment. Work surfaces of Biological Safety Cabinets[3] and other equipment shall be decontaminated following the completion of the experimental activity contained within them. Mechanical pipetting devices shall be used. All other practices listed above with the exception of (vi) shall apply.

C. *Shipment.* To protect product, personnel, and the environment, all recombinant DNA material will be shipped in containers that meet the requirements issued by the U.S. Public Health Service (Section 72.25 of Part 72, Title 42, Code of Federal Regulations), Department of Transportation (Section 173.387 (b) of Part 173, Title 49, Code of Federal Regulations) and the Civil Aeronautics Board (C.A.B. No. 82, Official Air Transport Restricted Articles Tariff No. 6-D) for shipment of etiologic agents. Labeling requirements specified in these Federal regulations and tariffs will apply to all viable recombinant DNA materials in which any portion of the material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25. Additional information on packing and shipping is given in a supplement to the guidelines (Appendix D, part X).

D. *Biological containment levels.* Biological barriers are specific to each host-vector system. Hence the criteria for this mechanism of containment cannot be generalized to the same extent as for physical containment. This is particularly true at the present time when our experience with existing host-vector systems and our predictive knowledge about projected systems are sparse. The classification of experiments with recombinant DNAs that is necessary for the construction of the experimental guidelines (Section III) can be accomplished with least confusion if we use the host-vector system as the primary element and the source of the inserted DNA as the secondary element in the classification. It is therefore convenient to specify the nature of the biological containment under host-vector headings such as those given below for *Escherichia coli* K-12.

III. Experimental Guidelines

A general rule that, though obvious, deserves statement is that the level of containment required for any experiment on DNA recombinants shall never be less than that required for the most hazardous component used to construct and clone the recombinant DNA (i.e. vector, host, and inserted DNA). In most cases the level of containment will be greater, particularly when the recombinant DNA is formed from species that ordinarily do not exchange genetic information. Handling the purified DNA will generally require less stringent precautions than will propagating the DNA. However, the DNA itself should be handled at least as carefully as one would handle the most dangerous of the DNAs used to make it.

The above rule by itself effectively precludes certain experiments—namely, those in which one of the components is in Class 5 of the "Classification of Etiologic Agents on the Basis of Hazard" (5), as these are excluded from the United States by law and USDA administrative policy. There are additional experiments which may engender such serious biohazards that they are not to be performed at this time. These are considered prior to presentation of the containment guidelines for permissible experiments.

A. *Experiments that are not to be performed.* We recognize that it can be argued that certain of the recombinants placed in this category could be adequately contained at this time. Nonetheless, our estimates of the possible dangers that may ensue if that containment fails are of such magnitude that we consider it the wisest policy to at least defer experiments on these recombinant DNAs until there is more information to accurately assess that danger and to allow the construction of more effective biological barriers. In this respect, these guidelines are more stringent than those initially recommended (1).

The following experiments are not to be initiated at the present time: (i) Cloning of recombinant DNAs derived from the pathogenic organisms in Classes 3, 4, and 5 of "Classification of Etiologic Agents on the Basis of Hazard" (5), or oncogenic viruses classified by NCI as moderate risk (6), or cells known to be infected with such agents, regardless of the host-vector system used. (ii) Deliberate formation of recombinant DNAs containing genes for the biosynthesis of potent toxins (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.). (iii) Deliberate creation from plant pathogens of recombinant DNAs that are likely to increase virulence and host range. (iv) Deliberate release into the environment of any organism containing a recombinant DNA molecule. (v) Transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

In addition, at this time large-scale experiments (e.g., more than 10 liters of culture) with recombinant DNAs known to make harmful products are not to be carried out. We differentiate between small- and large-scale experiments with such DNAs because the probability of escape from containment barriers normally increases with increasing scale. However, specific experiments in this category that are of direct societal benefit may be excepted from this rule if special biological containment precautions and equipment designed for large-scale operations are used, and provided that these experiments are expressly approved by the Recombinant DNA Molecule Program Advisory Committee of NIH.

B. *Containment guidelines for permissible experiments.* It is anticipated that most recombinant DNA experiments initiated before these guidelines are next reviewed (i.e., within the year) will employ *E. coli* K-12 host-vector systems. These are also the systems for which we have the most experience and knowledge regarding the effectiveness of the containment provided by existing hosts and vectors necessary for the construction of more effective biological barriers.

For these reasons, *E. coli* K-12 appears to be the system of choice at this time, although we have carefully considered arguments that many of the potential dangers are compounded by using an organism as intimately connected with a man as is *E. coli*. Thus, while proceeding cautiously with *E. coli*, serious efforts should be made toward developing alternate host-vector systems; this subject is discussed in considerable detail in Appendix A.

We therefore consider DNA recombinants in *E. coli* K-12 before proceeding to other host-vector systems.

1. *Biological containment criteria using E. coli K-12 host-vectors EK1 host-vectors.* These are host-vector systems that can be estimated to already provide a moderate level of containment, and include most of the presently available systems. The host is always *E. coli* K-12, and the vectors include nonconjugative plasmids [e.g., pSC101, ColE1 or derivatives thereof (19-26)] and variants of bacteriophage γ (27-29).

The *E. coli* K-12 nonconjugative plasmid system is taken as an example to illustrate the approximate level of containment referred to here. The available data from experiments involving the feeding of bacteria to humans and calves (30-32) indicate that *E. coli* K-12 did not usually colonize the normal bowel, and exhibited little, if any, multiplication while passing through the alimentary tract even after feeding high doses (i.e., 10^9 to 10^{10} bacteria per human or calf). However, general extrapolation of these results may not be warranted because the implantation of bacteria into the intestinal tract depends on a number of parameters, such as the nature of the intestinal flora present in a given individual and the physiological state of the inoculum. Moreover, since viable *E. coli* K-12 can be found in the feces after humans are fed 10^8 bacteria in broth (30) or 3×10^4 bacteria protected by suspension in milk (31), transductional and conjugational transfer of the plasmid vectors from *E. coli* K-12 to resident bacteria in the fecal matter before and after excretion must also be considered.

The nonconjugative plasmid vectors cannot promote their own transfer, but require the presence of a conjugative plasmid for mobilization and transfer to other bacteria. When present in the same cell with derepressed conjugative plasmids such as F or R*drd*19, the nonconjugative ColE1, ColE1-trp and pSC101 plasmids are transferred to suitable recipient strains under ideal laboratory conditions at frequencies of about 0.5, 10^{-4} to 10^{-5}, and 10^{-4} per donor cell, respectively. These frequencies are reduced by another factor of 10^2 to 10^4 if the conjugative plasmid employed is repressed with respect to expression of donor fertility.

The experimental transfer system which most closely resembles nonconjugative plasmid transfer in nature is a triparental mating. In such matings, the bacterial cell possessing the nonconjugative plasmid must first acquire a conjugative plasmid from another cell before it can transfer the nonconjugative plasmid to a secondary recipient. With ColE1, the frequencies of transfer are 10^{-2} and 10^{-4} to 10^{-5} when using conjugative plasmid donors possessing derepressed and repressed plasmids, respectively. Mobilization of ColE1-trp and pSC101 under similar laboratory conditions is so low as to be usually undetectable (33). Since most conjugative plasmids in nature are repressed for expres-

sion of donor fertility, the frequency at which nonconjugative plasmids are mobilized and transferred by this sequence of events *in vivo* is difficult to estimate. However, in calves fed on an antibiotic-supplemented diet, it has been estimated that such triparental nonconjugative R plasmid transfer occurs at frequencies of no more than 10^{-10} to 10^{-13} per 24 hours per calf (32). In terms of considering other means for plasmid transmission in nature, it should be noted that transduction does operate *in vivo* for *Staphylococcus aureus* (34) and probably for *E. coli* as well. However, no data are available to indicate the frequencies of plasmid transfer *in vivo* by either transduction or transformation.

These observations indicate the low probabilities for possible dissemination of such plasmid vectors by accidental ingestion, which would probably involve only a few hundred or thousand bacteria provided that at least the standard practices (Section II–A above) are followed, particularly the avoidance of mouth pipetting. The possibility of colonization and hence of transfer are increased, however, if the normal flora in the bowel is disrupted by, for example, antibiotic therapy (35). For this reason, persons receiving such therapy must not work with DNA recombinants formed with any *E. coli* K–12 host-vector system during the therapy period and for seven days thereafter; similarly, persons who have achlorhydria or who have had surgical removal of part of the stomach or bowel should avoid such work, as should those who require large doses of antacids.

The observations on the fate of *E. coli* K–12 in the human alimentary tract are also relevant to the containment of recombinant DNA formed with bacteriophage λ variants. Bacteriophage can escape from the laboratory either as mature infectious phage particles or in bacterial host cells in which the phage genome is carried as a plasmid or prophage. The fate of *E. coli* K–12 host cells carrying the phage genome as a plasmid or prophage is similar to that for plasmid-containing host cells as discussed above. The survival of the λ phage genome when released as infectious particles depends on their stability in nature, their infectivity and on the probability of subsequent encounters with naturally occurring λ-sensitive *E. coli* strains. Although the probability of survival of λ and its infection of resident intestinal *E. coli* in animals and humans has not been measured, it is estimated to be small given the high sensitivity of λ to the low pH of the stomach, the insusceptibility to λ infection of smooth *E. coli* cells (the type that normally resides in the gut), the infrequency of naturally occurring λ-sensitive *E. coli* (36) and the failure to detect infective λ particles in human feces after ingestion of up to 10^{11} λ particles (37).

Moreover, λ particles are very sensitive to desiccation.

Establishment of λ as a stable lysogen is a frequent event (10^{0} to 10^{-1}) for the att^{+} int^{+} cI^{+} phage so that this mode of escape would be the preponderant laboratory hazard; however, most EK1 λ vectors currently in use lack the att and int functions (27–29) thus reducing the probability of lysogenization to about 10^{-2} to 10^{-4} (38–40). The frequency for the conversion of a λ to a plasmid state for persistence and replication is also only about 10^{-4} (41). Moreover, the routine treatment of phage lysates with chloroform (42) should eliminate all surviving bacteria including lysogens and λ plasmid carriers. Lysogenization could also occur when an infectious λ containing cloned DNA infects a λ-sensitive cell in nature, and recombines with a resident lambdoid prophage. Although λ-sensitive *E. coli* strains seem to be rare, a significant fraction do carry lambdoid prophages (43–44) and thus this route of escape should be considered.

While not exact, the estimates for containment afforded by using these host-vectors are at least as accurate as those for physical containment, and are sufficient to indicate that currently employed plasmid and λ vector systems provide a moderate level of biological containment. Other nonconjugative plasmids and bacteriophages that, in association with *E. coli* K–12 can be estimated to provide the same approximate level of moderate containment are included in the EK1 class.

EK2 host-vectors. These are host-vector systems that have been genetically constructed and shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. The genetic modifications of the *E. coli* K–12 host and/or the plasmid or phage vector should not permit survival of a genetic marker carried on the vector, preferably a marker within an inserted DNA fragment, in other than specially designed and carefully regulated laboratory environments at a frequency greater than 10^{-8}. This measure of biological containment has been selected because it is a measurable entity. Indeed, by testing the contributions of pre-existing and newly introduced genetic properties of vectors and hosts, individually or in various combinations, it should be possible to estimate with considerable precision, that the specially designed host-vector system can provide a margin of biological containment in excess of that required. For the time being, no host-vector system will be considered to be a bona fide EK2 host-vector system until it is so certified by the NIH Recombinant DNA Molecule Program Advisory Committee.

For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^{8} host cells should be able to perpetuate the vector and/or a cloned DNA fragment under non-permissive conditions designed to represent the natural environment either by survival of the original host or as a consequence of transmission of the vector and/or a cloned DNA fragment by transformation, transduction or conjugation to a host with properties common to those in the natural environment.

In terms of potential EK2 plasmid-host systems, the following types of genetic modifications should reduce survival of cloned DNA. The examples given are for illustrative purposes and should not be contrued to encompass all possibilities. The presence of the non-conjugative plasmids ColE1-trp and pS101 in an *E. coli* K–12 strain possessing a mutation eliminating host-controlled restriction and modification (hsdS) results in about 10^{3}-fold reduction in mobilization to restriction-proficient recipients. The combination of the dapD8, ΔbioH-asd, Δgal-chl^{r} and rfb mutations in *E. coli* K–12 results in no detectable survivors in feces of rats following feeding by stomach tube of 10^{10} cells in milk and similarly leads to complete lysis of cells suspended in broth medium lacking diaminopimelic acid. *E. coli* K–12 strains with ΔthyA and deoC(dra) mutations undergo thymineless death in growth medium lacking thymine and give a 10^{5}-fold reduced survival during passage through the rat intestine compared to wild-type thy^{+} *E. coli* K–12. (However, the ΔthyA mutation alone or in combination with a deoB(drm) mutation only reduces *in vivo* survival by a factor of 10^{2}.) Other host mutations, as yet untested, that might further reduce survival of the plasmid-host system or reduce plasmid transmission are: the combination polA(TS) recA(TS) ΔthyA which might interfere with ColE1 replication and lead to DNA degradation at body temperatures; Con mutations that reduce the ability of conjugative plasmids to enter the plasmid-host complex and thus should reduce mobilization of the cloned DNA to other strains; and mutations that confer resistance to known transducing phages. Mutations can also be introduced into the plasmid to cause it to be dependent on a specific host, to make its replication thermosensitive and/or to endow it with a killer capability such that all cells (other than its host) into which it might be transferred will not survive.

In the construction of EK2 plasmid-host systems it is important to use the most stable mutations available, preferably deletions. Obviously, the presence of all mutations contributing to higher degrees of biological containment must be verified periodically by appropriate tests. In testing the level of biological containment afforded by a proposed EK2 plasmid-host system, it is important to design relevant tests to evaluate the survival of the vector and/or a cloned DNA fragment under conditions that are possible in nature and that are also most advantageous for its perpetuation. For example, one might conduct a triparental mating with a primary donor possessing a redepressed F-type or I-type conjugative plasmid, the safor host with ΔbioH-asd, dapD8, Δgal-chl^{r}, rfb, ΔthyA, deoC, trp and hsdS mutations and a plasmid vector carrying an easily detectable marker such as for ampicillin resistance or an inserted gene such as trp^{+}, and a secondary recipient that is Su^{+} hsdS trp (i.e., permissive for the recombinant plasmid). Such matings would be conducted in a medium lacking diamino pimelic acid and thymine and survival of the Ap^{r} or trp marker in any of the three strains followed as a function of time. Survival of the vector and/or a cloned marker by transduction could also be evaluated by introducing a known generalized transducing phage into the system. Similar experiments should also be done using a secondary recipient that is restrictive for the plasmid vector as well as with primary donors possessing repressed conjugative plasmids with incompatibility group properties like those commonly found in enteric microorganisms. Since a common route of escape of plasmid-host systems in the laboratory might be by accidental ingestion, it is suggested that the same types of experiments be conducted in suitable animal-model systems. In addition to these tests on survival of the vector and/or a cloned DNA fragment, it would be useful to determine the survival of the host strain under nongrowth conditions such as in water and as a function of drying time after a culture has been spilled on a lab bench.

For EK2 host-vector systems in which the vector is a phage, no more than one in 10^{8} phage particles should be able to perpetuate itself and/or a cloned DNA fragment under non-permissive conditions designed to represent the natural environment either (a) as a prophage or plasmid in the laboratory host used for phage propagation or (b) by surviving in natural environments and transferring itself and/or a cloned DNA fragment to a host (or its resident lambdoid prophage) with properties common to those in the natural environment.

In terms of potential EK2 λ-host systems, the following types of genetic modification should reduce survival of cloned DNA. The examples given are for illustrative purposes and should not be construed to encompass all possibilities. The probability of establishing λ lysogeny in the normal laboratory host should be reduced by removal of the phage att site, the Int function, the repressor gene(s) and adding virulence-enhancing mutations. The frequency of plasmid formation, although normally already less than 10^{-8}, could be further reduced by defects in

the P_R–Q region, including mutations such as *Sir–s*, *cro*(TS), *cI17*, *rt*^{1}, O(TS), P(TS), and *nin*. Moreover, chloroform treatment used routinely following cell lysis would reduce the number of surviving cells, including possible lysogens or plasmid carriers, by more than 10^3. The host may also be modified by deletion of the host *λatt* site and inclusion of one or more of the mutations described above for plasmid-host systems to further reduce the chance of formation and survival of any lysogen or plasmid carrier cell.

The survival of escaping phage and the chance of encountering a sensitive host in nature are very low, as discussed for EK1 systems. The infectivity of the phage particles could be further reduced by introducing mutations (e.g., suppressed ambers) which would make the phage particles extremely unstable except under special laboratory conditions (e.g., high concentrations of salts or putrescine). Another means would be to make the phage itself a two-component system, by eliminating the tail genes and reproducing the phage as heads packed with DNA; when necessary and under specially controlled conditions, these heads could be made infective by adding tail preparations. An additional safety factor in this regimen is the extreme instability of the heads, unless they are stored in 10mM putrescine, a condition easy to obtain in the laboratory but not in nature. The propagation of the escaping phage in nature could further be blocked by adding various conditional mutations which would permit growth only under special laboratory conditions or in a special permissive laboratory host with suppressor or *gro*-type (*mop, dnaB, rpoB*) mutations. An additional safety feature would be the use of an r- m- (*hsdS*) laboratory host, which produces phage with unmodified DNA which should be restricted in r+ m+ bacteria that are probably prevalent in nature. The likelihood of recombination between the λ vector and lambdoid prophages which are present in some *E. coli* strains might be reduced by elimination of the Red function and the presence of the recombination-reducing Gam function together with mutations contributing to the high lethality of the λ phage. However, these second-order precautions might not be relevant if the stability and infectivity of the escaping λ particles are reduced by special mutations or by propagating the highly unstable heads.

Despite multiple mutations in the phage vectors and laboratory hosts, the yield of phage particles under suitable laboratory conditions should be high (10^{12} —10^{13} particles/ml). This permits phage propagation in relatively small volumes and constitutes an additional safety feature.

The phenotypes and genetic stabilities of the mutations and chromosome alterations included in these λ-host systems indicate that containment well in excess of the required 10^{-8} or lower *survival frequency* for the λ vector with or without a cloned DNA fragment should be attained. Obviously the presence of all mutations contributing to this high degree of biological containment must be verified periodically by appropriate tests. Laboratory tests should be performed with the bacterial host to measure all possible routes of escape such as the frequency of lysogen formation, the frequency of plasmid formation and the survival of the lysogen or carrier bacterium. Similarly, the potential for perpetuation of a cloned DNA fragment carried by infectious phage particles can be tested by challenging typical wild-type *E. coli* strains or a λ-sensitive nonpermissive laboratory K–12 strain, especially one lysogenic for a lambdoid phage.

In view of the fact that accurate assessment of the probabilities for escape of infectious λ grown on r- m- Su+ hosts is dependent upon the frequencies of r+, Su+, and λ-sensitive strains in nature, investigators need to screen *E. coli* strains for these properties. These data will also be useful in predicting frequencies of successful escape of plasmid cloning vectors harbored in r+ m+ Su+ strains.

When any investigator has obtained data on the level of containment provided by a proposed EK2 system, these should be reported as rapidly as possible to permit general awareness and evaluation of the safety features of the new system. Investigators are also encouraged to make such new safer cloning systems generally available to other scientists. NIH will take appropriate steps to aid in the distribution of these safer vectors and hosts.

EK3 host-vectors. These are EK2 systems for which the specified containment shown by laboratory tests has been independently confirmed by appropriate tests in animals, including humans or primates, and in other relevant environments in order to provide additional data to validate the levels of containment afforded by the EK2 host-vector systems. Evaluation of the effects of individual or combinations of mutations contributing to the biological containment should be performed as a means to confirm the degree of safety provided and to further advance the technology of developing even safer vectors and hosts. For the time being, no host-vector system will be considered to be a bona fide EK3 host-vector system, until it is so certified by the NIH Recombinant DNA Molecule Program Advisory Committee.

2. Classification of experiments using the E. coli K–2 containment systems. In the following classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimums. Higher levels of biological containment (EK3→EK2→EK1) are to be used if they are available and are equally appropriate for the purposes of the experiment.

<a>*Shotgun experiments.* These experiments involve the production of recombinant DNAs between the vector and the total DNA or (preferably) any partially purified fraction thereof from the specified cellular source.

(i) *Eukaryotic DNA recombinants—Primates.* P3 physical containment+an EK3 host-vector, or P4 physical containment+an EK2 host-vector, except for DNA from uncontaminated embryonic tissue or primary tissue cultures therefrom, and germ-line cells for which P3 physical containment+an EK2 host-vector can be used. The basis for the lower estimated hazard in the case of DNA from the latter tissues (if freed of adult tissue) is their relative freedom from horizontally acquired adventitious viruses.

Other mammals. P3 physical containment +an EK2 host-vector.

Birds. P3 physical containment+an EK1 host-vector.

Cold-blooded vertebrates. P2 physical containment+an EK2 host-vector except for embryonic or germ-line DNA which require P2 physical containment+an EK1 host-vector. If the eukaryote is known to produce a potent toxin, the containment shall be increased to P3+EK2.

Other cold-blooded animals and lower eukaryotes. This large class of eukaryotes is divided into the following two groups:

(*i*) Species that are known to produce a potent toxin or are known pathogens (i.e., an agent listed in Class 2 of ref. 5 or a plant pathogen) or are known to carry such pathogenic agents must use P3 physical containment+an EK2 host-vector. Any species that has a demonstrated capacity for carrying particular pathogenic agents is included in this group unless it has been shown that those organisms used as the source of DNA do not *contain these agents; in this case they may* be placed in the second group.

(2) The remainder of the species in this class can use P2+EK1. However, any insect in this group should have been grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA.

Plants. P2 physical containment+an EK1 host-vector. If the plant carries a known pathogenic agent or makes a product known to be dangerous to any species, the containment must be raised to P3 physical containment+an EK2 host-vector.

(ii) *Prokaryotes DNA recombinants—Prokaryotes that exchange genetic information with E. coli*[7].

The level of physical containment is directly determined by the rule of the most dangerous component (see Introduction to Section III). Thus P1 conditions can be used for DNAs from those bacteria in Class 1 of ref. 5, ("Agents of no or minimal hazard " " "") which naturally exchange genes with *E. coli*; and P2 conditions should be used for such bacteria if they fall in Class 2 of ref. 5 ("Agents of ordinary potential hazard " " "), or plant pathogens or symbionts. EK1 host-vectors can be used for all experiments requiring only P1 physical containment; in fact, experiments in this category can be performed with *E. coli* K–12 vectors exhibiting a lesser containment (e.g., conjugative plasmids) than EK1 vectors. Experiments with DNA from species requiring P2 physical containment which are of low pathogenicity (for example, enteropathogenic *Escherichia coli, Salmonella typhimurium,* and *Klebsiella pneumoniae*) can use EK1 host-vectors, but those of moderate pathogenicity (for example, *Salmonella typhi, Shigella dysenteriae* type I, and *Vibrio cholerae*) must use EK2 host-vectors.[8] A specific example of an experiment with a plant pathogen requiring P2 physical containment+an EK2 host-vector, would be cloning the tumor gene of *Agrobacterium tumefaciens.*

Prokaryotes that do not exchange genetic information with E. coli. The minimum containment conditions for this class consist of P2 physical containment+an EK2 host-vector or P3 physical containment+an EK1 host-vector, and apply when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal. Experiments with DNAs from pathogenic species (Class 2 ref. 5 plus plant pathogens) must use P3+EK2.

(iii) *Characterized clones of DNA recombinants derived from shotgun experiments.* When a cloned DNA recombinant has been rigorously characterized[4] and there is sufficient evidence that it is free of harmful genes,[4] then experiments involving this recombinant DNA can be carried out under *P1+EK1 conditions if the inserted DNA is* from a species that exchanges genes with *E. coli*, and under P2+EK1 conditions if not.

<b> *Purified cellular DNAs other than plasmids, bacteriophages, and other viruses.* The formation of DNA recombinants from cellular DNAs that have been enriched[4] by physical and chemical techniques (i.e., not by cloning) and which are free of harmful genes can be carried out under lower containment conditions than used for the corresponding shotgun experiment. In general, the containment can be decreased one step in physical containment (P4→P3→P2→P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (EK3→EK2→EK1) while maintaining the specified physical containment—provided that the

See footnotes on p. 38459.

new condition is not less than that specified above for characterized clones from shotgun experiments (Section <a> —iii).

<c> Plasmids, bacteriophages, and other viruses. Recombinants formed between EK-type vectors and other plasmid or virus DNAs have in common the potential for acting as double vectors because of the replication functions in these DNAs. The containment conditions given below apply only to propagation of the DNA recombinants in *E. coli* K-12 hosts. They do not apply to other hosts where they may be able to replicate as a result of functions provided by the DNA inserted into the EK vectors. These are considered under other host-vector systems.

(i) *Animal viruses.* P4+EK2 or P3+EK3 shall be used to isolate DNA recombinants that include all or part of the genome of an animal virus. This recommendation applies not only to experiments of the "shotgun" type but also to those involving partially characterized subgenomic segments of viral DNAs (for example, the genome of defective viruses, DNA fragments isolated after treatment of viral genomes with restriction enzymes, etc). When cloned recombinants have been shown by suitable biochemical and biological tests to be free of harmful regions, they can be handled in P3+EK2 conditions. In the case of DNA viruses, harmless regions include the late region of the genome; in the case of DNA copies of RNA viruses, they might include the genes coding for capsid proteins or envelope proteins.

(ii) *Plant viruses.* P3+EK1 or P2+EK2 conditions shall be used to form DNA recombinants that include all or part of the genome of a plant virus.

(iii) *Eukaryotic organelle DNAs.* The containment conditions given below apply only when the organelle DNA has been purified from isolated organelles. Mitochondrial DNA from primates: P3+EK1 or P2+EK2. Mitochondrial or chloroplast DNA from other eukaryotes: P2+EK1. Otherwise, the conditions given under shotgun experiments apply.

(iv) *Prokaryotic plasmid and phage DNAs. Plasmids and phage from hosts that exchange genetic information with E. coli.* Experiments with DNA recombinants formed from plasmids or phage genomes that have not been characterized with regard to presence of harmful genes or are known to contribute significantly to the pathogenicity of their normal hosts must use the containment conditions specified for shotgun experiments with DNAs from the respective host. If the DNA recombinants are formed from plasmids or phage that are known not to contain harmful genes, or from purified and characterized plasmid or phage DNA segments known not to contain harmful genes, the experiments can be performed with P1 physical containment+an EK1 host-vector.

Plasmids and phage from hosts that do not exchange genetic information with E. coli. The rules for shotgun experiments with DNA from the host apply to their plasmids or phages. The minimum containment conditions for this category (P2+EK2, or P2+EK1) can be used for plasmid and phage, or for purified and characterized segments of plasmid and phage DNAs, when the risk that the recombinant DNAs will increase the pathogenicity or ecological potential of the host is judged to be minimal.

NOTE: Where applicable, cDNAs (i.e., complementary DNAs) synthesized in vitro from cellular or viral RNAs are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under <c>, shotgun experiments; cDNAs formed from purified and characterized RNAs are included under <b>; cDNAs formed from viral RNAs are included under <c>; etc.

3. *Experiments with other prokaryotic host-vectors.* Other prokaryotic host-vector systems are at the speculative, planning, or developmental stage, and consequently do not warrant detailed treatment here at this time. However, the containment criteria for different types of DNA recombinants formed with *E. coli* K-12 host-vectors can, with the aid of some general principles given here, serve as a guide for containment conditions with other host-vectors when appropriate adjustment is made for their different habitats and characteristics. The newly developed host-vector systems should offer some distinct advantage over the *E. coli* K-12 host-vectors—for instance, thermophilic organism or other host-vectors whose major habitats do not include humans and/or economically important animals and plants. In general, the strain of any prokaryotic species used as the host is to conform to the definition of Class 1 etiologic agents given in ref. 5 (i.e., "Agents for no or minimal hazard * * *"), and the plasmid or phage vector should not make the host more hazardous. Appendix A gives a detailed discussion of the *B. subtilis* system, the most promising alternative to date.

At the initial stage, the host-vector must exhibit at least a moderate level of biological containment comparable to EK1 systems, and should be capable of modification to obtain high levels of containment comparable to EK2 and EK3. The type of confirmation test(s) required to move a host-vector from an EK2-type classification to an EK3-type will clearly depend upon the preponderant habitat of the host-vector. For example, if the unmodified host-vector propagates mostly in, on, or around higher plants, but not appreciably in warm-blooded animals, modification should be designed to reduce the probability that the host-vector can escape to and propagate in, on, or around such plants, or transmit recombinant DNA to other bacterial hosts that are able to occupy these ecological niches, and it is these lower probabilities which must be confirmed. The following principles are to be followed in using the containment criteria given for experiments with *E. coli* K-12 host-vectors as a guide for other prokaryotic systems. Experiments with DNA from prokaryotes (and their plasmids or viruses) are classified according to whether the prokaryote in question exchanges genetic information with the host-vector or not, and the containment conditions given for these two classes with *E. coli* K-12 host-vectors applied. Experiments with recombinants between plasmid or phage vectors and DNA that extends the range of resistance of the recipient species to therapeutically useful drugs must use P3 physical containment + a host-vector comparable to EK1 or P2 physical containment + a host-vector comparable to EK2. Transfer of recombinant DNA to plant pathogens can be made safer by using nonreverting, doubly auxotrophic, non-pathogenic variants. Experiments using a plant pathogen that affects an element of the local flora will require more stringent containment than if carried out in areas where the host plant is not common.

Experiments with DNAs from eukaryotes (and their plasmids or viruses) can also follow the criteria for the corresponding experiments with *E. coli* K-12 vectors if the major habitats of the given host-vector overlap those of *E. coli*. If the host-vector has a major habitat that does not overlap those of *E. coli* (e.g., root nodules in plants), then the containment conditions for some eukaryotic recombinant DNAs need to be increased (for instance, higher plants and their viruses in the preceding example) while others can be reduced.

4. *Experiments with eukaryotic host-vectors.—<a> Animal host-vector systems.* Because host cell lines generally have little if any capacity for propagation outside the laboratory, the primary focus for containment is the vector, although cells should also be derived from cultures expected to be of minimal hazard. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by humans; thus vectors should be chosen that have little or no ability to replicate in human cells. To be used as a vector in a eukaryotic host, a DNA molecule needs to display all of the following properties:

(1) It shall not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture.

(2) Its functional anatomy should be known—that is, there should be a clear idea of the location within the molecule of:

(a) The sites at which DNA synthesis originates and terminates.

(b) The sites that are cleaved by restriction endonucleases.

(c) The template regions for the major gene products.

(3) It should be well studied genetically. It is desirable that mutants be available in adequate number and variety, and that quantitative studies of recombination have been performed.

(4) The recombinant must be defective, that is, its propagation as a virus is dependent upon the presence of a complementing helper genome. This helper should either (a) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector to that particular cell line) or (b) consist of a defective genome or an appropriate conditional lethal mutant virus (in which case the experiments would be done under nonpermissive conditions), making vector and helper dependent upon each other for propagation. However, if none of these is available, the use of a non-defective genome as helper would be acceptable.

Currently only two viral DNAs can be considered as meeting these requirements: These are the genomes of polyoma virus and SV40.

Of these, polyoma virus is highly to be preferred. SV40 is known to propagate in human cells, both *in vivo* and *in vitro*, and to infect laboratory personnel, as evidenced by the frequency of their conversion to producing SV40 antibodies. Also, SV40 and related viruses have been found in association with certain human neurological and malignant diseases. SV40 shares many properties, and gives complementation, with the common human papova viruses. By contrast, there is no evidence that polyoma infects humans, nor does it replicate to any significant extent in human cells *in vitro*. However, this system still needs to be studied more extensively. Appendix B gives further details and documentation.

Taking account of all these factors:

1. Polyoma virus, a Recombinant DNA molecules consisting of defective polyoma virus genomes plus DNA sequences of any nonpathogenic organism, including Class 1 viruses (5), can be propagated in or used to transform cultured cells. P3 conditions are required. Appropriate helper virus can be used if needed. Whenever there is a choice, it is urged that mouse cells, derived preferably from embryos, be used as the source of eukaryotic DNA. Polyma virus is a mouse virus and recombinant DNA molecules containing both viral and cellular sequences are already known to be present in virus stocks grown at a high multiplicity. Thus, recombinants formed *in vitro* between polyoma virus DNA

See footnotes on p. 38459.

and mouse DNA are presumably not novel from an evolutionary point of view.

b. Such experiments are to be done under P4 conditions if the recombinant DNA contains segments of the genomes or Class 2 animal viruses (5). Once it has been shown by suitable biochemical and biological tests that the cloned recombinant contains only harmless regions of the viral genome (see Section IIIB-2-c-1) and that the host range of the polyoma virus vector has not been altered, experiments can be continued under P3 conditions.

2. SV40 Virus. a. Defective SV40 genomes, with appropriate helper, can be used as a vector for recombinant DNA molecules containing sequences of any non-pathogenic organism or Class I virus (5), (i.e., a shotgun type experiment). P4 conditions are required. Established lines of cultured cells should be used.

b. Such experiments are to be carried out in P3 (or P4) conditions if the non-SV40 DNA segment is (a) a purified[4] segment of prokaryotic DNA lacking toxigenic genes, or (b) a segment of eukaryotic DNA whose function has been established, which does not code for a toxic product, and which has been previously cloned in a prokaryotic host-vector system. It shall be confirmed that the defective virus—helper virus system does not replicate significantly more efficiently in human cells in tissue culture than does SV40, following infection at a multiplicity of infection of one or more helper SV40 viruses per cell.

c. A recombinant DNA molecule consisting of defective SV40 DNA lacking substantial segments of the late region, plus DNA from non-pathogenic organisms or Class I viruses (5), can be propagated as an autonomous cellular element in established lines of cells under P3 conditions provided that there is no exogenous or endogenous helper, and that it is demonstrated that no infectious virus particles are being produced. Until this has been demonstrated, the appropriate containment conditions specified in *2. a,* and *2. b.* shall be used.

d. Recombinant DNA molecules consisting of defective SV40 DNA and sequences from non-pathogenic prokaryotic or eukaryotic organisms or Class I viruses (5) can be used to transform established lines of non-permissive cells under P3 conditions. It must be demonstrated that no infectious virus particles are being produced; rescue of SV40 from such transformed cells by co-cultivation or transfection techniques must be carried out in P4 conditions.

3. Efforts are to be made to ensure that all cell lines are free of virus particles and mycoplasma.

Since SV40 and polyoma are limited in their scope to act as vectors, chiefly because the amount of foreign DNA that the normal virions can carry probably cannot exceed 2×10^6 daltons, the development of systems in which recombinants can be cloned and propagated purely in the form of DNA, rather than in the coats of infectious agents is necessary. Plasmid forms of viral genomes or organelle DNA need to be explored as possible cloning vehicles in eukaryotic cells.

<b> Plant host-vector systems. For cells in tissue cultures, seedlings, or plant parts (e.g., tubers, stems, fruits, and detached leaves) or whole mature plants of small species (e.g., *Arabidopsis*) the P1–P4 containment conditions that we have specified previously are relevant concepts. However, work with most plants poses additional problems. The greenhouse facilities accompanying P2 laboratory physical containment conditions can be provided by: (i) Insect-proof greenhouses, (ii) appropriate sterilization of contaminated plants, pots, soil, and runoff water, and (iii) adoption of the other standard practices for microbiological work. P3 physical containment can be sufficiently approximated by confining the operations with whole plants to growth chambers like those used for work with radioactive isotopes: *Provided,* That (i) such chambers are modified to produce a negative pressure environment with the exhaust air appropriately filtered, (ii) that other operations with infectious materials are carried out under the specified P3 conditions, and (iii) to guard against inadvertent insect transmission of recombinant DNA, growth chambers are to be routinely fumigated and only used in insect proof rooms. The P2 and P3 conditions specified earlier are therefore extended to include those cases for work on higher plants.

The host cells for experiments on recombinant DNAs may be cells in culture, in seedling or plant parts. Whole plants or plant parts that cannot be adequately contained shall not be used as hosts for shotgun experiments at this time, and attempts to infect whole plants with recombinant DNA shall not be initiated until the effects on host cells in culture, seedlings or plant parts have been thoroughly studied.

Organelle or plasmid DNAs and DNAs of viruses of restricted host range may be used as vectors. In general, similar criteria for selecting host-vectors to those given in the preceding section on animal systems are to apply to plant systems.

DNA recombinants formed between the initial moderately contained vectors and DNA from cells of species in which the vector DNA can replicate, require P2 physical containment. However, if the source of the NA is itself pathogenic or known to carry pathogenic agents, or to produce products dangerous to plants, or if the vector is an unmodified virus of unrestricted host range, the experiments shall be carried out under P3 conditions.

Experiments on recombinant DNAs formed between the above vectors and DNAs from other species can also be carried out under P2 if that DNA has been purified[4] and determined not to contain harmful genes. Otherwise, the experiments shall be carried out under P3 conditions if the source of the inserted DNA is not itself a pathogen, or known to carry such pathogenic agents, or to produce harmful products—and under P4 conditions if these conditions are not met.

The development and use of host-vector systems that exhibit a high level of biological containment permit a decrease of one step in the physician containment specified above (P4→P3→P2→P1).

<c> Fungal or similar lower eukaryotic host-vector systems. The containment criteria for experiments on recombinant DNAs using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, in that the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. We therefore consider that the containment guidelines given for experiments with *E. coli* K-12 and other prokaryotic host-vectors (Sections IIIB-1 and -2, respectively) provide adequate direction for experiments with these lower eukaryotic host-vectors. This is particularly true at this time since the development of these host-vectors is presently in the speculative stage.

IV. Roles and Responsibilities

Safety in research involving recombinant DNA molecules depends upon how the research team applies these guidelines. Motivation and critical judgment are necessary, in addition to specific safety knowledge, to ensure protection of personnel, the public, and the environment.

The guidelines given here are to help the principal investigator determine the nature of the safeguards that should be implemented. These guidelines will be incomplete in some respects because all conceivable experiments with recombinant DNAs cannot now be anticipated. Therefore, they cannot substitute for the investigator's own knowledgeable and discriminating evaluation. Whenever this evaluation calls for an increase in containment over that indicated in the guidelines, the investigator has a responsibility to institute such an increase. In contrast, the containment conditions called for in the guidelines should not be decreased without review and approval at the institutional and NIH levels.

The following roles and responsibilities define an administrative framework in which safety is an essential and integrated function of research involving recombinant DNA molecules.

A. Principal investigator. The principal investigator has the primary responsibility for: (i) Determining the real and potential biohazards of the proposed research, (ii) determining the appropriate level of biological and physical containment, (iii) selecting the microbiological practices and laboratory techniques for handling recombinant DNA materials, (iv) preparing procedures for dealing with accidental spills and overt personnel contamination, (v) determining the applicability of various precautionary medical practises, serological monitoring, and immunization, when available, (vi) securing approval of the proposed research prior to initiation of work, (vii) submitting information on purported EK2 and EK3 systems to the NIH Recombinant DNA Molecule Program Advisory Committee and making the strains available to others, (viii) reporting to the institutional biohazards committee and the NIH Office of Recombinant DNA Activities new information bearing on the guidelines, such as technical information relating to hazards and new safety procedures or innovations, (ix) applying for approval from the NIH Recombinant DNA Molecule Program Advisory Committee for large scale experiments with recombinant DNAs known to make harmful products (i.e., more than 10 liters of culture), and (x) applying to NIH for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes.

Before work is begun, the principal investigator is responsible for: (i) Making available to program and support staff copies of those portions of the approved grant application that describe the biohazards and the precautions to be taken, (ii) advising the program and support staff of the nature and assessment of the real and potential biohazards, (iii) instructing and training the staff in the practices and techniques required to ensure safety, and in the procedures for dealing with accidentally created biohazards, and (iv) informing the staff of the reasons and provisions for any advised or requested precautionary medical practises, vaccinations, or serum collection.

During the conduct of the research, the principal investigator is responsible for: (i)

See footnotes on p. 38459.

Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed, (ii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities and the institutional biohazards committee any serious or extended illness of a worker or any accident that results in (a) inoculation of recombinant DNA materials through cutaneous penetration, (b) ingestion of recombinant DNA materials, (c) probable inhalation of recombinant DNA materials following gross aerosolization, or (d) any incident causing serious exposure to personnel or danger of environmental contamination, (iii) investigating and reporting in writing to the NIH Office of Recombinant DNA Activities and the institutional biohazards committee any problems pertaining to operation and implementation of biological and physical containment safety practices and procedures, or equipment or facility failure, (iv) correcting work errors and conditions that may result in the release of recombinant DNA materials, and (v) ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., genotypic and phenotypic characteristics, purity, etc.).

B. *Institution.* Since in almost all cases, NIH grants are made to institutions rather than to individuals, all the responsibilities of the principal investigator listed above are the responsibilities of the institution under the grant, fulfilled on its behalf by the principal investigator. In addition, the institution is responsible for establishing an institutional biohazards committee[1] to: (i) Advise the institution on policies, (ii) create and maintain a central reference file and library of catalogs, books, articles, newsletters, and other communications as a source of advice and reference regarding, for example, the availability and quality of the safety equipment, the availability and level of biological containment for various host-vector systems, suitable training of personnel and data on the potential biohazards associated with certain recombinant DNAs, (iii) develop a safety and operations manual for any P4 facility maintained by the institution and used in support of recombinant DNA research, (iv) certify to the NIH on applications for research support and annually thereafter, that facilities, procedures, and practices and the training and expertise of the personnel involved have been reviewed and approved by the institutional biohazards committee.

The biohazards committee must be sufficiently qualified through the experience and expertise of its membership and the diversity of its membership to ensure respect for its advice and counsel. Its membership should include individuals from the institution or consultants, selected so as to provide a diversity of disciplines relevant to recombinant DNA technology, biological safety, and engineering. In addition to possessing the professional competence necessary to assess and review specific activities and facilities, the committee should possess or have available to it, the competence to determine the acceptability of its findings in terms of applicable laws, regulations, standards of practices, community attitudes, and health and environmental considerations. Minutes of the meetings should be kept and made available for public inspection. The institution is responsible for reporting names of and relevant background information on the members of its biohazards committee to the NIH.

C. *NIH Initial Review Groups (Study Sections).* The NIH Study Sections, in addition to reviewing the scientific merit of each grant application involving recombinant DNA molecules, are responsible for: (i) Making an independent evaluation of the moral and potential biohazards of the proposed research on the basis of these guidelines, (ii) determining whether the proposed physical containment safeguards certified by the institutional biohazards committee are appropriate for control of these biohazards, (iii) determining whether the proposed biological containment safeguards are appropriate, (iv) referring to the NIH Recombinant DNA Molecule Program Advisory Committee or the NIH Office of Recombinant DNA Activities those problems pertaining to assessment of biohazards or safeguard determination that cannot be resolved by the Study Sections.

The membership of the Study Sections will be selected in the usual manner. Biological safety expertise, however, will be available to the Study Sections for consultation and guidance.

D. *NIH Recombinant DNA Molecule Program Advisory Committee.* The Recombinant DNA Molecule Program Advisory Committee advises the Secretary, Department of Health, Education, and Welfare, the Assistant Secretary for Health, Department of Health, Education, and Welfare, and the Director, National Institutes of Health, on a program for the evaluation of potential biological and ecological hazards of recombinant DNAs (molecules resulting from different segments of DNA that have been joined together in cell-free systems, and which have the capacity to infect and replicate in some host cell, either autonomously or as an integrated part of their host's genome), on the development of procedures which are designed to prevent the spread of such molecules within human and other populations, and on guidelines to be followed by investigators working with potentially hazardous recombinants.

The NIH Recombinant DNA Molecule Program Advisory Committee has responsibility for: (i) Revising and updating guidelines to be followed by investigators working with DNA recombinants, (ii) for the time being, receiving information on purported EK2 and EK3 systems and evaluating and certifying that host-vector systems meet EK2 or EK3 criteria, (iii) resolving questions concerning potential biohazard and adequacy of containment capability if NIH staff or NIH Initial Review Group so request, and (iv) reviewing and approving large scale experiments with recombinant DNAs known to make harmful products (e.g., more than 10 liters of culture).

E. *NIH Staff.* NIH Staff has responsibility for: (i) assuring that no NIH grants or contracts are awarded for DNA recombinant research unless they (a) conform to these guidelines, (b) have been properly reviewed and recommended for approval, and (c) include a properly executed Memorandum of Understanding and Agreement, (ii) reviewing and responding to questions or problems or reports submitted by institutional biohazards committees or principal investigators, and disseminating findings, as appropriate, (iii) receiving and reviewing applications for approval to lower containment levels when a cloned DNA recombinant derived from a shotgun experiment has been rigorously characterized and there is sufficient evidence that it is free of harmful genes, (iv) referring items covered under (ii) and (iii) above to the NIH Recombinant DNA Molecule Program Advisory Committee, as deemed necessary, and (v) performing site inspections of all P4 physical containment facilities, engaged in DNA recombinant research, and of other facilities as deemed necessary.

APPENDIX D

V. FOOTOTES

[1] Biological Safety Cabinets referred to in this section are classified as *Class I, Class II* or *Class III* cabinets. A *Class I* cabinet is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency or high efficiency particulate air (HEPA) filter before discharged to the outside atmosphere. This cabinet is used in three operational modes: (1) with an 8 inch high full width open front, (2) with an installed front closure panel (having four eight inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm length rubber gloves. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all Biohazard Safety Cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through HEPA filters or incinerated before being discharged to the outside environment.

[2] Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection and/or conjugation with transfer of phage, plasmid and/or chromosomal genetic information.

[3] The bacteria which constitute Class 2 of ref. 5 ("Agents of ordinary potential hazard . . .") represent a broad spectrum of etiologic agents which possess different levels of virulence and degrees of communicability. We think it appropriate for our specific purpose to further subdivide the agents of Class 2 into those which we believe to be of relatively low pathogenicity and those which are moderately pathogenic. The several specific examples given may suffice to illustrate the principle.

[4] The terms "characterized" and "free of harmful genes" are unavoidably vague. But in this instance, before containment conditions lower than the ones used to clone the DNA can be adopted, the investigator must obtain approval from the National Institutes of Health. Such approval would be contingent upon data concerning: (a) The absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or which code for toxic substances), (b) the relation between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable), and (c) maintenance of the biological properties of the vector.

[5] A DNA preparation is defined as enriched if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation. The reason for lowering the containment level when this degree of enrichment has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is

markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more that 10^6-fold when a non-repetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

[4] The DNA preparation is defined as purified if the desired DNA represents at least 99 percent (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

[5] In special circumstances, in consultation with the NIH Office of Recombinant DNA Activities, an area biohazards committee may be formed, composed of members from the institution and/or other organizations beyond its own staff, as an alternative when additional expertise outside the institution is needed for the indicated reviews.

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APPENDIX A TO APPENDIX D

STATEMENT ON THE USE OF BACILLUS SUBTILIS IN RECOMBINANT MOLECULE TECHNOLOGY

Unquestionably, *Escherichia coli* is the most well characterized unicellular organism. Years of basic research have enabled investigators to develop a well characterized genetic map, to obtain detailed knowledge of virulent and temperate bacteriophages, and to explore the physiology, genetics, and regulation of plasmids. More recently, the development of DNA-mediated transformation has permitted exogenous fragments or molecules of DNA to be incorporated into the genome or to reside as self-replicating units. The discovery of transformation of *Bacillus subtilis* by Spizizen (1) stimulated the development of an alternative model system. The purpose of this report is to summarize the current status of this genetic system and to describe the actual and potential vectors and vehicles available for recombinant molecule technology.

A. *Current knowledge of the chromosomal architecture and mechanisms of genetic exchange in B. subtilis.* Two mechanisms of genetic exchange have been utilized to establish the linkage map of *B. subtilis*, DNA-mediated transformation (capable of transferring approximately 1 percent of the genome) and transduction with bacteriophage PBSI (capable of transferring 5–8 percent of the chromosome). Recent detailed genetic studies with PBSI by Lepesant-Kejzlorova *et al.* (2) have resulted in the development of a circular genetic map for this organism. The current edition of the map (3) contains 196 loci. Biophysical analyses have established that the chromosome is circular (4) and replicates bidirectionally (5).

Transformation with purified fragments of DNA is a highly efficient process in *B. subtilis* with frequencies of 1 to 4 percent usually attained for any auxotrophic or antibiotic resistance markers. Frequencies of approximately 10 percent transformation can be achieved with DNA prepared from gently lysed L-forms or protoplasts (6). These large fragments of DNA are readily incorporated by the recipient cell. Generalized transduction occurs with bacteriophages SP10 (7), PBSI (8), and SPP1 (9), while a low frequency of specialized transduction has been reported with bacteriophage ϕ105 (10).

Although transformation is most efficient in homologous crosses (*B. subtilis* into *B. subtilis*), it has also been possible to exchange DNA among closely related species (11). The most extensively studied members of the *B. subtilis* genospecies include *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, and *B. globigii* (refer to reference 12 for a review and references 13–15 for examples of this heterologous exchange). This exchange occurs even though there is a surprisingly wide discrepancy between DNA-DNA hybridization among these organisms (16). Even though the frequency of transformation is low in the heterologous cross [e.g., *B. amyloliquefaciens* (donor)/*B subtilis* (recipient)], the newly acquired DNA from *B. amyloliquefaciens* in the *B. subtilis* background can be readily transferred at high efficiencies to other recipient strains of *B. subtilis* (14). Therefore, the extremely high frequency of transformation permits the recognition and selection of rare events.

B. *Current and potential vectors for recombinant molecule experiments.* Lovett and coworkers have recently described cryptic plasmids in *B. pumilus* (17) and *B. subtilis* (18). Of these organisms, *B. subtilis* ATCC 7003 appears to be the most useful since it carries one to two copies of a plasmid with a molecular weight of 46×10^6. This strain is also closely related to *B. subtilis* 168. Another strain of *B. subtilis* (ATCC 15841) contains 16 copies of a plasmid with a molecular weight of 4.6×10^6. Currently it is not known whether genetic markers can be readily introduced into these plasmids. To date it has not been possible to readily stabilize plasmids derived from *B. pumilus* in *B. subtilis* even with heavy selective pressure (P. Lovett, personal communication).

Two temperate bacteriophages are under development as vectors in *B. subtilis*, ϕ3T and SPO2. Lysogeny of thymine auxotrophs (strains carrying *thyA thyB*) by bacteriophage ϕ3T results in "conversion" to a Thy$^+$ phenotype. The attachment site for this bacteriophage and the bacteriophage gene for thymidylate synthetase (*thyP*) map between the bacterial *thyA* and *thyB* loci in the terminal region of the chromosome of *B. subtilis* (19). The viral genome is readily cleaved by the site-specific endonuclease, Bam I (20), to produce 5 fragments (one of which carries the *thyP* gene). The *thyP* carrying gene can be integrated into the bacterial genome in the absence of the intact viral genome. Because deletions are available that include the *thyP* region, it is theoretically possible to introduce *thyP* at many sites on the chromosome. The *thyP* gene can be readily purified for insertion into plasmids or utilized as a readily integrative other heterologous DNA into the chromosome of *B. subtilis*. Alternatively, it is possible to purify fragments of the chromosome by gel electrophoresis (21, 22), for insertion into bacteriophage ϕ3T or SPO2. At present, unfortunately, only the former carries a selective marker, i.e., the gene for thymidylate synthetase, *thyP*.

C. *Development of vehicles. B. subtilis* is a Gram-positive sporulating rod that usually inhabits soil. Although it can exist on cutaneous surfaces of man (23) and experimental animals, it rarely produces disease. To develop a suitable vehicle it is imperative to have a host that is asporogenic. The most appropriate deletion mutation is deletion 29 (cit D). In addition to a deficiency in sporulation this mutant rapidly lyses when it has reached the end of its growth cycle. Presumably this is due to the failure to inactivate one of the autolytic enzymes (24). Through the introduction of a D-alanine requirement (34 ug/ml) it is possible to block transport of compounds that are transported by active transport (25,26). The further introduction of thymine auxotrophy (defects in the *thyA thyB* loci) will enable the strain to survive only with a plasmid vector carrying the purified *thyP* gene from bacteriophage ϕ3T or a defective bacteriophage ϕ3T carrying the *thyP* gene but attached to the chromosome at an alternative site (due to the presence of deletion 29 in the host). We have recently isolated temperature-sensitive *thyP* mutants. If we can isolate a temperature-dependent lysogen that will grow only at 48°C it should be possible to make an unusual vehicle.

D. *Site-specific endonucleases.* Recently two restriction modification systems have been observed between *B. subtilis* 168 and other bacilli. Trautner *et al.* have isolated an effective system that inhibits infection of the R strain of *B. subtilis* by bacteriophage SPPI propagated on *B. subtilis* 168 (27). The site-specific nuclease recognizes the sequence GGCC.
CCGG
Young, Radnay, and Wilson observed a restriction modification system between *B. amyloliquefaciens* and *B. subtilis* 168 (28). The endonuclease from *B. amyloliquefaciens* (20) recognizes the sequence GGTAC (29).
CCTACG
More recently, two additional enzymes have been isolated from *B. globigii* (30). The recognition sequence is not known.

E. *Advantages and liabilities of the B. subtilis system*—a. *Advantages.* 1. *B. subtilis* is nonpathogenic. Asporogenic deletion mutants are available to preclude the problem of persistence through sporulation.

2. The circular chromosomal map is well defined. At least 196 loci have been positioned.

3. The organism is commercially important in the fermentation industry.

4. Large numbers of organisms can be disposed of readily with minimal environmental impact.

5. Unlike *E. coli*, it lacks endotoxin in the cell wall. Therefore the cells can be used as a single cell protein source.

6. The frequency of transformation is very high, facilitating the detection of rare events.

7. A unique bacteriophage, ϕ3T, exists that carries a gene that can be readily purified for "scaffolding" experiments.

b. *Disadvantages.* 1. The knowledge of genetics and physiology of plasmids and viruses is primitive compared with *E. coli*.

2. High-frequency, specialized transduction is not available as a means of gene enrichment.

Based on its promise, it seems appropriate, and not chauvinistic, to urge development of this system.

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APPENDIX B TO APPENDIX D

POLYOMA AND SV40 VIRUS

Polyoma virus is a virus of mice, and infection of wild mouse populations is a common events, for the virus has often been isolated from a high proportion of healthy adult animals, both wild and laboratory bred, of many colonies (Gross, L., Proc. Soc. Exp. Biol. *88*, 362–368, 1955; Rowe, W. P., Bact. Rev. *25*, 18–31, 1961). As far as is known the virus almost never causes a disease in these animals. However, when large quantities of the virus are inoculated into newborn or suckling mice or hamsters, a variety of solid tumors is induced (Gross, L., Oncogenic Viruses, Second Edition, Pergamon Press, NY).

Polyoma virus grows lytically in mouse cells in tissue culture. Thus mouse cells in culture are probably transformed only by virus particles that contain certain kinds of defective genomes. Cells of other rodent species, however, can be transformed by polyoma virus particles that contain complete genomes (Folk, W., J. Virol., *11*, 424–431, 1973). The virus does not replicate to a significant extent in human cells in tissue culture (Eddy, B.E., Virol. Monogr., *7*, 1–114, 1969; Pollack, R. E., Salas, J., Wang R. Kusano T., and Green, H., J. Cell Physiol. *77*, 117–120, 1971). The resistance of the cells seems to be a consequence of the failure of the virus to absorb or uncoat. However even when naked viral DNA is introduced into the cells only an abortive cycle of replication ensues; early viral proteins are made, there is induction of cellular DNA synthesis, but no expression of late viral proteins is detectable (Gruen, H., Grossmann, M. and Grassmann, A. Virology, *58*, 290–293, 1974).

There is no evidence that polyoma virus can infect humans (Hartley, J., Huebner, R., Parker, J. and Rowe, W. P., unpublished data). Thus no antibodies to the virus have been detected in people living in buildings that are infested with virus-infected mice, nor in laboratory workers who have been exposed to the virus for a number of years.

At most a small segment of polyoma virus DNA shows weak homology with a portion of the late region of SV40 DNA (Ferguson, J. and Davis, R. W., J. Mol. Biol., *94*, 135–150, 1975). However, there appears to be no genetic interaction between the two viruses and there is no immunological cross-reaction between the gene products of the two viruses.

SV40 causes persistent but apparently harmless infections of the kidneys of virtually all adult rhesus monkeys. (Hsiung, G. D., Bact. Revs. *32*, 185–205, 1968); it causes tumors when injected into newborn hamsters (Girardi, A. J., Sweet, B. H., Slotnick, V. B. and Hillemann, M. R., Proc. Soc. Exp. Biol. Med., *105*, 420–427, 1964) and transforms cells of several mammalian species (including human). SV40 is able to infect humans since antibodies to the virus are found in a small proportion of the human population (Shah, K. V., Goverdhan, M. K. and Ozer, H. L., Am. J. Epid. *93*, 291–298, 1970) and serum conversions have been noted in many laboratory personnel who have been exposed to the virus (Horvath, L. B., Acta Microbiol. Acta Sci. Hung. *12*, 201–206, 1965).

Isolations of SV40 have been reported from humans, twice from patients suffering from the rare demyelinating disease, progressive multifocal leukoencephalopathy (Weiner, L., Herndon, R., Narayon, O., Johnson, R. T., Shah, K., Rubinstein L. G. Prezozisi T. J. and Conley, F. K., New England J. Med *286*, 385–390, 1972) and apparently from a tumor of a person with metastatic melanoma (Soriano, F., Shelburne, C. E. and Gokeen, M., Nature, *249*, 421–424, 1974). In other studies a nonstructural antigen characteristic of papovaviruses, T antigen, has been detected in the nuclei of cells cultured from 2 meningiomas, while another SV40-specific antigen, U antigen, has been found in the cells of a third tumor of the same type (Weiss, A. F., Portman, R., Fisher, H., Simon, J. and Zang, K. D., Proc. Nat. Acad. Sci. USA *72*, 609–613, 1975). Furthermore new papovaviruses have been isolated from the brains of patients with PML (JC virus-Padgett, B. L., Walker, D. L., zuRhein, G. M., Eckroade, R. I. and Dessel, B. H., Lancet *1*, 1257–1260, 1971), from the urine of a patient carrying a renal allograft (BK virus-Gardner, S. D., Field, A. M., Coleman, D. V. and Hulme, B. Lancet *1*, 1253–1257, 1971) and from a reticulum cell sarcoma and the urine of patients with the sex-linked recessive disorder, Wiskott-Aldrich syndrome (Takemoto, K. K., Rabson, A. S., Mullarkey, M. F., Blaese, R. M. Garon, C. F. and Nelson, D. J., Nat. Cancer Inst., *53*, 1205–1207, 1974). All of these viruses which are distributed widely throughout human populations share antigenic and biological properties with SV 40; the virus particles are identical in size and architecture (Madeley, C. R., In Virus Morphology, Churchill-Livingstone, London, 134–135, 1973); the non-structural intracellular T antigen, which appears to be coded by the A gene of SV40 cross reacts extensively with antigens found in cells infected or transformed by BK or JC viruses; both JC and BK viruses induce tumors in newborn hamsters (Walter, D. L., Padgett, B. L., zuRhein, B. M., Albert, A. E. and Marsh, R. F., Science *181*, 674–676, 1973; Shah, K. V., Daniel, R. W. and Strandberg, J., J. Nat. Cancer Inst. *54*, 945–950, 1975); BK virus causes transformation of hamster cells in culture (Major, E. D., and DiMayorca, G., Proc. Nat. Acad. Sci. US *70*, 3210–3212, 1973; Portolani, M., Barbanti, A., Brodano, G. and LaPlaca, M.J., Virol. *15*, 420–422, 1975) and is able to complement the growth of certain temperature-sensitive mutants of SV40 (Mason, D. H. and Takemoto, K. K., submitted for publication).

FURTHER WORK

At present, a potential eukaryotic vector of choice is polyoma virus. And while available information indicates that it fulfills all the necessary criteria, we recommend that the following subjects be further investigated:

1. The molecular mechanism of resistance of human cells to the virus.

2. The extent of homology between polyoma virus DNA and the DNAs of human papovaviruses.

3. The ability of human papovaviruses to complement defective polyoma virus genomes.

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APPENDIX C TO APPENDIX D

SUMMARY OF THE WORKSHOP ON THE DESIGN AND TESTING OF SAFER PROKARYOTIC VEHICLES AND BACTERIAL HOSTS FOR RESEARCH ON RECOMBINANT DNA MOLECULES

Torrey Pines Inn, La Jolla, California

The development of techniques for the cloning of DNA from both prokaryotic and eukaryotic organisms in bacteria has had great impact on research in biology and medicine and promises extraordinary social benefits. The biohazards involved in the use of this technology in many instances are very difficult to assess. For this reason codes of practice are being formulated in the United States and other countries for the conduct of those experiments that present a potential biohazard. One of the requirements for conducting certain cloning experiments is the use of safer vector (bacteriophage or plasmid) -host systems, i.e., vector-bacterium systems that have restricted capacity to survive outside of controlled conditions in the laboratory. Approximately sixty scientists from the United States and several foreign countries participated in a workshop on the Design and Testing of Safer Prokaryotic Vehicles and Bacterial Hosts for Research on Recombinant DNA Molecules at La Jolla, California, on December 1 to 3, 1975. The workshop was sponsored by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases. The purposes of the meeting were the exchange of recent data on the development of safer prokaryotic host-vector systems, devising methods of testing the level of containment provided by these systems and exploring the various directions that future research should take in the construction of safer bacterial systems for the cloning of foreign DNA.

The first session of the workshop, chaired by W. Szybalski (University of Wisconsin), was devoted to bacteriophage vectors. Szybalski outlined the main safety features of the two-component, phage-bacterial system, in which the host bacteria offer the safety feature of not carrying the cloned DNA, and the phage vectors cannot be propagated in the absence of an appropriate host. There are two primary escape routes for the clones of foreign DNA carried by the phage vector: (1) Establishment of a stable prophage or plasmid in the laboratory host used for phage propagation, and subsequent escape of this self replicating lysogen or carrier system, and (2) escape of the phage vector which carries the cloned DNA and its subsequent productive encounter with a suitable host in the natural environment. The general consensus was that to ensure safety, both routes should be blocked by appropriate genetic modifications. For phage λ, route (1) can be blocked by phage mutations that interfere with lysogenization (*att-*, *int-*, *cI-*, *cIII-*, *vir*) and plasmid formation (*N-*, *ninR*, *cS*, *ri-*, *cI7*, *Ots*, *crots*), and by mutations on the *Escherichia coli* host that affect these processes (*attB-*, *dncAts*) and host survival. Route (2), (which is of low probability since λ phages do not survive well in natural environments (no *lcI* phage was recovered after ingestion of 10^8–10^{12} particles), are killed by desiccation, and have a low chance to encounter a naturally sensitive host) can be blocked further by the following phage modifications: (a) Mutations which result in extreme instability of the infectious phage particles under all conditions other than those specially designed for phage propagation in the laboratory (e.g., high concentrations of putrescine or some other compound), or (b) employing phage vectors in which the tail genes are deleted and which permit propagation of only the DNA-packed heads; only under laboratory conditions could such heads be made transiently infectious by rejoining them with separately prepared tails. The high instability of the phage would minimize the possibility of transfer of the cloned genes into receptive bacteria found in nature. Moreover, the propagation of the phage can be blocked by many conditional mutations, which would be designed to block any secondary route of escape, mainly depending on transfer of the cloned DNA into another phage or bacterial host. It was recommended further that the vector be designed in such a manner as to permit easy insertion and monitoring of the foreign DNA and rapid assay of the safety features and give a high yield of cloned DNA (not less than 10^{11} molecules per ml). There also was general agreement that host-phage systems other than *E. coli* should be considered, especially those restricted to very rare and unusual environments. Also, plasmids derived from phage vectors and which give very high DNA yields while exhibiting safety features, e.g., *Adv*crots, should be considered as vehicles for cloned DNA.

Szybalski and S. Brenner (Cambridge University) stressed that research on recombinant DNA molecules may lend itself to very simple and inexpensive mechanical containment, e.g., a small sealed glove box, since all the vectors that carry such recombinant molecules possibly can be both created and destroyed in such a box, while development of special methods might permit study of many properties of the recombinant DNA, without ever removing it from the box.

These safety features were reflected in the subsequent presentations. F. Blattner and W. Williams (University of Wisconsin) described four specially constructed λ-ϕ80 phages which incorporate many of these safety features, and which they named Charon phages, for the mythical boatman of the river Styx. Some of these highly contained phages give yields of over 10^{13} particles/ml. R. Davis, J. Cameron and K. Struhl (Stanford University) found that λ phages that carry foreign DNA never grow as well as the parental vector, which would select against their survival in nature. They also reported that some eukaryotic genes could be expressed in *E. coli*, partially compensating for deficiencies in the histidine pathway or in *polA* or *lig* functions. These investigators surveyed over 1000 strains of *E. coli* isolated in the natural environment and did not find a single strain that could support propagation of the *λvir* vector.

V. Bode (Kansas State University) discussed the possibility of growing tail-free λ heads. Such heads, which are packed with DNA, are very fragile, unless stored in 0.1 M putrescine buffer. Head yields close to 10^{14}/ml could easily be attained and, when required, heads could be quantitatively rejoined with separately supplied tails under special laboratory conditions. W. Arber, D. Scandella and J. Elliott (University of Basel) described bacterial host mutants that permit efficient infection only by phages with a full complement of DNA. This permits selecting for vectors that carry long fragments of foreign DNA.

K. Matsubara, T. Mukai and Y. Takagi (University of Osaka and Kyushu University), and G. Hobom and P. Phillippsen (University of Freiburg and Stanford University) described various defective λ plasmids (λdv) that could be used as efficient vectors. Matsubara has shown that temperature-sensitive *cro* mutations permit obtaining between 1000 and 3000 cloned molecules per cell and at the same time result in killing of the carrier cells at body temperature. The mutations *Ots* and *Pts* were also evaluated as safety features. Phillippsen described many new λdv plasmids created by cutting λ DNA with *HindIII* and *BamI* restriction endonucleases followed by ligation. The final talk by F. Young, G. Wilson and M. Williams (University of Rochester) summarized the progress on the development of safer *Bacillus subtilis* host mutants and phages, especially ϕ3, as vectors. New restriction nucleases, Bgl-1 and Bgl-2, were also described.

The morning session on bacteriophage vectors was followed by a session on plasmid vectors that was chaired by D. Helinski (University of California, San Diego). Helinski presented the following properties as highly desirable characteristics of a safer plasmid vehicle: (a) Non-conjugative; (b) non-mobilizable or poorly mobilizable by a conjugative plasmid; (c) possesses little or no extraneous genetic information; (d) poorly recombines or does not recombine with the chromosome of the host cell; (e) provides no selective advantage to the host cell or the selective property is conditional; and (f) possesses mutations that restrict its maintenance to a specific host, prevent replication at mammalian body temperature and/or provide with the capability of killing any cell to which it might be transmitted other than the host cell. V. Hershfield (University of California, San Diego) described the properties of a variety of derivatives of the ColE1 derivatives, ColE1-*trp*, constructed in collaboration with C. Yanofsky and N. Franklin (Stanford University) provides the means to use the tryptophan genes of *E. coli* as a selective marker in transformation with recombinant DNA in situations where it is desirable to avoid antibiotic resistance genes. In addition, Hershfield described collaborative work with H. Boyer that resulted in the development of a mini-ColE1 plasmid and derivatives of this plasmid (mini-ColE1-*kan* and mini-ColE1-*trp*) as cloning vehicles. Finally, she described the temperature-sensitivity properties of *trp* and *kan* derivatives of a temperature-sensitive replication mutant of ColE1 isolated by J. Collins (Molecular Biology Institute, Stockholm) and hybrid ColE1 plasmids carrying the *Eco*RI generated *Cts* fragment of bacteriophage λ-trp81.

J. Carbon (University of California, Santa Barbara) described a replica plating method that greatly facilitates the detection of *E. coli* clones bearing ColE1 plasmids. The procedure, which utilizes the F, plasmid to promote the transfer of a hybrid ColE1 plasmid to a suitable auxotrophic recipient, was successful in identifying clones bearing hybrid plasmids carrying a number of different regions of the *E. coli* chromosome. The contributions of A. J. Clark and collaborators (University of California, Berkeley) were relevant to the problem of the mobilization and subsequent transfer of non-conjugative plasmids carrying foreign DNA of a potentially hazardous nature. Clark described the variations in transmission frequencies between the nonconjugative plasmids pSC101, pML31, pSC138 and a number of pSC101 hybrids containing various *Eco*RI fragments of F when the conjugal transfer of these plasmids was promoted by several different conjugative plasmids.

I. C. Gunsalus and collaborators (University of Illinois) and A. Chakraberty (General Electric Research and Development Center)

described the properties of a variety of plasmids isolated from *Pseudomonas putida*. These contributions were followed by a discussion on the merits of developing plasmid-host systems involving *Pseudomonas* strains that naturally exhibit unusual growth requirements. Similar studies with plasmids isolated from *Bacillus megaterium* by B. Carlton (University of Georgia) from *B. subtilis* by P. Lovett (University of Maryland) and other naturally occurring *Bacillus* species by W. Gosbel and K. Bernhard (Microbiology Institute, Wurzburg) were discussed and their further development as plasmid-host cloning systems was explored. It was clear from these presentations that considerable progress has meen made recently in the identification and characterization of a variety of plasmid elements that occur naturally in *Pseudomonas* and *Bacillus* species. Several of the plasmids described show considerable promise as plasmid cloning systems involving a host other than *E. coli*.

A third session on the ecology and epidemiology of vector-host systems was chaired by S. Falkow (University of Washington). This workshop emerged, in part, from expressed fears that microorganisms containing cloned fragments of foreign DNA may potentially pose a threat to health or disrupt the normal ecological chain in some manner. Consequently, this session was devoted to a review of currently available information on the ecology and epidemiology of *E. coli* and related bacterial species since it was recognized that *E. coli* K–12 would be the prokaryote host most commonly employed in the cloning of DNA molecules in the immediate future. F. Ørskov (Escherichia Reference Center, Copenhagen) reviewed the state of *E. coli* serotyping and what has been learned about the distribution of *E. coli* types in health and disease. Only certain *E. coli* types are generally recognized as good colonizers of the human gut and such strains come from a handful of the 160 well defined O (lipopolysaccharide) antigen types and invariably possess K (acidic polysaccharide capsule) antigens. Some serotypes apparently have become disseminated worldwide and possibly represent the proliferation of a bacterial clone because of, as yet unknown, selective pressures. In contrast, *E. coli* K–12 has no detectable O or K antigens and is considered to be rough. This may account, at least in part, for its demonstrate poor ability to colonize the human or animal gut. However, R. Freter (University of Michigan) pointed out that we still remain largely ignorant of the factors which control intestinal *E. coli* populations. Freter also noted that while adherence to the mucosal surface of the small intestine is important in the pathogenesis of *E. coli* diarrheal disease, the 'normal' long-lasting symbiotic relationship between a mammalian host and bacterium is established in the cecum and colon. It is in these locations that factors come into play to determine whether an *E. coli* strain passing through the intestine will become successfully implanted or whether it will be quickly eliminated in the feces. The factors controlling implantation include competition for substrates, inhibitors and the physiological state of the organism when it reaches the large bowel. For example, ingested *E. coli* previously grown under usual laboratory conditions fare poorly while cells of the same strain 'pre-adapted' in Eh, pH, etc., often colonize well. Freter has developed a continuous flow culture model which may be useful in studying the mechanisms of implantation. Falkow reviewed the pathogenicity of *E. coli*. *E. coli* causes diarrheal disease either by direct invasion of the bowel epithelium or by elaboration of enterotoxin(s). While invasive *E. coli* appear to owe their pathogenicity to a constellation of at least five unlinked chromosomal gene clusters, toxigenic *E. coli* species generally owe their pathogenicity to the possession of two species, Ent and K. The introduction of Ent and K plasmids may be sufficient to convert a normal wild-type *E. coli* into a strain now capable of causing overt clinical disease. However, the introduction of these plasmids into *E. coli* K–12 sublines had no discernable effect on their ability to cause disease, although the K–12 strains could now better colonize calves. Despite the observation that *E. coli* K–12 did not appear to offer a significant hazard as a potential enteric pathogen even when it possessed well-defined determinants of pathogenicity it was emphasized by Ørskov, Freter and Falkow that *E. coli* K–12 strains carrying recombinant DNA molecules could still act as effective genetic donors *in vivo* and still posed a significant problem requiring control. E. Geldreich (U.S. Environmental Protection Agency, Cincinnati, Ohio) discussed the possible outcomes of the release of *E. coli* containing recombinant DNA molecules into the aquatic environment and concluded that total reliance cannot be placed on sewage treatment and the natural self-purification capacity of receiving waters to limit potential hazards. While these are realistic barriers to the dissemination of *E. coli* and associated fecal organisms via the water route, they are not infallible because of technological limitations, improper operational practices and system overloading. Finally, M. Starr (University of California, Davis) described the numerous genera of gram-negative bacteria found naturally occurring in the soil and on plants. He stated that most of these organisms do not appear to be a reasonable alternative to *E. coli* K–12 as a host for recombinant DNA molecules. Indeed, Starr pointed out that since such genera as *Erwinia*, *Rhizobium* and *Agrobacterium* are known to conjugate with *E. coli*, the potential dissemination of recombinant DNA molecule includes a greater spectrum of microorganisms than just enteric species.

The fourth session of the workshop, chaired by R. Curtiss III (University of Alabama), was concerned with the construction of safer bacterial hosts for DNA cloning. The goals in constructing safer host strains enunciated at the beginning of the session included introduction of mutations that would: (a) Preclude colonization in normal ecological niches; (b) preclude cell wall biosynthesis except in specially defined media; (c) cause degradation of genetic information in normal ecological niches; (d) cause vectors to be host-dependent; (e) minimize transmission of recombinant DNA to other strains in normal ecological niches; (f) increase usefulness for recombinant DNA molecule research; and (g) permit monitoring.

Most of the progress in developing safer hosts has been achieved with *E. coli* K–12, although F. Young described a *B. subtilis* strain with a deletion for sporulation genes which readily undergoes autolysis. The strain also has defects in genes for purine and TTP biosynthesis and a mutation conferring a D-alanine requirement can be introduced to cause cell wall biosynthesis to be defective. This strain may be defective in transformation, however, and therefore might be useful only with a phage vector which has yet to be developed and/or discovered.

A. I. Bukhari (Cold Spring Harbor Laboratory) described the use of the *dapD8* mutation in *E. coli* K–12 to block cell wall biosynthesis and another non-reverting mutation which causes sensitivity to bile salts and detergents. The *dapD8* allele is the most stable *dap* point mutation known, although it does revert at frequencies of 10^{-8} to 10^{-9}. The mutation conferring bile salts sensivity was obtained after Mu-1 infection of an Hfr strain and, although exhibiting the theoretically useful properties of ease of DNA isolation and inability to survive in the intestinal tract, might be due to Mu insertion which would compromise its use for safe strain construction.

Curtiss reported on the work performed by him and his coworkers in constructing and testing numerous strains with different mutations. Survival of strains *in vivo* was tested by feeding rats 10^{10} cells in milk by stomach tube. *Apur* mutations did not reduce strain titers in feces whereas *AthyA; AthyA dra*; and *AthyA dra* mutations gave 10^{1}-fold, 10^{3}-fold and 10^{8}-fold reductions, respectively, in strain titers in feces. Strains with *AthyA* mutations also exhibited thymineless death in *in vitro* tests. Since strains with *dapD8* allele can revert to Dap^{+}, strains were constructed with both *dapD8* and *AbioH-asd* mutations. These strains have not been observed to revert to Dap^{+} but can survive passage through the rat intestine and in growth media lacking diaminopimelic acid but containing NaCl and 0.5% usable carbon sources. This survival was due to the production of the mucopolysaccharide, colanic acid, which permits many of the cells to grow and survive as spheroplasts. A *Agal-chl*^{r} mutation (also deletes *λatt, bio* and *uvrB* genes) was introduced which blocks colanic acid biosynthesis and leads to no detectable survivors in media lacking diaminopimelic acid or following passage through the rat intestine. The *dapD8 AbioH-asd Agal-chl* strains are more readily lysed, transform at higher frequencies and are conjugation-defective in matings with donors possessing conjugative plasmids in the F, W and O incompatability groups but Con^{+} as recipients for F, I and T group plasmids when compared to the *dap*$^{+}$ *gal*$^{+}$ parent strain. Strains with *endA* mutations were also observed to exhibit increased transformation frequencies. Attempts to introduce temperature-sensitive *polA* alleles into strains to block replication of ColE1 cloning vectors at elevated temperatures and to cause DNA degradation at elevated temperatures in the presence of *recA* and *AthyA* alleles often do not have the same properties in the constructed strains as in the strains in which the allele was originally induced. Many mutations causing a Con phenotype have been investigated, but many of these revert and/or do not exhibit a Con phenotype in matings with donors possessing conjugative plasmids of the incompatibility groups commonly found in enteric microorganisms. Some Con- mutants exhibit increased sensitivity to bile salts; thus, the mutant described by Bukhari may also exhibit a Con- phenotype. All of the strains constructed by the Curtiss group are SuII^{+} and most have mutations abolishing restriction alone or both restriction and modification. Thus, sufficient information is now known to construct a usable safer *E. coli* K–12 host. Curtiss and collaborators are now introducing *AthyA* and *dna* mutations into their *dapD8 AbioH-asd Agal-chl*^{r} *uvrB hsr malA*^{r} (for ease in monitoring) Su^{+} λ^{r} 480^{r} strain to accomplish this objective.

The final session involved a general discussion of some of the major points raised previously in the workshop. There was general agreement at this session that both plasmid-host and phage-host systems have been developed that should meet the criteria of an EK3 system specified by the National Institutes of Health guidelines for research on recombinant DNA molecules. Additional testing is required to confirm the EK2 properties of these available systems, but it is anticipated that these vector-host systems will meet these tests.

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APPENDIX D TO APPENDIX D

SUPPLEMENTARY INFORMATION ON PHYSICAL CONTAINMENT

CONTENTS

I. Biological safety cabinets.
Table I.
II. Universal biohazard warning symbol.
III. Laboratory techniques for biohazard control.
A. Pipetting.
B. Syringes and needles.
C. Opening culture plates, tubes, bottles, and ampoules.
D. Centrifuging.
E. High-speed centrifuges.
F. Blenders, ultrasonic disintegrators, colloid mills, ball mills, jet mills, grinders, mortar and pestle.
G. Miscellaneous precautions and recommendations.
IV. Personal hygiene, habits, and practices.
V. Care and use of laboratory animals.
A. Care and handling.
B. Cages housing infected animals.
C. General guidelines that apply to animal room maintenance.
D. Necropsy rules for infected animals.
VI. Decontamination and disposal.
A. Introduction.
B. Decontamination methods.
C. Laboratory spills.
D. Disposal.
E. Characteristics of chemical decontaminants in common use in laboratory operations.
F. Properties of some common decontaminants.
G. Vapors and gases.
H. Residual action of decontaminants
I. Selecting chemical decontaminants for research on recombinant DNA molecules.
Table II.
VII. Housekeeping.
A. Introduction.
B. Floor care.
C. Dry sweeping.
D. Vacuum cleaning.
E. Selection of a cleaning solution.
F. Wet mopping—two-bucket method.
G. Alternative floor cleaning method for animal care areas and areas with monolithic floors.
VIII. Clean-up of biohazardous spills.
A. Biohazardous spill in a biological safety cabinet.
B. Biohazard spill outside a biological safety cabinet.
C. Radioactive biohazard spill outside a biological safety cabinet.
IX. A secondary reservoir and filtration apparatus for vacuum systems.
X. Packaging and shipping.
A. Introduction.
B. Packaging of recombinant DNA materials.
C. Labeling of packages containing recombinant DNA materials.
D. Additional shipping requirements and limitations for recombinant DNA materials.
Table III.
Table IV.
XI. Training aids, materials and courses.
A. Slide-tape cassettes.
B. Films.
C. Courses.
XII. Outline of a safety and operation manual for a P4 facility.
References.

I. BIOLOGICAL SAFETY CABINETS

Biological Safety Cabinets suitable for confining operations involving recombinant DNA molecules are described below:

1. *Class I.* A ventilated cabinet for personnel protection only, with an unrecirculated inward flow of air away from the operator. The exhaust air from this cabinet may be filtered through a high-efficiency or high-efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is suitable for research work with the Center for Disease Control (CDC) classes of etiologic agents 1, 2 and 3 where no product protection is required. This cabinet may be used in three operational modes: (i) With an eight-inch high, full-width open front; (ii) with an installed front closure panel (having four, eight-inch diameter openings) without gloves; and (iii) with an installed front closure panel equipped with arm length rubber gloves. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

2. *Class II.* A ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA-filtered recirculated mass air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. Two models of this cabinet are available, Type 1 and Type 2.

(i) *Type 1.* The Type 1 recirculates approximately 70 percent of the air. The exhaust air from this cabinet may discharge into the laboratory or be diverted out of the laboratory. This cabinet is suitable for CDC classes of etiologic agents 1, 2, and 3. Vapors or gases which are hazardous from a toxic, radioactive, or flammability standpoint should not be used in this cabinet because of the high quantity of recirculated air.

(ii) *Type 2.* The Type 2 cabinet recirculates approximately 30 percent of the air. The exhaust air from this cabinet is normally ducted out of the laboratory through a HEPA filter and, occasionally, an activated charcoal filter depending on the operation. The cabinet may be used with gases or vapors that are hazardous from a toxic, radioactive, or flammability standpoint. However, any consideration of use of such materials should be evaluated carefully from the standpoint of build-up to dangerous levels and problems of decontamination of the cabinet. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

3. *Class III.* A closed front ventilated cabinet of gastight construction providing total protection for personnel and product from contaminants exterior to the cabinet. The cabinet is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is HEPA-filtered. Exhaust air is HEPA-filtered or incinerated to protect the environment. This cabinet, fitted with arm length rubber gloves, provides the highest containment of these three classes of cabinets and is utilized for all activities involving high risk agents (i.e., CDC etiologic agents, class 4). See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

The integrity of any cabinet depends on initial and periodic evaluation to meet established performance tests. Table I outlines the minimum performance required to assure that the cabinets will provide protection of personnel and the environment.

TABLE I

BIOLOGICAL SAFETY CABINETS

SAFETY PERFORMANCE REQUIREMENTS AND SPECIFICATIONS
JUNE 1976

Cabinet	USE CLASSIFICATION		PERFORMANCE REQUIREMENTS				
	BSC[a]	CDC[b]	FACE VELOCITY (linear feet per minute)	EXHAUST AIR (CFM)[c]		LEAK TIGHTNESS	EXHAUST FILTER EFFICIENCY
				4'hood	6'hood		
Class I	P1-P3	1-3	75	300	300	Not applicable	99.97%
Class II, Type 1	P1-P3	1-3	75	360	400	Gas tight; Leak rate < $1x10^{-7}$ cc/sec at 2"wg pressure	99.97%
Class II, Type 2	P1-P3	1-3	100	250	360	Pressure tight; No air/soap bubble at 2"wg pressure	99.97%
Class III	P4	4	Not applicable	d	d	Gas tight; Leak rate < $1x10^{-6}$ cc/sec at 2"wg pressure	99.97%

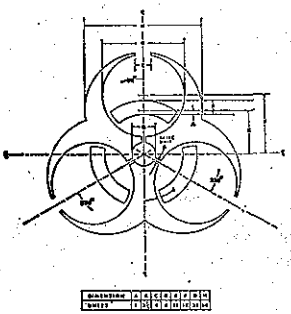
a = For work with recombinant DNA molecules.
b = Center for Disease Control (US Public Health Service).
c = Cubic feet per minute.
d = Based on one volume of air change each 5 minutes, in the absence of unusual heat or moisture that would require more air changes.

APPENDIX D, Page D-4

II. UNIVERSAL BIOHAZARD WARNING SYMBOL (1)

The biological hazard warning symbol (biohazard symbol) specified herein shall be used to signify the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals or combinations thereof which contain or are contaminated with viable hazardous agents.

The biohazard symbol shall be designed and proportioned as illustrated here:



minimum	A	B	C	D	E	F	G
units	7	32	4	9	11	12	14

The symbol shall be as prominent as practical, and of a size consistent with the size of the equipment or material to which it is affixed, provided the proportions shown above are maintained, and, in any case, that the symbol can be easily seen from as many directions as possible.

Except when circumstances do not permit, the symbol shall be oriented with one of the three open circles pointed up and the other two forming a base.

The symbol color shall be a fluorescent orange or orange-red color.* Background color is optional as long as there is sufficient contrast for the symbol to be clearly defined.

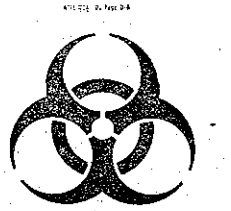


Revised 3-9-76

*Hopeful® Fire Orange of the Seltzer Brothers, Inc. is cited as an example, not an endorsement.

The biohazard symbol shall be used or displayed only to signify the actual or potential presence of biological hazard.

Appropriate wording may be used in association with the symbol to indicate the nature or identity of the hazard, name of individual responsible for its control, precautionary information, etc., but never should this information be superimposed on the symbol. (See next page)



ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Hazard Identity: _____

Responsible Investigator _____

In case of emergency call:

Daytime phone _____ Home phone _____

Authorization for entrance must be obtained from the Responsible Investigator named above.

III. LABORATORY TECHNIQUES FOR BIOHAZARD CONTROL

A. *Pipetting.* 1. No infectious or toxic materials should be pipetted by mouth (2, 3, 4).

2. No infectious mixtures should be prepared by bubbling expiratory air through a liquid with a pipette (2, 3, 4).

3. No infectious material should be blown out of pipettes (2, 3, 4).

4. Pipettes used for the pipetting of infectious or toxic materials should be plugged with cotton (2, 3, 4).

5. Contaminated pipettes should be placed horizontally in a pan containing enough suitable disinfectant to allow complete immersion (2, 3, 4). They should not be placed vertically in a cylinder.

6. The pan and pipettes should be autoclaved as a unit and replaced by a clean pan with fresh disinfectant (2, 3, 4).

7. Infectious material should not be mixed by alternate suction and expulsion through a pipette (2, 3, 4).

8. Mark-to-mark pipettes are preferable to other types, as they do not require expulsion of the last drop (5).

9. Discharge should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle whenever possible—not dropped from a height (5).

10. A disinfectant-wetted towel over the immediate work surface is useful in some cases to minimize the splash from accidental droppage (9).

B. *Syringes and Needles* (9). 1. To lessen the chance of accidental injection, aerosol production or spills, avoid unnecessary use of the syringe and needle. For instance:

(i) Use the needle for parenteral injections but use a blunt needle or a cannula on the syringe for oral or intranasal inoculations.

(ii) Do not use a syringe and needle as a substitute for a pipette in making dilutions of dangerous fluids.

2. Use the syringe and needle in a Biological Safety Cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.

3. Examine glass syringes for chips and cracks, and needles for barbs and plugs.

NOTE: This should be done *prior* to sterilization before use.

4. Use needle-locking (Luer-Lok® type) syringes only, and be sure that the needle is locked securely into the barrel. A disposable syringe-needle unit (where the needle is an integral part of the unit) is preferred.

5. Wear surgical or other type rubber gloves for all manipulations with needles and syringes.

6. Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.

7. Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with the proper disinfectant, or into a small bottle of sterile cotton.

8. Do not use the syringe to expel forcefully a stream of infectious fluid into an open vial or tube for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the needle is held below the surface of the fluid in the tube.

9. If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in transfer of infectious material to the fingers.

10. When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with the proper disinfectant. If there is danger of the disinfectant contaminating sensitive experiments, a sterile dry pledget may be used and discarded immediately into disinfectant solution.

11. Inoculate animals with the hand "behind" the needle to avoid punctures.

12. Be sure the animal is properly restrained prior to the inoculation, and be on the alert for any unexpected movements of the animal.

13. Before and after injection of an animal, swab the site of injection with a disinfectant.

14. Discard syringes into a pan of disinfectant without removing the needle. The syringe first may be filled with disinfectant by immersing the needle and slowly withdrawing the plunger, and finally removing the plunger and placing it separately into the disinfectant. The filling action cleans the needle and dilutes the contents of the syringe. Autoclave syringes and needles in the pan of disinfectant.

15. Use separate pans of disinfectant for disposable and nondisposable syringes and needles to eliminate a sorting problem in the service area.

16. Do not discard syringes and needles into pans containing pipettes or other glassware that must be sorted out from the syringes and needles.

C. *Opening Culture Plates, Tubes, Bottles, and Ampoules.* 1. Plates, tubes and bottles of fungi may release spores in large numbers when opened. Such cultures should be manipulated in a Biological Safety Cabinet (8,15).

2. In the absence of definite accidents or obvious spillage, it is not certain that opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents, some infections have occurred by this means and are represented in the 80% for which no known act or accident is ascribable (3).

3. Water of syneresis in petri dish cultures is usually infected and forms a film between the rim and lid of the inverted

plate. Aerosols are dispersed when this film is broken by opening the plate. Vented plastic petri dishes where the lid touches the rim at only three points are less likely to offer this hazard (3,19).

4. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent, dispersal. If plates are obviously wet they should be opened in the Biological Safety Cabinet (8).

5. Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of infected liquid which may collect between the rim and the liner is broken during removal of the closure (8).

6. Dried, infected culture material may also collect at or near the rim or neck of culture tubes and may be disposed into the air when disturbed (18). Containers of dry powdered hazardous materials, (e.g., Class 3 fungal agents in the spore phase of growth) should be opened only in a Biological Safety Cabinet (6, 14).

7. When the neck of an ampoule containing liquid is broken after nicking with a file, the snapping action creates aerosols. The following methods have been recommended:

(i) After nicking the ampoule with a file, wrap the ampoule in disinfectant-wetted cotton before breaking. Wear gloves (2).

(ii) The bottom of the ampoule should be held in several layers of tissue paper to protect the hands, and a file mark made at the neck. A hot glass rod should be carefully applied to the mark. The glass will crack, allowing air to enter the ampoule and equalize the pressures. After a few seconds the ampoule should be wrapped in a few layers of tissue and broken along the crack. The tissues and ampoule neck can then be discarded into disinfectant, and the contents of the ampoule removed with a syringe. If the ampoule contains dried cultures, about 0.5 cm³ of broth should be added slowly to avoid blowing dried material out. The contents may then be mixed without bubbling and withdrawn into a culture tube (8).

(iii) The researcher uses an intense, but tiny, gas-oxygen flame and heats the tip of the hard glass ampoule until the expanding internal air pressure blows a bubble. After allowing this to cool, he breaks the bubble while holding it in a large low temperature flame; this immediately incinerates any infectious dust which may come from the ampoule when the glass is broken (16). Preliminary practice with a simulant ampoule of the same type actually in use is necessary to develop a technique that will not cause explosion of the ampoule.

(iv) A simple device has been recommended consisting of a sleeve of rubber tubing into which the ampoule is inserted before it is broken (17,18).

D. Centrifuging. 1. A safety centrifuge cabinet or safety centrifuge cup (3,7,8,14,22) may be used to house or safeguard all centrifuging of infectious substances. When bench type centrifuges are used in a Biological Safety Cabinet, the glove panel should be in place with the glove ports covered. The centrifuge operation creates air currents that may cause escape of agent from an open cabinet (2,3,4,13).

2. In some situations, in the absence of O-ring cap sealed trunnion cups, specimens can be enclosed in sealed plastic bags before centrifugation (12).

3. Before centrifuging, inspect tubes for cracks, inspect the inside of the trunnion cup for rough walls caused by erosion or adhering matter, and carefully remove bits of glass from the rubber cushion (4,10).

4. A germicidal solution should be added between the tube and trunnion cup to disinfect the materials in case of accidental breakage. This practice also provides an excellent cushion against shocks that might otherwise break the tube (4,10).

5. Avoid decanting centrifuge tubes. If you must do so, afterwards wipe off the outer rim with a disinfectant; otherwise the infectious fluid will spin off as an aerosol (4, 10).

6. Avoid filling the tube to the point that the rim, cap or cotton plug ever becomes wet with culture (4, 10).

7. Screw caps, or caps which fit over the rim outside the centrifuge tube are safer than plug-in closures. Some fluid usually collects betwen a plug-in closure and the rim of the tube. Even screw-capped bottles are not without risk, however; if the rim is soiled some fluid will escape down the outside of the tube. Screw-capped bottles may jam in the bucket, and removing them is hazardous. Propping such bottles higher in the bucket with additional rubber buffers is mechanically unsound (8).

8. Kitchen foil is often used to cap centrifuge tubes. This creates more risk then the screw cap. Foil caps often become detached in handling and centrifuging (8).

9. The balancing of buckets is often mismanaged. Care must be taken to ensure that matched sets of trunnios, buckets and plastic inserts do not become mixed. If the components are not inscribed with their weights by the manufacturer, colored stains can be applied to avoid confusion. When the tubes are balanced, the buckets, trunnions and inserts should be included in the procedure; and care must be taken to ensure that the centers of gravity of the tubes are equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20g of mercury and 20g of water respectively will balance perfectly on the scales; but their performance in motion is totally different, leading to violent vibration with all its attendant hazards (5).

10. Fill and open centrifuge tubes or trunnion cups in a Biological Safety Cabinet (10).

E. High-Speed Centrifuges (22). 1. In high-speed centrifuges the bowl is connected to a vacuum pump. If there is a breakage or accidental dispersion of infected particles the pump and the oil in it will become contaminated. A high efficiency filter should be placed between the centrifuge and the pump (8).

2. High speed rotor heads are prone to metal fatigue, and where there is a chance that they may be used on more than one machine each rotor should be accompanied by its own log book indicating the number of hours run at top or de-rated speeds. Failure to observe this precaution can result in dangerous and expensive disintegration. Frequent inspection, cleaning and drying are important to ensure absence of corrosion or other traumata which may lead to creeping cracks. Rubber O-rings and tube closures must be examined for deterioration and be kept lubricated with the material recommended by the makers. Where tubes of different materials are provided (e.g., celluloid, polypropylene, stainless steel), care must be taken that the tube closures designed specifically for the type of tube in use are employed. These caps are often similar in appearance, but are prone to leakage if applied to tubes of the wrong material. When properly designed tubes and rotors are well maintained and handled, leaking should never occur (5).

3. Cleaning and disinfection of tubes, rotors and other components requires considerable care. It is unfortunate that no single process is suitable for all items, and the various manufacturers' recommendations must be followed meticulously if fatigue, distortion and corrosion are to be avoided. This is not the place to catalogue recommended methods, but one less well appreciated fact is worthy of mention. Celluloid (cellulose nitrate) centrifuge tubes are not only highly inflammable and prone to shrinkage with age and distortion on boiling, but can behave as high explosive in an autoclave (5). Large-scale zonal centrifugation requires special attention (11).

F. Blenders, ultrasonic disintegrators, colloid mills, ball mills, jet mills, grinders, motar and pestle. All these devices release considerable aerosols during their operation. For maximum protection to the operator during the blending of infectious materials, the following practices should be observed:

1. Operate blending and cell-disruption and grinding equipment in a Biological Safety Cabinet (9).

2. Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl (9).

3. In the absence of a leak-proof rotor, inspect the rotor bearing at the bottom of the blender bowl for leakage prior to operation. Test it in a preliminary run with sterile saline or methylene blue solution prior to use with infected material (9).

4. Sterilize the device and residual infectious contents promptly after use. Use a towel moistened with disinfectant over the top of the blender (9).

5. Glass blender bowls are undesirable for use with infectious material because of potential breakage. If used, they should be covered with a polypropylene jar to prevent dispersal of glass (8).

6. A new machine, the Colworth Stomacker (England), in which material is homogenized in a plastic bag in a closed container, would appear to be safer than some of the other blenders (8).

7. A heat-sealed flexible plastic film enclosure for a grinder or blender can be used, but it must be opened in a Biological Safety Cabinet (7).

8. Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal efforts on the product (7).

9. Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.

10. Clinical or other laboratories handling human blood should be aware of the aerosols produced by the microhaematocrit centrifuge, the autoanalyzer stirrer, and the micotonometer, inasmuch as it seems that airborne transmission of infectious hepatitis may occur in the laboratory (20).

G. Miscellaneous precautions and recommendations. 1. Water baths and Warburg baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70 percent propylene glycol is recommended (4, 10).

2. Deepfreeze, liquid nitroger, and dry ice chests and refrigerators should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deepfreezes should be properly labelled. Security measures should be commensurate with the hazards (4,10,21).

3. Freeze-dried culture ampoules should always be opened in a Biological Safety Cabinet. The ampoule should be wrapped in a disinfectant-soaked swab before breaking it open to minimize the risk of cutting the hands, and to a lesser extent of releasing

aerosol of dried material. Whenever possible, ampoules should be filled with dry nitrogen after freeze-drying, thus avoiding implosion that may occur during the sealing as well as opening of evacuated ampoules. The whole process of freeze-drying itself should be performed in a Biological Safety Cabinet. Filtration of the effluent air from the vacuum pump is desirable either up (preferably), or down stream of the pump (5).

4. Ensure that all virulent fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, nonbreakable leak-proof containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel (4,10).

5. All inoculated petri plates or other inoculated solid media should be transported and incubated in leak-proof pans or leak-proof containers (4,10).

6. Care must be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as noninfectious until culture or other tests have proved their sterility (4,10).

7. Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.

8. No person should work alone on an extremely hazardous operation (4,10).

IV. PERSONAL HYGIENE, HABITS, AND PRACTICES

Personal hygienic practices in the laboratory are directed, in most part, toward the prevention of occupationally acquired physical injury or disease. To a less obvious extent, they can raise the quality of the laboratory work by reducing the possibilities for contamination of experimental materials. The reasons for many of the recommended precautions and practices are obvious, but, in some instances, amplification will permit a better review of the applicability to any one specific laboratory.

Consequently, what might be forbidden in one laboratory might be only discouraged in another, and be permissible in a third. Nevertheless, adherence to safe practices that become habitual, even when seemingly not essential, provides a margin of safety in situations where the hazard is unrecognized. The history of occupational injury is replete with examples of hazards unrecognized until too late. The following guidelines, recommendations, and comments are presented with this in mind:

1. Food, candy, gum, and beverages for human consumption will be stored and consumed only outside the laboratory (5, 10).

2. Foot-operated drinking fountains should be the sole source of water for drinking by human occupants of the laboratory (27).

3. Smoking is not permitted in the laboratory or animal quarters. Cigarettes, pipes, and tobacco will be kept only in clean areas (5, 10, 26).

4. Shaving and brushing of teeth are not permitted in the laboratory. Razors, toothbrushes, toiletry supplies, and cosmetics are permissible only in clean change rooms or other clean areas, and should never be used until after showering or thorough washing of the face and hands (27).

5. A beard may be undesirable in the laboratory in the presence of actual or potential airborne contamination, because it retains particulate contamination more persistently than clean-shaven skin. A clean-shaven face is essential to the adequate facial fit of a face mask or respirator when the work requires respiratory protection (10,27,31).

6. Develop the habit of keeping hands away from mouth, nose, eyes, face, and hair. This may prevent self-inoculation (10,27).

7. For product protection, persons with long hair should wear a suitable hair net or head cover that can be decontaminated. This has long been a requirement in hospital operating rooms and in the manufacture of biological pharmaceutical products. A head cover also will protect the hair from fluid splashes, from swinging into Bunsen flames and petri dishes, and will reduce facial contamination caused by habitual repetitive manual adjustment of the hair (5).

8. Long-flowing hair and loose-flapping clothing are dangerous in the presence of open flame or moving machinery. Rings and wrist watches also are a mechanical hazard during operation of some types of machines (5, 10).

9. Contact lenses do not provide eye protection. The capillary space between the contact lenses and the cornea may trap any material present on the surface of the eye. Caustic chemicals trapped in this space cannot be washed off the surface of the cornea. If the material in the eye is painful or the contact lens is displaced, muscle spasms will make it very difficult, if not impossible, to remove the lens. For this reason, contact lenses must not be worn by persons exposed to caustic chemicals unless safety glasses with side shields, goggles, or plastic face masks are also worn to provide full protection. It is the responsibility of supervisors to identify employees who wear contact lenses (25, 26).

10. Personal items, such as coats, hats, storm rubbers or overshoes, umbrellas, purses, etc., do not belong in the laboratory. These articles should be kept elsewhere (25).

11. Plants, cut flowers, an aquarium, and pets of any kind are undesirable sources of yeast, molds, and other potential microbial contaminants of biological experimental materials (25).

12. Books and journals returnable to the institutional library should be used only in the clean areas as much as possible (10,27).

13. When change rooms with showers are provided, the employer should furnish skin lotion (27).

14. When employees are subject to potential occupational infection, the shower and/or face/hand-washing facilities should be provided with germicidal soap (8,27).

15. Personal cloth handkerchiefs should not be used in the laboratory. Cleansing tissue should be available instead.

16. Hand washing for personal protection:

(i) This should be done promptly after removing protective gloves. Tests show it is not unusual for microbial or chemical contamination to be present despite use of gloves, due to unrecognized small holes, abrasions, tears, or entry at the wrist.

(ii) Throughout the day, at intervals dictated by the nature of the work, the hands should be washed. Presence of a wrist watch discourages adequate washing of the wrist (10,25).

(iii) Hands should be washed after removing soiled protective clothing, before leaving the laboratory area, before eating, and before smoking. The provision of hand cream by the employer encourages these practices (5,8,10).

(iv) A disinfectant-wash or dip may be desirable in some cases, but its use must not be carried to the point of causing roughening, desiccation or sensitization of the skin.

17. Anyone with a fresh or healing cut, abrasion, or skin lesion should not work with infective material unless the injured area is completely protected (8,25).

18. Persons vaccinated for smallpox may be shedders of vaccinia virus during the phase of cutaneous reaction. Therefore, vaccination requires permission of the appropriate supervisor, because two weeks' absence may be necessary before returning to work with normal cell cultures or with susceptible animals, especially the normal mouse colony (25).

19. The surgeon's mask of gauze or filter paper is of little value for personal respiratory protection (29). It is designed to prevent escape of droplets from the nose or mouth (23G). If biohazards demand respiratory protection, then nothing but a full face respirator or ventilated hood will suffice. A half-mask respirator does not protect the eyes, which are an unevaluated avenue of infection through the conjuctiva and the nasolacrimal duct (5,8).

20. Nonspecific contamination by environmental organisms from humans, animals, equipment, containers for specimens or supplies, and outside air is a complication that may affect or invalidate the results of an experiment. The human sources of this contamination are evaluated as follows:

(i) Sneezing, coughing and talking (23A, 24A). Sneezing, variously reported to generate as many as 32,000 or 1,000,000 droplets below 100 microns in diameter; coughing, which produces fewer and larger droplets; and talking, which has been reported to average only 250 droplets when speaking 100 words, show great differences between persons in regard to the number of microorganisms aerosolized. As a general rule, it may be said that these actions by normal healthy persons may play a less important role in transmission of airborne infection to humans or experimental materials than does liberation of microorganisms from human skin.

(ii) Dispersal of bacteria from human skin. There is a tremendous variation in the number of bacteria shed from the skin by a clothed subject. For instance, in one study, the number varied from 6,000 to 60,000 per minute (23C). These bacteria were released on skin scales which were of a size that could penetrate the coarse fabric used for the laboratory and surgical clothing in the test (23D). Dispersal of skin bacteria was several times greater from below the waist than from upper parts of the body (24D). Effective reduction is accomplished by use of closely-woven or impervious clothing fitted tightly at the neck, wrists, and ankles to prevent the clothing from acting as a bellows that disperses air carrying skin scales laden with bacteria (23B). Such clothing sometimes is too warm to work in. It was found that a significant reduction in dispersal of bacteria occurred with the wearing of close-fitting and closely-woven underpants beneath the usual laboratory clothing (23D). The purpose of this summary is to alert laboratory personnel to the existence of this source of contamination (9).

(iii) Pyolific dispersal of bacteria occurs from infected abrasions, small pustules, boils, and skin disease (23F, 24B). Washing the lesions with germicidal soap will greatly decrease the number of organisms on the skin and dispersal into the air. Healthy nasal carriers who generate aerosolized staphylococci usually can be identified by the presence of heavy contamination of their fingers, face, and hair (23E). This point may be useful in investigating the source of staphylococcal contamination of cell lines.

(iv) Footwear. In moderate and high risk situations, shoes reserved for only laboratory use have been recommended as a precaution against transporting spilled infectious agents outside the laboratory. However, in experiments during which reduction of potential contamination of experimental materials is important, laboratory-only shoes can reduce the microbial load brought into the

laboratory each day by street shoes. Shoes are efficient transporters. In one study, there were 4 to 850 times as many bacteria per square centimeter on the laboratory footwear as on the floor itself (30).

V. CARE AND USE OF LABORATORY ANIMALS (10,32–37)

A. *Care and handling.* 1. Special attention must be given to the humane treatment of all laboratory animals in accordance with the Animal Welfare Act of 1970. The implementing rules and regulations appear in the Code of Federal Regulations (CFR) Title 9, Chapter I, Subchapter A, Parts 1, 2, 3. Recommended provisions and practices that meet the requirements of the Act have been published by the U.S. Public Health Service (32).

2. There are specific minimum requirements (33) concerning the caging, feeding, watering, and sanitation for dogs, cats, guinea pigs, hamsters, rabbits, and nonhuman primates. To meet these requirements, the animal room supervisor must have a copy of 9 CFR Chapter I, Subchapter A, Parts 1, 2, 3.

3. Each laboratory should establish procedures to ensure the use of animals that are free of diseases prejudicial to the proposed experiments and free from carriers of disease or vectors, such as ectoparasites, which endanger other experimental animals or personnel (10).

B. *Cages housing infected animals* (10). 1. Careful handling procedures should be employed to minimize the dissemination of dust from cage refuse and animals.

2. Cages should be sterilized by autoclaving. Refuse, bowls and watering devices should remain in the cage during sterilization.

3. All watering devices should be of the "non-drip" type.

4. Cages should be examined each morning and at each feeding time so that dead animals can be removed.

5. Heavy gloves should be worn when feeding, watering, handling, or removing infected animals. Bare hands should NEVER be placed in the cage to move any object therein.

6. When animals are to be injected with biohazardous material, the animal caretaker should wear protective gloves and the laboratory workers should wear surgeons gloves. Animals should be properly restrained to avoid accidents that might result in disseminating biohazardous material, as well as to prevent injury to the animal and to personnel.

7. Animals exposed to biohazardous aerosols should be housed in ventilated cages, in gas-tight cabinet systems, or in rooms designed for protection of personnel by use of ventilated suits.

8. Animals inoculated by means other than by aerosols should be housed in equipment suitable for the level of risk involved.

9. Infected animals to be transferred between buildings should be placed in ventilated cages or other aerosol-proof containers.

10. The oversize canine teeth of large monkeys present a particular biting hazard; these are important in the potential transmission of naturally-occurring, and very dangerous, monkey virus infections. Such teeth should be blunted or surgically removed by a veterinarian.

11. Presently available epidemiological evidence indicates that infectious hepatitis may be transmitted from non-human primates (typically chimpanzees) to man. Newly imported animals may be naturally infected with this disease, and persons in close contact with such animals may become infected. After six months residence in this country, chimpanzees apparently no longer transmit the disease. A record should be maintained for each newly imported animal. A sign should be posted at rooms housing these animals to warn that the animals are potentially infectious.

C. *General Guidelines that Apply to Animal Room Maintenance* (10). 1. Doors to animal rooms should be kept closed at all times except for necessary entrance and exit.

2. Unauthorized persons should not be permitted to enter animal rooms.

3. A container of disinfectant should be kept in each animal room for disinfecting gloves and hands, and for general decontamination, even though no infectious animals are present. Hands, floors, walls, and cage racks should be washed with an approved disinfectant at the recommended strength as frequently as the supervisor directs.

4. Floor drains in animal rooms, as well as floor drains throughout the building should be flooded with water or disinfectant periodically to prevent backup of sewer gases.

5. Shavings or other refuse on floors should not be washed down the floor drain because such refuse clogs the sewer lines.

6. An insect and rodent control program should be maintained in all animal rooms and in animal food storage areas.

7. Special care should be taken to prevent live animals, especially mice, from finding their way into disposable trash.

D. *Necropsy rules for infected animals* (10). 1. Necropsy of infected animals should be carried out by trained personnel in Biological Safety Cabinets with the hinged glass panel down. The glove port panel with or without attached gloves, and a respirator should be used at the discretion of the supervisor.

2. Surgeons gowns should be worn over laboratory clothing during necropsies.

3. Rubber gloves should be worn when performing necropsies.

4. The fur of the animal should be wetted with a suitable disinfectant.

5. Small animals should be pinned down or fastened on wood or metal in a metal tray.

6. Upon completion of necropsy, all potentially biohazardous material should be placed in suitable containers and sterilized immediately.

7. Contaminated instruments should be placed in a horizontal bath containing a suitable disinfectant.

8. The inside of the Biological Safety Cabinets and other potentially contaminated surfaces should be disinfected with a suitable germicide.

9. Grossly contaminated rubber gloves should be cleaned in disinfectant before removal from the hands, preparatory to sterilization.

10. Dead animals should be placed in proper leak-proof containers, autoclaved and properly tagged before being placed outside for removal and incineration.

VI. DECONTAMINATION AND DISPOSAL (7, 10, 38–42)

A. *Introduction.* Available data on the efficacy of various decontaminants for etiologic agents indicate that no major surprises will be forthcoming regarding the susceptibility of organisms containing recombinant DNA molecules. In the absence of adequate information, tests to determine the efficacy of candidate decontaminants should be conducted with the specific agent of interest. The goal of decontamination is not only the protection of personnel and the environment from exposure to infectious agents, but also the prevention of contamination of experimental materials by a variable, persistent, and unwanted background of microorganisms. This additional factor should be considered in selecting decontamination materials and methods.

B. *Decontamination Methods.* Physical and chemical means of decontamination fall into four main categories: Heat; Liquid Decontaminants; Vapors and Gases; and UV Radiation.

1. *Heat.* The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121 C under pressure in the autoclave is the most convenient method of rapidly achieving sterility. Dry heat at 160 to 170 C for periods of 2 to 4 hours is suitable for destruction of viable agents on impermeable non-organic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat in the decontamination of microorganisms and also serves as an efficient means for disposal.

2. *Liquid Decontaminants.* In general, the liquid decontaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems. There are many misconceptions concerning the use of liquid decontaminants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, time of contact, pH, concentration, and the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in effectiveness of decontamination. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants when the end result must be sterility.

There are many liquid decontaminants available under a wide variety of trade names. In general, these can be categorized as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols and amines. Unfortunately, the more active the decontaminant the more likely it is that the decontaminant will possess undesirable characteristics, such as the possession of corrosive properties. None is equally useful or effective under all conditions.

3. *Vapors and Gases.* A variety of vapors and gases possess decontamination properties. The most useful of these are formaldehyde and ethylene oxide. When these can be employed in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas decontaminants are primarily useful in decontaminating: (i) Biological Safety Cabinets and associated effluent air-handling systems and air filters; (ii) bulky or stationary equipment that resists pentration by liquid surface decontaminants; (iii) instruments and optics that might be damaged by other decontamination methods; and (iv) rooms and buildings and associated air-handling systems.

4. *Radiation.* The usefulness of ultraviolet (UV) irradiation as a decontaminant is limited by its low penetrating power. No information is available regarding the effectiveness of UV irradiation for decontaminating microorganisms containing recombinant DNA molecules. Dependence on UV must be based on the results of experiments imitating particular anticipated environmental conditions and applications. Ultraviolet light is generally of limited application and is primarily useful in air locks and animal holding areas for controlling low levels of airborne contaminants.

No one procedure or material will solve all decontamination problems. The only method of assuring the efficacy of selected methodologies is to critically examine the results

obtained in practical tests with the microorganism(s) of interest.

C. *Laboratory spills.* A troublesome problem that may occur in the laboratory is the decontamination of an overt *biological spill.* The occurrence of a spill poses less of a problem if it occurs in a Biological Safety Cabinet provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid decontaminant and a thorough wipe down of the internal surfaces of such cabinetry will usually be effective for decontaminating the work zone but gaseous decontaminants would be required to rid the interior sections of the cabinet of contaminants. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be lost to the experiment.

The greater problem arises if the incident occurs in the open laboratory. All laboratory protocols should be designed to prevent such occurrences. The first action in the event of an overt laboratory spill is evacuation of the affected area to minimize the exposure of personnel involved. Next, the spill area must be isolated to prevent exposure of personnel and experimental materials beyond those involved in the immediate area of the spill. The procedures adopted must be rapidly effective *and must not create additional aerosol* or foster mechanical transfer of materials to unaffected areas. Personnel carrying out the procedures must be provided with protective clothing and equipment, including respiratory protection. Consideration must be given to the safe disposal of all materials and liquids resulting from cleanup procedures. Reentry of personnel to the area should be avoided until it can be reasonably established that the area has been effectively *decontaminated. Further specific details are* provided in Section VIII.

D. *Disposal.* Decontamination and disposal in infectious disease laboratories are closely interrelated acts in which decontamination constitutes the introductory phase of disposal. All materials and equipment used in research on recombinant DNA molecules will ultimately be disposed of; however, in the sense of daily use, only a portion of these will require actual removal from the laboratory complex or *on-site destruction.* The remainder will be recycled for use either within the same laboratory or in other laboratories that may or may not engage in DNA recombinant research. Examples of the latter that immediately come to mind are: Reusable laboratory glassware, instruments used in necropsy of infected animals, and laboratory clothing. Disposal should therefore be interpreted in the broadest sense of the word, rather than in the restrictive sense of dealing solely with a destructive process.

The principal questions to be answered prior to disposal of any objects or materials from laboratories dealing with potentially infectious microorganisms or animal tissues are:

1. Have the objects or materials been effectively decontaminated by an approved procedure?

2. If not, have the objects or materials been packaged in an approved manner for immediate on-site incineration or transfer to another laboratory?

3. Does disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to *personnel either:* (i) *Those carrying out* the immediate disposal procedures or (ii) Those who might come into contact with the objects or materials outside the laboratory complex?

Laboratory materials requiring disposal will normally occur as liquid, solid, and animal room wastes. The volume of these can become a major problem when there is the requirement that all wastes be decontaminated prior to disposal. It is most evident that a significant portion of this *problem can be eliminated if the kinds of* materials initially entering the laboratory are reduced. In any case, and wherever possible, materials not essential to the research should be retained in the nonresearch areas for disposal by conventional methods. Examples are the packaging materials in which goods are delivered, disposable carton-cages for transport of animals, and large carboys or tanks of fluids which can be left outside and drawn from as required. Reduction of *this bulk will free* autoclaves and other decontamination and disposal processes within the laboratory for the more rapid and efficient handling of materials known to be contaminated.

Inevitably, disposal of materials raises the question, "How can we be sure that the materials have been treated adequately to assure that their disposal does not constitute a hazard?" In the small laboratory, the problem is often solved by requiring that each investigator decontaminate all contaminated *materials* not of immediate use at the end of each day and place them in suitable containers for routine disposal. In larger laboratories where the mass of materials for *disposal becomes much greater and sterilization* and decontamination bottlenecks occur, materials handling and disposal will likely be the chore of personnel not engaged in the actual research. In either situation, a case can be made for establishing a positive method of designating the state of materials to be disposed of. This may consist of a tagging system stating that the materials are either *sterile* or contaminated.

Disposal of materials from the laboratory and animal *holding areas will be required for* research projects ranging in size from an individual researcher to those involving large numbers of personnel of many disciplines. Procedures and facilities to accomplish this will range from the simplest to the most elaborate. The primary consideration in any of these is to dispel the notion that laboratory wastes can be disposed of in the same manner and with as little thought as household wastes. Selection and enforcement of safe procedures for disposal of laboratory *materials* are of no less importance than the consideration given to any other methodology for the accomplishment of research objectives.

Materials of dissimilar nature will be common in laboratories studying recombinant DNA molecules. Examples are combinations of common flammable solvents, chemical carcinogens, radioactive isotopes, and concentrated viruses or nucleic acids. These may require input from a number of disciplines in arriving at the most practical approach for their decontamination.

E. Characteristics of chemical decontaminants in common use in laboratory operations. Every person actively working with viable microorganisms, no matter how remote the field of specialization, will, from time to time, find it necessary to decontaminate by chemical methods work areas and materials, equipment, and specialized instruments. Chemical decontamination is necessary because the use of pressurized steam, the most rapid and reliable method of sterilization, is not normally feasible for decontaminating large spaces, surfaces, and stationary equipment. *Moreover, high temperatures* and moisture often damage delicate instruments, particularly those having complex optical and electronic components.

Chemicals with decontaminant properties are, for the most part, available as powders, crystals, and liquid concentrates. These may be added to tap water for application as surface decontaminants, and some, when added in sufficient quantity, find use as decontaminants of bulk liquid wastes. Chemical decontaminants that are gaseous at room temperatures *are useful as space-penetrating* decontaminants. Others become gases at reasonably elevated temperatures and can act as either aqueous surface or gaseous space-penetrating decontaminants.

Inactivation of microorganisms by chemical decontaminants may occur in one or more of the following ways: (1) Coagulation and denaturation of protein, (2) Lysis, (3) Binding to enzymes, or inactivation of an essential enzyme by either oxidation, binding, or destruction of enzyme substrate.

The relative resistance to the action of chemical decontaminants can be substantially altered by such factors as: Concentration of active ingredient, duration of contact, pH, temperature, humidity and presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms *to an indicated sterility within the limits of* sensitivity of the assay systems employed.

There are dozens of contaminants available under a wide variety of trade names. In general, these decontaminants can be classified as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the decontaminant the more likely it will possess undesirable char*acteristics. For example, peracetic acid is a* fast-acting, universal decontaminant. However, in the concentrated state it is a hazardous compound that can readily decompose with explosive violence. When diluted for use, it has a short half-life, produces strong, pungent, irritating odors, and is extremely corrosive to metals. Nevertheless, it is such an outstanding decontaminant that it is commonly used in germ-free animal studies despite these undesirable characteristics.

The halogens are probably the second most active group of decontaminants. Chlorine, iodine, bromine, and fluorine will rapidly kill bacterial spores, viruses, rickettsiae, and fungi. These decontaminants are effective over a wide range of temperatures. In fact, chlorine has been shown to be effective at −40 F. (On the other hand, phenols and formaldehyde have high temperature coefficients). The halogens have several undesirable features. They readily combine with *protein, so that an excess of the halogen* must be used if proteins are present. Also, the halogens are relatively unstable so that fresh solutions must be prepared at frequent intervals. Finally, the halogens corrode metals. A number of manufacturers of decontaminants have treated the halogens to remove some of the undesirable features. For example, sodium hypochlorite reacts with p-toluenesulfonamide to form Chloramine T, and iodine reacts with certain surface-active agents to form the popular *Iodophors.* These "tamed" halogens are stable, non-toxic, odorless, and relatively noncorrosive to metals. However, the halogens are highly reactive elements, and, because they are reactive they are good germicides. When a halogen acts as a decontaminant, free halogen is the effective agent. Raising the pH or combining the halogen with other compounds to decrease the corrosive effect will also decrease the germicidal power. A trade-off *situation* occurs.

Ineffectiveness of a decontaminant is due primarily to the failure of the decontaminant to contact the microorganisms *rather than failure of the decon*taminant to act. If one places an item in

a liquid decontaminant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. Also, if there are spots of grease, rust or dirt on the object, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful, and a decontaminant should have, and most do have, incorporated surface-active agents.

F. *Properties of some common decontaminants*—1. *Alcohol.* Ethyl or isopropyl alcohol in a concentration of 70–80 percent by weight is often used. Alcohols denature proteins and are somewhat slow in their germicidal action. However, they are effective decontaminants against lipid-containing viruses.

2. *Ether and Chloroform.* These compounds are not ordinarily used as decontaminants, but they do demonstrate the fact that lipid-containing viruses are inactivated by these organic solvents, whereas non-lipid-containing viruses are quite resistant.

3. *Formaldehyde.* Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37 percent concentration referred to as formalin or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5 percent active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures and the pungent, irritating odors make formaldehyde solutions difficult to use in the laboratory. Formaldehyde vapor generated from formaldehyde solution is an effective space decontaminant for decontaminating rooms or buildings, but in the vapor state with water it tends to polymerize out on surfaces to form paraformaldehyde, which is persistent and unpleasant. Formaldehyde gas can be liberated by heating paraformaldehyde to depolymerize it. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than formaldehyde released in the vapor state.

4. *Phenol.* Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against some viruses, rickettsiae, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.

5. *Quaternary Ammonium Compounds or Quats.* After 30 years of testing and use, there is still a considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses. The Quats will attach to protein so that dilute solutions of Quats will quickly lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The Quats have the advantages of being nontoxic, odorless, nonstaining, noncorrosive to metals, stable, and inexpensive.

6. *Chlorine.* This halogen is a universal decontaminant active against all microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently. Sodium hypochloride is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25 percent available chlorine or 52,500 ppm. If one dilutes them 1 to 100, the solution will contain 525 ppm of available chlorine, and, if a nonionic detergent such as Naccanol is added in a concentration of about 0.7 percent, a very good decontaminant is created.

7. *Iodine.* The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants used in the laboratory is the iodophors, and Wescodyne is perhaps the most popular. The *range of dilution of Wescodyne recommended* by the manufacturer is 1 oz. in 5 gals. of water giving 25 ppm of available iodine to 3 oz. in 5 gal. giving 75 ppm. At 75 ppm, the concentration of free iodine is .0075 percent. This small amount can be rapidly taken up by any extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For bacterial spores, a dilution of 1 to 40 giving 750 ppm is recommended by the manufacturer. For washing the hands, it is recommended that Wescodyne be diluted 1 to 10 or 10 percent in 50 percent ethyl alcohol (a reasonably good decontaminant itself) which will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

G. *Vapors and gases.* The use of formaldehyde as a vapor or gas has already been discussed. Other chemical decontaminants which have been used this way included ethylene oxide, peracetic acid, beta-propiolactone (BPL), methyl bromide, and ethylene amine. When these can be used in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can be obtained. Residues from ethylene oxide must be removed by aeration; but otherwise it is convenient to use, versatile, and noncorrosive. Paracetic acid is corrosive for metals and rubber. BPL in the vapor form acts rapidly against bacteria, rickettsiae, and viruses. It has a half-life of 3.5 hours when mixed with water, is easily neutralized with water, and lends itself to removal by aeration. The National Institutes of Health does not recommend BPL as a decontaminant because it has been identified as a suspect carcinogen.

H. *Residual action of decontaminants.* As noted in the preceding discussion of decontaminant properties, many of the chemical decontaminants often have residual properties that may be considered a desirable feature in terms of aiding in the control of background contamination. One is cautioned, however, to consider residual properties carefully. Ethylene oxide used to sterilize laboratory shoes can leave residues which cause skin irritation. Animal cell cultures, as well as viruses of interest, are also inhibited or inactivated by decontaminants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in liquid decontaminant prior to autoclaving and cleaning should receive particular attention in rinse cycles. Similarly, during general area decontamination with gases or vapors, it may be necessary to protect new and used clean items by removing them from the area or by enclosing them in gastight bags or by insuring adequate aeration following decontamination.

I. *Selecting chemical decontaminants for research on recombinant DNA molecules.* No single chemical decontaminant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical decontaminants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

1. What is the target microorganism(s)?

2. What decontaminants in what form are known to, or can be expected to, inactivate the target microorganism(s)?

3. What degree of inactivation is required?

4. In what menstruum is the microorganism suspended; i.e., simple or complex, on solid or porous surfaces, and/or airborne?

5. What is the highest concentration of *cells anticipated to be encountered?*

6. Can the decontaminant either as an aqueous solution, a vapor, or a gas reasonably be expected to contact the microorganisms, and can effective duration of contact be maintained?

7. What restrictions apply with respect to compatibility of materials?

8. Does the anticipated use situation require immediate availability of an effective concentration of the decontaminant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the infectious disease laboratory is the microorganism under active investigation. Laboratory preparations or infectious agents usually have titers grossly in excess of those normally observed in nature. The decontamination of these high-titer materials presents certain problems. Maintenance systems for bacteria or viruses are specifically selected to preserve viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interferences with the desired action of decontaminants may require the use of decontaminant concentrations and contact times in excess of those shown to be effective in the test tube. Similarly, a major portion of decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a *relatively small number of the* more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these survivors. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting decontaminants and procedures for their use.

Microorganisms exhibit a range of resistance to chemical decontaminants. In terms of practical decontamination, most vegetative bacteria, fungi and lipid-containing viruses, are relatively susceptible to chemical decontamination. The non-lipid-containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Spore forms are the most resistant.

A decontaminant selected on the basis of its effectiveness against microorganisms on any range of the resistance scale will be ef-

fective against microorganisms lower on the scale. Therefore, if decontaminants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other microorganisms generated by laboratory operations, even in high concentrations, would also be inactivated.

An additional area that must be considered and for which there is little definitive information available is the "inactivation" of nucleic acids. Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Strong oxidizers, strong acids and bases, and either gaseous or aqueous formaldehyde should react readily with nucleic acids. Their ability to destroy the nucleic acid being studied, however, should be confirmed in the experimenter's laboratory. Because of innate differences in the chemistry of RNA and DNA the effectiveness of a decontaminant for one cannot be extrapolated to the other. For example, RNA molecules are susceptible to mild alkaline hydrolysis by virtue of the free hydroxyl group in the 2' position, whereas DNA molecules are not susceptible to mild alkaline hydrolysis.

Table II summarizes pertinent characteristics and potential applications for several categories of chemical decontaminants most likely to be used in the biological laboratory. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstruums. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. The efficacy of any of the decontaminants should be conclusively determined by individual investigators. It is readily evident that each of the decontaminants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflor. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of decontaminants.

APPENDIX B

TABLE II

SUMMARY OF PRACTICAL RECOMMENDATIONS FOR USE IN THE LABORATORY

Recommendations		Practical requirements			Inactivates				Important characteristics															Potential applications								Suggested decontaminant dosages
		Concentration and contact time (minutes)	Contact time (minutes)	Room temperature	Gram-positive bacteria	Gram-negative bacteria	Mycobacterium tuberculosis	Spores	Effective over a wide pH range	Corrosive	Flammable	Explosive mixtures	Residue	Inactivated by organic matter	Can complicate	Electrolyte effects	Safe to handle	Safe to use	Stable	Relatively inexpensive	Toxic to humans	Irritating to skin	Irritating to eyes	Odor	Hard surfaces	Rugs, upholstery	Lab. and sink surfaces	Air	Animal cages, bedding, waste, etc.	Equipment, tools, surface work areas, floors, etc.	Animal quarters, surface work areas, floors, etc.	Laundry, instruments, equipment, etc.	Living tissue (skin, eyes)	Soil, waste	
Type	Chemical																															
Halogens	Sod. hypo. CP/T	2%	10	R.T.	+	+	+		+					+		+		+		+				+	✓	✓			✓				0.1%, CH, HO-HC, H-HC, HCHO-CHO
	Hypochl. CP/T	2%	10	R.T.	+	+	+		+					+		+		+		+				+	✓	✓			✓				HC-HCHO, HCHO, HCHO-CHO, H-HC,
	Chloramine CP/T	2%	10	R.T.	+	+	+		+					+		+		+		+				+	✓	✓			✓				CHLORINE T, CLOROX, PUREX
	Iodophor	2%	10	R.T.	+	+	+		+					+		+		+		+				+	✓	✓			✓				H-HC, HCHO, HCHO-CHO, HCHO-CHO
	Phenol, Tech.	5%	10	R.T.	+	+	?		+									+		+				+	✓	✓			✓				
	Phenol, Isopropyl	70%	10	R.T.	+	+	?	+						+		+		+		+				+	✓	✓			✓				
	Phenolsulfonate	2%	10	R.T.	+	+	+	+						+		+		+		+				+	✓	✓			✓				PHENOL
	Hydrochloride	2%	10	R.T.	+	+	+	+						+		+		+		+				+	✓	✓			✓				CHEM
Gas	Ethylene Oxide	12g/l	60	60	10	10	+	+																					✓				CHEMICAL, CHEMICAL, METHYLENE
	Paraformaldehyde	3g/ft³	60	60 - 75	+	+	+	+																					✓				

1. + = Effective; ? = Not effective.
2. Recently reported dependable as virucide.
3. Prepared from 37% formalin by 50% dilution with water.
4. Prepared from 100% acid.
5. Be aware of brands to check or refer to manufacturer's literature and/or Merck Index.
6. Spore destruction possible but not all products available. Individual literature on substances do not imply endorsement or rejection of any product by the NIH.
7. Refers to microscope and camera lenses.

VII. HOUSEKEEPING

A. *Introduction.* Well-defined housekeeping procedures and schedules are essential in reducing the risks of working with etiologic agents and in protecting the integrity of the research program. This is particularly true in the biological laboratory operating under less than total containment concepts and in all areas used for the housing of animals, whether or not they have been intentionally infected. A well-conceived and well-executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that promotes the efficient use of decontaminants in the event of the inadvertent release of a harmful agent. Less immediately evident are the benefits of establishing, among personnel of widely varying levels of education, an appreciation of the nature and sources of biological contamination.

Housekeeping is an omnibus term that can be interpreted as broadly or as narrowly as one chooses. It can be seen that many of the procedures found under special headings, such as decontamination, disposal, and animal care, are, in reality, specific instructions for safely accomplishing otherwise routine housekeeping chores. In these safety suggestions for research on recombinant DNA molecules, it has been elected to address specifically only tasks of a janitorial nature under the subject of housekeeping.

The objectives of housekeeping in the biological laboratory are to:

1. Provide an orderly work area conducive to the accomplishment of the research program.

2. Provide work areas devoid of physical hazards.

3. Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems being researched.

4. Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.

5. Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of organic debris that (i) may harbor microorganisms that are a potential threat to the integrity of the biological systems under investigation, (ii) may enhance the survival of microorganisms inadvertently released in experimental procedures, (iii) may retard penetration of decontaminants, (iv) may be transferable from one area to another on clothing and shoes, (v) may, with sufficient buildup, become a biohazard as a consequence of secondary aerosolization by personnel and air movement, and (vi) may cause allergenic sensitization of personnel, e.g., to animal danders.

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other areas in the laboratory have the constant potential for creation of significant quantities of contaminated organic debris than do animal care facilities.

In all laboratories, efforts to achieve total decontamination and to conduct a major cleanup of the biological complex are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding.

The following checklist outlines a portion of the items requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed manner in which housekeeping in the biological laboratory complex must be viewed.

Administration Areas
Aisles
Animal Food Storage
Animal Bedding Storage
Biological Safety Cabinets
Bench Tops and Other Work Surfaces
Ceilings
Change Rooms
Cleaning Solution Disposal
Cages and Cage Racks
Dry Ice Chests
Deep Freeze Chests
Entry and Exit Ways
Equipment Storage
Floors
Glassware
General Laboratory Equipment Cleanup
Hallways
Incubators
Instruments
Insect and Rodent Control
Light Fixtures
Mechanical Equipment Areas
Mops
Pipes—Wall and Ceiling Hung
Refrigerators
Showers
Supply Storage
UV Lamps
Vacuum Cleaners
Waste Accumulations
Waste Water Disposal
Others

Housekeeping in the laboratory is one of the avenues that leads to accomplishing the research program safely. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research program and special hazards of the research environment. The recommended approach to housekeeping is the assignment of housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need be accomplished. He should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by an alien cleanup crew, delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements, and the location of concentrated biological preparations and contaminated equipment used in their preparation and application will be known.

B. *Floor care.* Avoidance of dry sweeping and dusting will reduce the formation of nonspecific environmental aerosols. Wet mopping or vacuum cleaning with a high-efficiency particulate air (HEPA) filter on the exhaust is recommended.

Careful consideration must be given to design and quality in the selection of cleaning equipment and materials and in their use to prevent the substitution of one hazard for another.

In the absence of overt hazardous spills, the cleaning process commonly will consist of an initial vacuuming to remove all gross particulate matter and a follow-up wet mopping with a solution of chemical decontaminant containing a detergent. Depending on the nature of the surfaces to be cleaned and availability of floor drains, removal of residual cleaning solutions can be accomplished by a number of methods. Among these are: Pickup with a partially dry mop, pickup with a wet vacuum that has an adequately filtered exhaust, or removal to a convenient floor drain by use of a floor squeegee.

After cleaning up a spill of infected material, the residual solution should not be discharged to a sanitary sewer until it has been autoclaved or given further chemical treatment, such as by the addition of sodium hypochlorite sufficient to provide a final concentration of 500 ppm chlorine. Most household bleaches are marketed with a chlorine content of 5.25%. These in a final dilution of 1:100, yield 525 ppm of available chlorine. After allowing a contact time of 15 minutes, these solutions may be flushed down any available drain. Chlorine solutions in these high concentrations may be too corrosive for general application to floors and equipment. In any event, if solutions are used in this way, after the contact time the area should be rinsed with water.

C. *Dry sweeping.*—While it is recommended that dry sweeping be minimized, this may be the only method available or practicable under certain circumstances. In such cases, sweeping compounds used with push brooms and dry-dust mop heads treated to suppress aerosolization of dust should be used.

Sweeping compounds available from the usual janitorial supply firms fall in three categories:

Wax-based compounds used on vinyl floors and waxed floor coverings.

Oil-based compounds for concrete floors.

Oil-based compounds with abrasives (such as sand) to achieve a dry scouring action where much soil is present.

Dry-dust mop heads can be purchased as treated disposable units or as reusable, washable heads that must be treated with appropriate sprays or by other means to improve their dust-capturing property.

D. *Vacuum cleaning.* In the absence of a HEPA filter on the exhaust, the usual wet and dry industrial-type vacuum cleaner is a potent aerosol generator. The HEPA-filtered exhaust used in conjunction with a well-sealed vacuum unit, however, can negate this factor because of its ability to pass large volumes of exhaust air while retaining particles with a minimum efficiency of 99.97 percent. Wet and dry units incorporating a HEPA filter on the exhaust are available from a number of manufacturers.

There are no particular requirements with respect to the manner in which the dry vacuuming is accomplished other than to emphasize that the objective is to remove all debris and particulate matter. The manufacturer's directions adequately detail the frequency of bag changes, filter changes, and mechanical adjustments.

Dry material vacuum-collected during these floor-cleaning activities is potentially contaminated, but the nature of the risk is probably greater to the experiment than to the experimenter. It is wise to effect bag and filter changes and to clean out collection tanks in a manner that will avoid or minimize aerosolizing the contents of the vacuum cleaner.

A vacuum machine that collects debris in a disposable bag is preferable to machines that collect the major debris in a tank and on an exposed prime filter. Even though it may serve as a primary filter, the disposable bag must be removed with caution. A bellows effect may pump dust out of the bag if its intake opening is not sealed before moving it to a plastic bag for transfer out of the area. In any event, the outer surface of the disposable bag will probably bear some dust contamination, which also may occur on inner surfaces of the machine.

To avoid contaminating experimental materials, the emptying of vacuum collection tanks and changing of bags and filters are best done away from the immediate laboratory area, for example, in a small area that can be easily cleaned afterwards. The use of heavy rubber gloves is recommended when removing wastes from tanks in case broken glass is present. After making the filter changes, all external surfaces of the immediate work area and the equipment should be wiped with a cloth moistened in decontaminant. The operator might plan for a change of laboratory clothing afterwards so as to minimize carrying contamination into other areas of the laboratory.

Avoid use of dry vacuum cleaning equipment in work with high risk agents in the open laboratory. Should it be necessary to use it, it is recommended that gaseous sterilization may be used to minimize aerosolization of microorganisms before waste is emptied from the vacuum container. Because complete penetration of sterilizing gases into the collected dry dust may be a problem, all wastes should be placed in a plastic bag, which then is tightly closed and incinerated or disposed of in an approved manner.

When dry vacuum cleaning equipment has been used within a gastight safety cabinet system, it can be treated in an attached double-door carboxyclave (an autoclave equipped with an ethylene oxide gas sterilization system) to allow for removal and emptying of the collection tank.

If a wet vacuum is to be used for pickup of the detergent-germicide solution from the floor, the manufacturer's recommendations on filter life should be followed. In addition, the operation of the vacuum should be closely observed for evidence of operating changes indicating restricted airflow or, conversely, increased flow indicating filter failure. Liquids collected in the vacuum cleaner after floor mopping will contain decontaminant materials. These liquids may be poured down a convenient floor drain, except in the case of cleanup wastes from an overt spill. The collected liquid should then be autoclaved or treated with chlorine solution before disposal.

Provisions should be made for regular decontamination of the entire vacuum cleaner with formaldehyde gas or vapor, or ethylene oxide. This should be done after use if the vacuum is used in any manner for cleanup of overt spills of infectious material.

E. *Selection of a cleaning solution.* The selection of a detergent-decontaminant combination for routine cleaning of the laboratory complex should be based on the requirements of the area of greatest potential for contamination by the widest spectrum of microorganisms. With rare exception, this will be identified as the animal holding area and the expected microorganisms may well include fungi, viruses, and the vegetative and spore forms of bacteria. A decontaminating solution for such a range of microorganisms would, however, be expensive and excessively corrosive for routine use. Except in those rare instances where it can be assumed that pathogenic spores are being shed by laboratory animals, the risks from the spores are more likely to affect the experiments than the personnel. The spores tend to be associated with organic debris from bedding and food, thus offering potential for removal or at least a large initial reduction in their numbers by vacuum cleaning. A wide range of cleaning solutions that are mildly sporicidal, reasonably residual, and are not destructive to the physical plant are available. Phenol derivatives in combination with a detergent have these characteristics and have been selected for routine use in a number of research facilities. There are numerous detergent-phenolic combinations available on the market. The phenols are one type of a broad spectrum of biocidal substances that includes the mercurials, quaternary ammonium compounds, chlorine compounds, iodophores, alcohols, formaldehyde, glutaraldehyde, and combinations of alcohol with either iodine or formaldehyde. These have been discussed in Section VI.

The laboratory supervisor should make a selection from those types most readily avail-

able which meet the general criteria of effectiveness, residual properties, and low corrosiveness.

F. *Wet mopping—two-bucket method.* Wet mopping of floors in laboratory and animal care areas is, *from a safety standpoint,* most conveniently and efficiently accomplished using a two-bucket system. The principal feature of such a system is that fresh detergent-decontaminant solution is always applied to the floor from one bucket, while all spent cleaning solution wrung from the mop *is collected in the second bucket. Compact* dolly-mounted double-bucket units with foot-operated wringers are available from most janitorial supply houses. A freshly laundered mop head of the cotton string type should be used daily. This requires that a mop with removable head be provided as opposed to a fixed-head type. In practice, the mop is saturated with fresh solution, very lightly wrung into the second bucket and applied to the floor using a figure eight motion of the mop head. After every four or five strokes, the mop head is turned over and the process continued until an area of approximately 100 ft² has been covered. After allowing a contact time of five minutes, the solution is removed with either a wet vacuum cleaner with HEPA-filtered exhaust or with the wrung-out mop. The mopping is continued in 100 ft² increments until the total floor area has been covered. Floor-cleaning procedures are most effectively completed after the majority of the work force has departed and should progress from areas of least potential contamination to those of greatest potential. Before a mop head is sent to a laundry, it should be autoclaved. Spent cleaning fluids are disposed of by flushing down the drain.

If the cleanup follows an overt spill of infectious material, the spent cleaning solution, after removal from the floor, should be autoclaved or treated with chlorine solution. Chlorine (as household bleach) should be *added to give 500 ppm and held for a contact* time of 15 minutes before dumping in the sanitary sewer.

G. *Alternative floor cleaning method for animal care areas and areas with monolithic floors.* The absence of permanently placed laboratory benches and fixed equipment, coupled with the mobility of modern cage racks, makes possible alternate floor-cleaning procedures in animal care facilities. As in all considerations of methodologies in biomedical laboratory facilities, it is necessary to assess the compatibility of procedures and facilities from the hazard point of view. The alternative floor-cleaning procedure to be discussed requires that floors are completely sealed or of monolithic construction so that liquid leakage to adjacent areas does not occur and that floor drains or wet vacuum cleaners are available.

Subsequent to the removal of all debris by dry vacuum, move the cage racks to one side of the room. Cover the floor of the remaining cleared portion of the room with detergent-decontaminant solution applied at a rate of approximately one gallon per 144 *ft²* from a one-gallon tank sprayer, using a setting on the nozzle which *will cause the solution to* flow on and not create a spray. The nozzle is placed close to the floor. Allow a fifteen-minute contact period; then push the cleaning solution to the floor drain with a large floor squeegee or pick it up with a wet vacuum. Allow the floor to air dry; move the cage racks into the cleaned area, and repeat the process for the remaining floor area. Floor drains in these areas should be *rim-flush,* at least six inches in diameter, and fitted with a screen or porous trap bucket to catch large debris that escapes the initial dry cleaning. Such screens and baskets should be emptied after treatment with a decontaminant. If space utilization does not require frequent floor washdown, pour a half-gallon of detergent-decontaminant solution into the drain each week to keep the trap in the waste line filled against backup of sewer gases.

VIII. CLEAN-UP OF BIOHAZARDOUS SPILLS (6, 9, 28)

A. *Biohazardous spill in a biological safety cabinet.* Chemical decontamination procedures should be initiated at once *while the cabinet continues to operate* to prevent escape of contaminants from the cabinet.

1. Spray or wipe walls, work surfaces, and *equipment with a 2 percent solution of an* iodophor-decontaminant (Wescodyne or equivalent). A decontaminant detergent has the advantage of detergent activity, which is important because extraneous organic substances frequently interfere with the reaction between the microorganisms and the active agent of the decontaminant. Operator should wear gloves during this procedure.

2. Flood the top work surface tray, and, if a Class II cabinet, the drain pans and catch basins below the work surface, with a decontaminant and allow to stand 10–15 minutes.

3. Remove excess decontaminant from the tray by wiping with a sponge or cloth soaked in a decontaminant. For Class II cabinets, drain the tray into the cabinet base, lift out tray and removable exhaust grille work, and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a decontaminant. Then replace in position and drain recontaminant from cabinet base into appropriate container and autoclave according to standard procedures. Gloves, cloth or sponge should be discarded in an autoclave pan and autoclaved.

B. Biohazard spill outside a biological safety cabinet. 1. Hold your breath. Leave the room immediately, and close the door.

2. Warn others not to enter the contaminated area.

3. Remove and put into a container contaminated garments for autoclaving and thoroughly wash hands and face.

4. *Wait* 20 minutes to allow dissipation of aerosols created by the spill.

5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. (For a high risk agent, a jumpsuit with tight-fitting wrists and use of a respirator should be considered).

6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.

7. Let stand 20 minutes to allow an adequate contact time.

8. Using an autoclavable dust pan and squeege, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) *into a deep autoclave pan.* Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

9. The dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable *items with* non autoclavable plastic bags should be avoided—separation of the plastic after autoclaving can be very difficult.

C. Radioactive biohazard spill outside a biological safety cabinet. In the event that a biohazardous spill also involves a radiation hazard, the clean-up procedure may have to be modified, depending on an evaluation of the risk assessment of relative biological and radiological hazard.

Laboratories handling radioactive substances must have the services of a designated radiation protection officer available for consultation.

The following procedure indicates suggested variations from the biohazard spill procedure (above) that should be considered when a radioactive biohazard spill occurs outside a Biological Safety Cabinet.[1]

1. Holding your breath, leave the room immediately and close the door.

2. *Warn others not to enter the contaminated area.*

3. Remove and put in a container contaminated garments for autoclaving and thoroughly wash hands and face.

4. Wait thirty minutes to allow dissipation of aerosols created by the spill.

**Before clean-up procedures begin, a radiation protection officer should survey the spill for external radiation hazard to determine the relative degree of risk.*

5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. (For a high risk agent, a jumpsuit with tight-fitting sleeves and a respirator should be considered).

6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.

7. Let stand 20 minutes to allow adequate disinfectant contact time.

8. **In most cases, the spill will involve α, or β, which present no external hazard. However, if more energetic beta or gamma emitters are involved, care must be taken to prevent hand and body radiation exposure. The radiation protection officer must make this determination before the clean-up operation is begun.*

If the radiation protection officer approves, the bio-hazard-handling procedure may begin: Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

**If the radiation protection officer determines that radioactive vapors may be released and thereby contaminate the autoclave, the material must not be autoclaved. In that case, sufficient decontaminant solution to immerse the contents should be added to the wastes container. The cover should be sealed with waterproof tape, and the container stored and handled for disposal as radioactive waste. Radioactive and biohazard warning symbols should be affixed to the waste container. As a general rule, autoclaving should be avoided.*

9. If autoclaving has been approved, the dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable items with plastic bags should be avoided—separation of the plastic after autoclaving can be very difficult.

**A final radioactive survey should be made of the spill area, dust pan, and squeegee with a Geiger counter, or a smear should be taken and counted in a liquid scintillation counter.*

IX. A SECONDARY RESERVOIR AND FILTRATION APPARATUS FOR VACUUM SYSTEMS

The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into collection vessels or reservoirs is a common procedure in many laboratories. To prevent the accidental contamination by aerosols or fluids of house vacuum systems or laboratory pumps, some investigators have installed side arm flasks containing cotton, sulfuric acid or decontaminant between the reservoir and the vacuum line. Cotton is not completely effective as a filtering agent,

[1] Changes in procedures have been starred and italicized.

sulfuric acid will corrode pipes, and contaminants may lose their inactivating ability upon standing. The introduction of a cartridge-type filter that is moisture resistant and has a rated capacity to remove particles 350 nm (0.35u) or larger in size provides an effective barrier to virus aerosols.

The secondary reservoir and filtration apparatus can be assembled from readily available units as shown in Figure 1. A length of plastic tubing ¼ inch I.D x ⅛ inch wall is attached at one end of the reservoir and at the other end to the lower arm of a filtration and media storage flask. These flasks vary in capacity from 250 to 4000 ml, the choice of flask depending on available space and amount of fluid that could be accidentally aspirated. A second tube of the same dimensions is attached from the upper arm of the flask to the inlet port of the disposable filter assembly. The third tube is attached from the filter assembly to a vacuum source. The tubes are securely held to the filter by fittings supplied with the filter and the other tubing connections can be secured by worm drive hose clamps.

Ideally the flask should be placed higher than the reservoir of collection vessel. If fluid is accidentally drawn into the flask, the liquid can drain back into the reservoir by gravity if the connection at the vacuum line is broken. This prevents the loss of fluid which the investigator needs to retain.

Should the flask be used only for the recovery and storage of waste fluids, then the addition of a few grams of Dow Corning Antifoam A to the flask will reduce violent foaming of fluids aspirated into it. Such fluids can be decontaminated by introducing into the reservoir a final 5% concentration of an iodophor or other appropriate decontaminant, holding for 30 minutes and draining as above.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter and flask should be autoclaved before the filter is discarded. A new filter can then be installed and the assembly replaced.

APPENDIX D, Page D-76

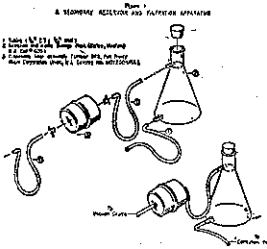


FIGURE I

X. PACKAGING AND SHIPPING

A. *Introduction.* Federal regulations and carrier tariffs have been promulgated to ensure the safe transport of hazardous biological materials. The NIH Guidelines specify that all DNA recombinant materials will be packaged and shipped in containers that meet the requirements of those regulations and carrier tariffs. In addition when any portion of the recombinant DNA material is derived from an etiologic agent listed in paragraph (c) of 42-CFR 72.25 (which is included at the end of this section, page D-85) the labeling requirements in these regulations and carrier tariffs shall apply.

B. *Packaging of recombinant DNA materials.* 1. *Volume less than 50 ml.* Material shall be placed in a securely closed, watertight container [primary container (test tube, vial, etc.)] which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). er(s).

Descriptions of this packaging method are given in Table III.

2. *Volumes of 50 ml or Greater.* Material shall be placed in a securely closed, watertight container (primary container) which shall be enclosed in a second, durable watertight container (secondary container). Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single secondary container. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength. A shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom, and sides between the secondary container and the outer shipping container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of materials which may be enclosed within a single outer shipping container should not exceed 4,000 ml.).

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

Descriptions of packages which comply with the regulations of the Department of Transportation (DOT) are given in Table IV.

C. Labeling of packages containing recombinant DNA materials. 1. Materials which do not contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. Place only the address label on the outer shipping container.

DO NOT USE THE LABEL FOR ETIOLOGIC AGENTS/BIOMEDICAL MATERIAL.

2. Materials which contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. In addition to the address label, the label for Etiologic Agents/Biomedical Material must be affixed to the outer shipping container. This label is described in paragraph (c)(4) of 42 CFR 72.25.

3. Materials which contain any portion of a plant pest (plant pathogens) which are so defined by the Department of Agriculture (USDA).

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. In addition to the address label, the shipping labels furnished by the USDA as part of the General, Courtesy, or Special Permits required for research with and shipment of such agents shall be affixed to the outer shipping container.

D. *Additional shipping requirements and limitations for recombinant DNA materials.*—1. *Domestic Transportation.* Civil Aeronautics Board Rule No. 82 (Air Transport Association Restricted Articles Tariff 6-D) requires that a Shipper's Certificate, depicted below, be completed and affixed to all shipments which bear the ETIOLOGIC AGENT/BIOMEDICAL MATERIALS label required under the provisions of the Interstate Quarantine regulations (42 CFR 72.25(c)). The Certificate must be completed in duplicate and affixed to the outer shipping container.

This is to certify that the contents of this consignment are properly classified, described by proper shipping name and are packed, marked and labelled and are in proper condition for carriage by air according to all applicable carrier and government regulations. (For international shipments add "and to the IATA Restricted Articles Regulations".) This consignment is within the limitations prescribed for: PASSENGER AIRCRAFT/CARGO ONLY (cross out nonapplicable).

Number of Packages	Specify Each Article Separately (Proper Shipping Name)	Classification	Net Quantity per Package
	ETIOLOGIC AGENT, n.o.s.	ETIO. AG.	

Shipper:

Date _____

Signature of Shipper

Shipments of recombinant DNA Materials exceeding 50 ml in volume and containing any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25 are restricted, by DOT regulations, to transport by cargo only aircraft. When the volume of a single primary container exceeds the 50 ml limitation, this restriction must be indicated on the Shipper's Certificate by crossing out "Passenger Aircraft".

When dry ice is used as a refrigerant an "ORA—Group A—DRY ICE LABEL" should be affixed to the outer shipping container. The amount of dry ice used and the date packed should be designated on the label.

2. *International Transportation.*—In addition to the packaging and labeling requirements of the regulations previously cited, international shipments of recombinant DNA materials in which any portion of the material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25 must have one or more of the following documents—depending on the country of destination:

(1) Parcel Post Customs Declaration (PS 2968) tag.

(2) Parcel Post Customs Declaration (PS 2966–A) label.

(3) International Parcel Post—Instructions Given by Sender (POD 2922) label.

(4) Dispatch note (POD 2972) tag.

(5) "Violet Label"

(6) Shipper's Certificate specified in the current International Air Transport Association Tariff. Individual country requirements are listed in "International Postage Rates and Fees" (USPO Publication 51).

APPENDIX D, PAGE D–83

TABLE III

Description of Packages for Material in Volumes less than 50 ml.

Volume (ml)	Primary Container	Packing	Secondary Container	Packing	Outer Shipping d/ Container
15 max.	Sealed vial(s) or small glass test tube, screw cap* or stopper, taped	a/	Metal can 1" diam. x 7" O.D. metal screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 4-1/2" diam. x 7 to 7-1/2" O.D.
30 or less	One 20 x 150 mm test tube, taped* stopper or multiple small vials	a/	Metal can 2-1/2" diam. x 6-1/2" high O.D. screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 5-1/4" diam. x 7 to 7-1/2" O.D.
50 or less	Plastic* screw cap* bottle or Pyrex glass with skirt rubber stopper	a/	Metal can 2-1/2" diam. x 6-1/2" high O.D. screw cap	None Required	Fiberbody; metal screw cap, top and bottom; 5-1/4" diam. x 7 to 7-1/2" O.D.
30 or less	Multiple watertight vials or * tubes, taped stoppers	a/	One or more friction-seal tin cans b/ 306 x 409 or larger	c/	Fiberboard box

*The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of enforeen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D. = outside dimensions.

a/ Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b/ 610 x 708 and 604 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-9/16" height, and 6-4/16" x 9-8/16",

c/ None required, but with the 306 x 409 cans or larger cans use sufficient nonparticulate shock-absorbent material to prevent rattling.

d/ If materials are to be refrigerated, it is recommended that an overpack be used to contain the refrigerant and the secured (original) outer shipping container. A leak proof outer container must be used for water ice. If dry ice is used the outer container must permit release of carbon dioxide. Interior supports must be provided to hold the container(s) in the original position(s) after wet or dry ice has dissipated.

TABLE IV

Description of Packages for Material in Volumes of 50 ml or greater

Volume (ml)	Primary Container	Packing	Secondary Container	Packing With Refrigerant	Packing Without Refrigerant	Outer Shipping Container With Refrigerant	Outer Shipping Container Without Refrigerant
51 to 100 ml	Plastic[a] or Pyrex glass screw cap[a] bottle; rubber or skirt rubber stopper, taped[a]	a/	Consists of metal container & outer container specified in Table III	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	Corrugated fiberboard or cardboard box, taped shut
100 max.	One 100 ml plastic[a] screw cap[a] narrow neck bottle or Pyrex glass, taped[a]	a/	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11-1/4" high C.D. taped shut with 3" type PS3 tape
200 max.	Two 100 ml plastic[a] screw cap[a] bottles or Pyrex glass, taped	a/	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11-1/4" high C.D. taped shut with 3" type PS3 tape
250 max.	One 250 ml, plastic[a] narrow mouth screw cap[a] bottle or Pyrex glass skirted rubber stopper, taped[a]	a/	No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11-1/4" high C.D. taped shut with 3" type PS3 tape
500 max.	Two 250 ml plastic[a] screw cap[a] bottles or Pyrex glass bottles, taped[a]	a/	2-gallon friction-seal tin can, 604 x 908, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box 12-1/4" x 12-1/4" x 10-3/16" high O.D. taped shut with 3" wide PS3 tape.
500 max.	500 ml Pyrex glass bottle, rubber-skirt stopper, taped, or 500 ml plastic[a] bottle, narrow or wide mouth, screw cap[a], taped	a/	No. 12 crimp seal tin can 603 x 810 2-gallon friction-seal tin can, 604 x 908, top soldered or clipped at 4 points b/	Styrofoam box shock-absorbent insulation	c/	Fiberboard box closely fitting the styrofoam box, taped shut	V3C cardboard box 12-1/4" x 12-1/4" x 10-3/16" high O.D. taped shut with 3" wide PS3 tape. For the No. 12 can a cardboard box is ok taped shut

[a]The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D. = outside dimensions.

a/ Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b/ 610 x 708 and 604 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-9/16" height, and 8-4/14" x 9-4/14".

c/ Shock absorbent material, in volume at least equal to that between the primary and secondary container(s), at the top, bottom, and sides between the secondary container and the outer shipping container. The shock absorbent material shall be so placed that the secondary container(s) does not become loose inside the outer shipping container as the water ice or dry ice is dissipated.

APPENDIX D, Page D-24

APPENDIX D, Page D-85 ATTACHMENT I

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333
Telephone: (404) 633-3311, Ext. 3183

TITLE 42—PUBLIC HEALTH

Chapter I—Public Health Service, Department of Health, Education, and Welfare

SUBCHAPTER F—QUARANTINE, INSPECTION, LICENSING

PART 72—INTERSTATE QUARANTINE

Subpart C—Shipment of Certain Things

Section 72.25 of Part 72, Title 42, Code of Federal Regulations, is amended to read as follows:

§ 72.25 Etiologic agents.[1]

(a) *Definitions.* As used in this section:

(1) An "etiologic agent" means a viable microorganism or its toxin which causes, or may cause, human disease.

(2) A "diagnostic specimen" means any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue, and tissue fluids being shipped for purposes of diagnosis.

(3) A "biological product" means a biological product prepared and manufactured in accordance with the provisions of 9 CFR Part 10, Licensed Veterinary Biological Products, 42 CFR Part 73, Licensed Human Biological Products, 21 CFR 130.3, *New drugs for investigational use in humans*, 9 CFR Part 103, Biological Products for Experimental Treatment of Animals, or 21 CFR 130.3(a), *New drugs for investigational use in animals*, and which, in accordance with such provisions, may be shipped in interstate traffic.

(b) *Transportation; etiologic agent minimum packaging requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, including but not limited to, diagnostic specimens and biological products, containing, or reasonably believed by such person to contain, an etiologic agent unless such material is packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.

(c) *Transportation; etiologic agents subject to additional requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, other than diagnostic specimens and biological products, containing, or reasonably believed by such person to contain, one or more of the following etiologic agents unless such material is packaged in accordance with the requirements specified in paragraph (b) of this section, and unless, in addition, such material is packaged and shipped in accordance with the requirements specified in subparagraphs (1)–(6) of this paragraph:

BACTERIAL AGENTS

Actinobacillus—all species.
Arizona hinshawii—all serotypes.
Bacillus anthracis.
Bartonella—all species.
Bordetella—all species.
Borrelia recurrentis, B. vincenti.
Brucella—all species.
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani.
Corynebacterium diphtheriae C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale.
Diplococcus (Streptococcus) pneumoniae.
Erysipelothrix insidiosa.
Escherichia coli, all enteropathogenic serotypes.
Francisella (Pasteurella) tularensis.
Haemophilus ducreyi, H. influenziae.
Herellea vaginicola.
Klebsiella—all species and all serotypes.
Leptospira interrogans—all serotypes.
Listeria—all species.
Mima polymorpha.
Moraxella—all species.
Mycobacterium—all species.
Mycoplasma—all species.
Neisseria gonorrhoeae, N. meningitidis.
Pasteurella—all species.
Pseudomonas pseudomallei.
Salmonella—all species and all serotypes.
Shigella—all species and all serotypes.
Sphaerophorus necrophorus.
Staphylococcus aureus.
Streptobacillus moniliformis.
Streptococcus pyogenes.
Treponema caretcum, T. pallidum, and *T. pertenue.*
Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus.*
Yersenia (Pasteurella) pestis.

FUNGAL AGENTS

Actinomycetes (including *Nocardia* species, *Actinomyces* species and *Arachnia propionica*).
Blastomyces dermatitidis.
Coccidioides immitis.
Cryptococcus neoformans.
Histoplasma capsulatum.
Paracoccidioides brasiliensis.

VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS

Adenoviruses—human—all types.
Arboviruses.
Coxiella burnetii.
Coxsackie A and *B viruses*—all types.
Cytamegolovirases.
Dengue virus.
Echoviruses—all types.
Encephalomyocarditis virus.
Hemorrhagic fever agents, including *Crimean hemorrhagic fever (Congo), Junin*, and *Machupo viruses*, and others as yet undefined.
Hepatitis-associated antigen.
Herpesvirus—all members.
Infectious bronchitis-like virus.
Influenza viruses—all types.
Lassa virus.
Lymphocytic choriomeningitis virus.
Marburg virus.
Measles virus.
Mumps virus.
Parainfluenza viruses—all types.
Poliioviruses—all types.
Poxviruses—all members.
Psittacosis = Ornithosis = Trachoma-Lymphogranuloma group of agents.
Rabies virus—all strains.
Reoviruses—all types.
Respiratory syncytial virus.
Rhinoviruses—all types.
Rickettsia—all species.
Rubella virus.
Simian viruses—all types.
Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever*, and *Central European encephalitis viruses.*
Vaccinia virus.
Varicella virus.
Variola major and *Variola minor viruses.*
Vesicular stomatis virus.
Yellow fever virus.

(1) *Volume less than 50 ml.* Material shall be placed in a securely closed, watertight container (primary container (test tube, vial, etc.)) which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container. If the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated, fiberboard, cardboard, wood, or other material of equivalent strength.

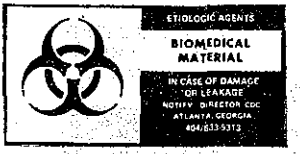
(2) *Volume 50 ml. or greater.* Packaging of material in volumes of 50 ml. or more shall include, in addition, a shock-absorbent material, in volume at least equal to that of the absorbent material

[1] The requirements of this section are in addition to and not in lieu of any other packaging or other requirements for the transportation of etiologic agents in interstate traffic prescribed by the Department of Transportation and other agencies of the Federal Government.

between the primary and secondary containers, at the top, bottom, and sides between the secondary container and the outer shipping container. Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single, secondary container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of etiologic agent which may be enclosed within a single outer shipping container shall not exceed 4,000 ml.)

(3) *Dry ice.* If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be so placed that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

(4) *Labels.* The label for Etiologic Agents/Biomedical Material, except for size and color, must be as shown:



(i) The color of material on which the label is printed must be white and the symbol and printing in red.

(ii) The label must be a rectangle measuring 51 mm. (2 inches) high by 102.5 mm. (4 inches) long.

(iii) The red symbol measuring 38 mm. (1½ inches) in diameter must be centered in a white square measuring 51 mm. (2 inches) on each side.

(iv) Type size of the letters of label shall be as follows:

ETIOLOGIC AGENT	10 pt. rev.
BIOMEDICAL MATERIAL	14 pt.
IN CASE OF DAMAGE OR LEAKAGE	10 pt. rev.
NOTIFY DIRECTOR CDC ATLANTA, GA	8 pt. rev.
404 633 5313	10 pt. rev.

(5) *Damaged packages.* Carriers shall promptly, upon discovery of damage to the package that indicates damage to the primary container, isolate the package and notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633-5313), and the sender.

(6) *Registered mail or equivalent system.* Transportation of the following etiologic agents shall be by registered mail or an equivalent system which requires or provides for sending notification to the shipper immediately upon delivery:

Actinobacillus mallei.
Coccidioides immitis.
Francisella (Pasteurella) tularensis.
Hemorrhagic fever agents, including, but not limited to, *Crimean hemorrhagic fever (Congo), Junin, Machupo viruses.*
Herpesvirus simiae (B virus).
Histoplasma capsulatum.
Lassa virus.
Marburg virus.
Pseudomonas pseudomallei.
Tick-borne encephalitis virus complex, including, but not limited to, *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever,* and *Central European encephalitis viruses, Variola minor* and *Variola major.*
Yersenia (Pasteurella) pes:

(d) *Notice of delivery, failure to receive.* When notice of delivery of agents containing, or suspected of containing, etiologic agents listed in paragraph (c) (6) of this section is not received by the sender within 5 days following anticipated delivery of the package, the shipper shall notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633-5313).

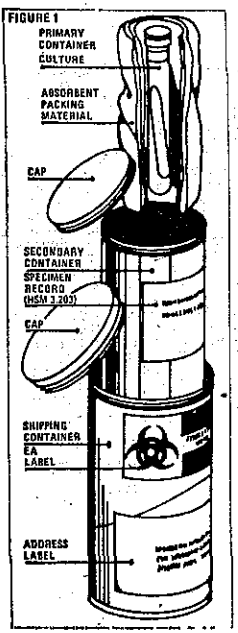
(e) *Requirements; variations.* The Administrator may approve variations from the requirements of this section if, upon review and evaluation, he finds that such variations provide protection at least equivalent to that provided by compliance with the requirements specified in this section and makes such findings a matter of official record.

(Sec. 361, 58 Stat. 703; 42 U.S.C. 264)

[redacted]

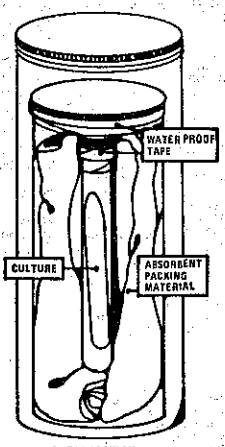
Effective July 30, 1972

FIGURE 1



PACKAGING AND LABELING OF ETIOLOGIC AGENTS

FIGURE 2



CROSS SECTION OF PROPER PACKING

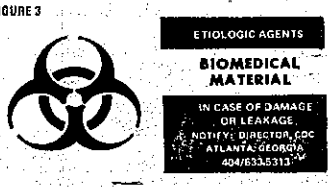
The Interstate Quarantine Regulations (42 CFR, Part 72 25 Etiologic Agents) was revised July 31, 1972 to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic.

Figures 1 and 2 diagram the packaging and labeling of etiologic agents in volumes of less than 50 ml. in accordance with the provisions of subparagraph (C) (1) of the cited regulation. Figure 3 illustrates the color and size of the label, described in subparagraph (C) (4) of the regulations, which shall be affixed to all shipments of etiologic agents.

For further information on any provision of this regulation contact:

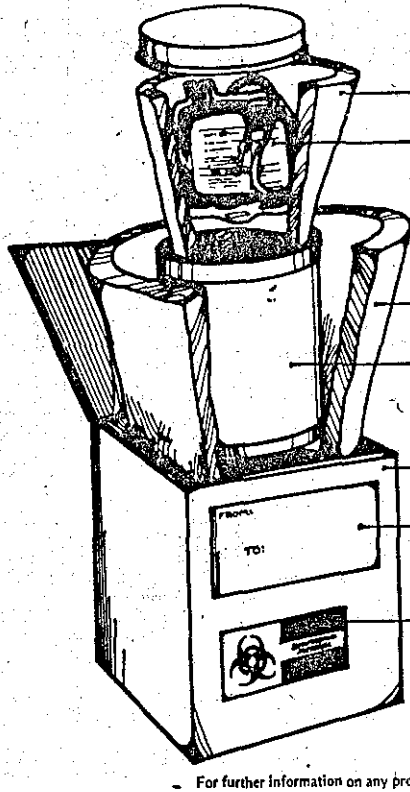
Center for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333
Telephone: 404 633 3311

FIGURE 3



APPENDIX D, Page D-88

PACKAGING AND LABELING OF ETIOLOGIC AGENTS



ABSORBENT PACKING MATERIAL

PRIMARY CONTAINER *(Bottle, blood bag, etc.)**

*NOTE: Single primary containers may not exceed 500 ml. of material. Two or more primary containers whose combined volumes do not exceed 500 ml. may be enclosed in a single, secondary container. The maximum volume of etiologic agent which may be enclosed in a single outer shipping container shall not exceed 4000 ml.

SHOCK ABSORBENT MATERIAL

SECONDARY CONTAINER *(Gasketed screwcap with waterproof tape or hermetically sealed can)*

OUTER SHIPPING CONTAINER

MAILING LABEL

ETIOLOGIC AGENT LABEL

The Interstate Quarantine Regulations (42 CFR, Part 72.25, Etiologic Agents) was revised July 31, 1972, to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic. The illustration shows acceptable packaging and labeling of etiologic agents in accordance with subparagraphs (c) (2) and (4) of the cited regulation.

For further information on any provision of this regulation contact:

Center for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333

Telephone: 404-633-3311

XI. TRAINING AIDS, MATERIALS AND COURSES

A. *Slide-tape cassettes.* 1. Assessment of Risk in the Cancer Virus Laboratory ($10).

2. Effective Use of The Laminar Flow Biological Safety Cabinet ($10).

3. Formaldehyde Decontamination of Laminar Flow Biological Safety Cabinets ($10).

4. Certification of Class II (Laminar Flow) Biological Safety Cabinets ($13).

5. Hazard Control in the Animal Laboratory ($10).

6. Basic Principles of Contamination Control (In preparation).

7. Selection of a Biological Safety Cabinet (In preparation).

These slide tape cassettes are available for purchase from the National Audiovisual Center. The price for each is given above after the title. Send your order prepaid with a check or money-order made payable to National Archives Trust Fund and mail to: Sales Branch, National Audiovisual Center (GSA), Washington, D.C. 20409.

8. Research Laboratory Safety.

This slide tape cassette, stock number 176.79, is available for $75 from the National Safety Council, 425 North Michigan Avenue, Chicago, Illinois 60611.

B. *Films.* 1. Air Sampling for Microbiological Particulates (M-926).

2. Handling the Laboratory Guinea Pig (T2618-X).

3. Handling the Laboratory Mouse (T2617-X).

4. Infectious Hazards of Bacteriological Techniques (M-382).

5. Laboratory Design for Microbiological Safety (M-1091).

6. Plastic Isolators: New Tools for Medical Research (M-899).

7. Safe Handling of Laboratory Animals (M-455).

8. Surface Sampling for Microorganisms (Rodac Method) (M-924).

9. Surface Sampling for Microorganisms (Swab Method) (M-925).

These films are available on loan without charge from: Media Resources Branch, National Medical Audiovisual Center (Annex), Station K, Atlanta, Georgia 30324. The same films (except 2 and 3) can be rented or bought from: National Audiovisual Center (GSA), (Rental Branch)—(Sales Branch), Washington, D.C. 20409.

C. *Courses.* 1. Biohazard and Injury Control in the Biomedical Laboratory. Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquiries to Dr. Donald Vesley, University of Minnesota, School of Public Health, 1325 Mayo Memorial Building, Minneapolis, Minnesota 55455. June 22-24, 1976, Los Angeles, CA; October 26-28, 1976, Boston, MA; December 7-9, 1976, Bethesda, MD.

2. Biohazard Containment and Control for Recombinant DNA Molecules. Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquires as above. September 8-9, 1976, Stanford, CA; September 21-11, 1976, Cold Spring Harbor, NY.

3. Safety in Laboratory. Presented by National Institute of Occupational Safety and Health, Division of Training and Manpower Development, by special arrangement. Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226.

4. Laboratory Safety Management. Presented by the Laboratory and Training Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia, September 14-16, 1976 and September 13-15, 1977.

XII. OUTLINE OF A SAFETY AND OPERATION MANUAL FOR A P4 FACILITY

A. *Purpose.*

B. *Policy.*

C. *Responsibility and Authority.* 1. Management.

2. Supervisor.

3. Each Employee.

4. Facility Safety Officer.

5. Biohazard Safety Committee.

D. *Facility Assignment Procedures.*

E. *Reporting of Major and Minor Accidents and Injuries, Exposure to Toxic or Infectious Materials, Unsafe Conditions and Property Damages, and Rendering First-Aid.*

F. *General Laboratory Safety.* 1. Fire.

2. Equipment.

3. Physical.

4. Chemical.

5. Radiological.

G. *Safety Procedures Associated with Biohazard Activities of the Laboratory.* 1. Personnel Practices.

2. Operational Practices.

H. *Medical Surveillance.*

I. *Facility Operations.* 1. Personnel Access Procedures.

2. Access Procedures for Equipment Materials and Supplies.

3. Maintenance and Support.

4. Zone Classification.

5. Facility Monitoring Procedures.

6. Housekeeping.

J. *Others.* 1. Packaging and Shipment of Biohazardous Materials.

2. Emergency Procedures.

3. Insect and Rodent Control.

4. Orientation and Training.

Appendix D was prepared by a Working Group Consisting of: W. Emmett Barkley (Chairman), National Cancer Institute, NIH; Manuel S. Barbeito, National Cancer Insitute, NIH; Everett Hanel, Jr., Frederick Cancer Research Center; George S. Michaelsen, School of Public Health, University of Minnesota; Vinson R. Oviatt, Division of Research Services, NIH; Warren V. Powell, Division of Research Services, NIH; John Richardson, Center for Disease Control; James F. Sullivan, National Animal Disease Laboratories; and Arnold G. Wedum, Frederick Cancer Research Center.

REFERENCES

1. American National Standards Institute. 1968. Z 35.1.

2. Wedum, A. G. 1964. Laboratory safety in research with infectious aerosols. Public Health Rep. 79:641-633.

3. Phillips, G. B. 1965. Microbiological hazards in the laboratory. J. Chem. Education. Part One—Control, 42:A43-A48. Part Two—Prevention, 42:A117-A130.

4. Hellman, A. 1969. Biohazard control and containment in oncogenic virus research. USPHS, NIH, NCI.

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APPENDIX 11

(Reproduced from Prism, November 1975, by permission of the publisher; the American Medical Association)

DNA Splicing: Will Fear Rob Us of Its Benefits?

We may be on the threshold of a technology of untold importance in diagnostic and therapeutic medicine, says this Nobel geneticist, if we have the courage to move ahead despite the risks involved.

(The Author: Joshua Lederberg, Ph.D., shared the Nobel Prize in medicine and physiology in 1958 for his work in genetics. In 1946, he and Professor E. L. Tatum showed that bacterial cells can transfer genetic material from one cell to another. Subsequently, Dr. Lederberg and D. N. Zinder discovered the phenomenon of transduction, the carriage of genes by viruses. Dr. Lederberg has been professor of genetics at Stanford University since 1959.)

Although our theoretical understanding of the cell has been completely transformed in the last 30 years, there has not yet been a corresponding advance in the practical application of our knowledge to medicine. Indeed, very little in the practice of medicine (even of clinical genetics) is directly related to the fundamental knowledge that DNA has a bihelical structure.

Nonetheless, our faith remains steadfast that further theoretical understanding of viruses, the neoplastic cell, the aging cell, the immune response mechanism, and the aberrant chromosome, will bring far-reaching changes to medicine. The human benefit from such understanding will someday surely match the theoretical impact that DNA study has already made on cell biology.

These expectations for a possibly long-delayed future benefit have been heightened and accelerated by new findings that give us much greater technical ability to manipulate microbial DNA. New methods of DNA splicing have already opened up many lines of investigation into the structure of eukaryotic (higher life form) chromosomes.

We can now fragment animal or human DNA into perhaps a million segments and transfer a single segment to a bacterial host for study in a microcosm or for production of large quantities of a specific DNA segment. This allows more elaborate analysis than has ever been possible with the enormously complex, original, unfragmented source material.

This technique of gene implantation can also be used to transfer the genetic information for a given product from the cell of one species to that of another; and this is the direction, in my own view, that will lead to a technology of untold importance in diagnostic and therapeutic medicine: the ready production of an unlimited variety of human proteins. Analogous applications may be foreseen in fermentation processes for the cheap manufacture of essential nutrients and in the improvement of microbes for the production of antibiotics and special industrial chemicals.

In the face of such a revolution, the primary concern of researchers in the field has been the public hazards that such a technology may create. While we may indeed inherit a Promethean dilemma, public policy decision can lead to social good only if we are equally well-informed about the potential risks and benefits of further work on DNA splicing. If substantial risk can be identified, there is no doubt of the need for ethical and operational safety standards; the only question must be whether the form and implementation of such standards are adequate.

Too often, the "easy" way to handle such a problem is to invoke a formal regulatory statute, ignoring how well the actual bureaucratic enforcement or policing of the rules meets the intended balance of risks and benefits. Before elaborating on the policy issues, it may be well for me to outline what is currently being done in DNA splicing, some promising applications, and also the risks of further work in this field.

DNA recombination, as the ultimate purpose of the sexual form of reproduction, is, of course, one of the major happenings in the natural world. Among higher life forms, DNA exchange is almost always limited to members of the same or closely related species. Bacteria and viruses, however, exhibit many exceptions to this rule, which perhaps reflects the fragility of the concept "species" when applied to these life forms.

For example, the entire group of enteric bacteria, includiug such forms as Shigella, Escherichia coli, Proteus and Serratia, can exchange genetic fragments without special intervention. Our own experiments in genetic exchange would not seriously increase the risks already latent in that natural process.

Convenient tools

An especially interestiug and important level of genetic organization in bacteria is the plasmid: a bit of circular DNA that behaves like an extra chromosome and seems to survive in nature by virtue of its easy transmissibility from one bacterial strain to another. Many different kinds of plasmids are known; in medicine, the most prominent are those which confer transmissible antibiotic resistance on human pathogens, notably staphylococci and some enteric pathogens such as Shigella.

These plasmids are a by-product of the evolution of their host organisms: the spread of antibiotic-resistance plasmids is the most formidable bacterial response yet to our widespread use of antibiotics. Other plasmids are undoubtedly involved in altering the pathogenicity and host-specificity of various bacteria; therefore, in simple self-defeuse, we must learn all we can about them, without delay.

Plasmids have also achieved special prominence for a technical reason—they are especially convenient tools for DNA splicing and for the transmission of DNA segments from one species to another, particularly in conjunction with another elegant tool: the R- (for restriction) enzyme. (The R-nucleases are widely distributed among cell types; they may be an important mechanism by which a cell fends off any "foreign" DNA while protecting its own.)

Stanley N. Cohen, M.D., of the Department of Medicine, Stanford University, has used an R-enzyme to simplify a naturally occurring plasmid to the point where it consisted of a small circle of DNA, embracing the minimum amount of genetic information needed to replicate, plus a single R-enzyme recognition site.

This artificial plasmid, pSC-101, has been an important tool in DNA splicing research. When exposed to R-enzyme, the circle is cut into a single open length with sticky ends. It is then possible to insert other sticky-ended pieces of DNA from divers sources into the plasmid, and finally to close it up with another enzyme, ligase. This process is the key to the convenient design and construction of new DNA molecules, which subsequently can be transferred to a bacterial host.

One important aspect of this research is that the new DNA does *not* have to come from the same bacterial species. For example, Dr. Cohen and his collaborators have already reported the successful transfer of DNA from a toad, Xenopus, to E. coli with evidence of the production of toad-like ribosomal nucleic acids in the modified bacteria.

In addition to these plasmids, bacterial viruses are being used in a similar fashion. Less elegantly, perhaps, segments of DNA from intact bacteria may also be used both for insertions and as the acceptors. So far, all of these techniques depend on the innate (and poorly understood) ability of bacterial cells to incorporate DNA furnished from without. There have beeu many published claims of similar phenomena with plant and animal cell acceptors, but to date the claims are unconfirmed.

The special power of the enzyme transfer techniques is that they depend on the basic chemical structure of DNA rather than on biological adaptation. Thus, laboratory manipulation may produce constructs that occur rarely, if ever, in the natural world. Most of these constructs would resemble hothouse plants, and be poorly adapted to competitive survival in the world outside the laboratory. But some, by chance, might be harbingers of new diseases, or the source of ecological upsets difficult to control—like the mongoose in Hawaii or the crabgrass in your lawn.

R-enzymes, mixed DNA, and acceptor bacteria surely bring about some DNA segment transfers in nature. Our knowledge of the extent of natural plasmid transmission among "unrelated" life forms was widened by recent discoveries of plasmids with extraordinarily broad host ranges. It is difficult, however, to assess just what *can* or *cannot* occur in nature.

Rapid advancement

DNA splicing is, however, merely the most powerful of several artificial techniques which bring together more-or-less natural assemblages of DNA. Indeed, it may prove to be less powerful than older methods (sexual crossing, transduction with bacteriophage, DNA-medicated transformation) for special constructions involving larger complexes than the segments yielded by R-enzymes.

These methods, in turn, are an extension of the artificial breeding of domestic animals and plants. In any event, the most efficient application of DNA splicing requires intimate knowledge of the genetic structure of both the donor and the acceptor strains, for which breeding methods are important if not indispensable.

Perhaps the single most important conclusion is that this technology is just in its infancy but has already advauced far—and that it is simple enough to be applied in any laboratory which can handle pure bacterial cultures. But it is just this simplicity, which makes for great convenieuce and speed of development, that has raised concern about the proliferation of such methods in the hands of people with perhaps less-than-mature professional and ethical judgment, and with insufficient skill to contain bacterial cultures in the laboratory.

Now that we have put the dangers of DNA splicing research into perspective, let us examine the promise that it holds. DNA segmentation and splicing is certain to play a vital role in the further domestication of microbes for such uses as the development of new antibiotics and the production of high-quality food protein supplements. However, the unique strength of this procedure is that it allows the large-scale production of gene products of a less easily domesticated species: man.

Hnman proteins already play a substantial role in medicine but a role which is hindered by scarce supply. Today, the most attractive candidates for such large-scale production are the human antibody globulins. Compared to the rare genetic defects in other proteins (as in the case of hemophilia), failure of error in the production of antibody globulin is quite prevalent and is known to play a major role in the breakdown of the body's defense against infectious disease, in autoimmune and allergic disease, and perhaps also in cancer.

The most comprehensive use for biosynthetic proteins would be in passive immunization against infectious disease. (Animal antisera were once used but had to be abandoned because of the anti-animal antibody that they provoked in man.) With wholesale production of biosynthetic proteins, passive globulin therapy could be targeted at those diseases for which either technical or social factors may bring about gaps in the protection provided by active immunization. Included in that group of diseases are influenza, hepatitis, smallpox, encephalitis, rubello, herpes, rabies, and perhaps also trypanosomiasis, malaria, schistossomiasis, tuberculosis, leprosy and many others.

Need for a ready defense

There is reason for special urgency in the development of a backup capability in passive immunization. Complacency about active immunization against diseases such as polio and the technical inadequacy of such vaccines as rubella and hepatitis have weakened our general posture of defense against viral pandemic. We have no assurance that the next influenza epidemic, slightly more virulent than the last one, will not take a million lives for lack of a ready defense.

A broader need for biosynthetic proteins lies in polyvalent prophylaxis for infants. The principal medical argument for breast feeding is that human milk provides the infant with colostrum and a continuing supply of maternal mixed globulins. In the future there might be a huge demand for polyvalent gamma globulin supplements for infants both in industrialized and in poorer countries. And an analogous veterinary use could bring about greater efficiency in livestock production.

Specific antibodies, of course, are already widely used as diagnostic reagents of high specificity and selectivity. But in sufficient quantity, blocking antibodies

might also play a useful role in helping protect transplanted tissues and organs from immunological attack by the new host. Conversely, tissue-specific ligating antibodies, although not necessarily cytotoxic themselves, may be useful in enhancing the cell-specific toxicity of certain cancer drugs. Cell-specific reagents would also be invaluable in diagnosis and in the specific separation of human cell types for either diagnostic or therapeutic applications.

Besides the specific antibody globulins, a number of important, but less specific, proteins (complement, properdin) play a major part in defense against infection. Fibrinolysin (plasmin) and urokinase (plasminogen-activator) represent a group of enzymes that experimentally have shown promise in the control of embolism. Besides these human proteins, many human hormones are also discouragingly scarce for use in clinical trials. The list of such bioproducts could be extended substantially. And perhaps the most important products are those that remain to be discovered.

Of course, microbial biosynthesis may well be supplemented by organic synthesis in human and hybrid somatic cell cultures and by cell-free ribosomal synthesis with m-RNA extracted from natural sources or synthesized. Each of these methods has its own peculiar difficulties and hazards, and the whole field will be advanced most rapidly by using the best available methods for any given problem.

At present, perhaps a half-dozen bacterial species are well enough understood to serve as prime vehicles in laboratory studies of DNA splicing. For safety and convenience, investigators have preferred not to use pathogenic forms. Yet many scientists are primarily concerned that DNA splicing may inadvertently generate a new pathogen inimicable to man or to some other species important to man's ecology. The most likely, but not necessarily the only, sources of such pathogenic genes are the organisms that most urgently need further study—the subtle and insidious killers not now amenable to medical treatment. These include slow virus infections that may be involved in a wide range of chronic diseases, including cancer and more familiar viruses, such as herpes, for which satisfactory vaccines are not available.

Speculating the hazards

The public debate over DNA splicing has focused on the possible hazards of new microorganisms, and away from their utilitarian prospects. The most urgent concern has been the danger of introducing potentially cancer-causing DNA into common bacteria. While this hazard is clearly speculative, the general territory is so poorly understood that no one can argue against the need for cautious laboratory procednres. A numher of workers—particularly those whose special experience or training has been in fields other than medical microbiology—have confessed giving almost no thought in the past to safety; some of them are now among the most zealous in demanding tighter regulation of such research. And that zeal has spread to create a sincere, almost frantic effort to ferret out and identify the most remote, conceivable hazards.

Viewed as a rather public soul-searching and self-education, these discussions are invaluable. The main danger is that some political imperative may forge these tentative questions into iron-clad regulations which will be with us long after their origins have been forgotten. After all, similar questions can be raised about the widest range of human activities: should it be lawful to keep domestic cats when we suspect that they harbor toxoplasmosis, and possibly leukemia as well? Similarly, what assurance do we have that artificial pollination will not produce a weed that could ruin the wheat crop a decade from now? Closer to home, should we forbid international travel simply because our quarantine procedures do not guarantee that exotic diseases will be kept out?

For each of these cases, and many more, the apparently innocuous doctrine, "As long as there is any risk, don't do it!" can only bring a loss to human welfare. We must instead make every feasible effort to assess both the risks and the benefits of a given course of action—only then will we be able to find the optimal balance. But individuals can hardly determine the best policy about their own future—includiug their expectations for what medicine will offer for the infirmities of their own later years—without expert assessment.

Such assessments are difficult, problematical, and controversial. But a committee of the National Academy of Sciences has made some headway in trying to classify different categories of hazard. Where such hazard is reasonably predictable, the committee has recommended laboratory containment precautions akin to those appropriate for known pathogens. This applies, for example, to experiments in the recombination of known tumor virus DNA with bacterial plasmids.

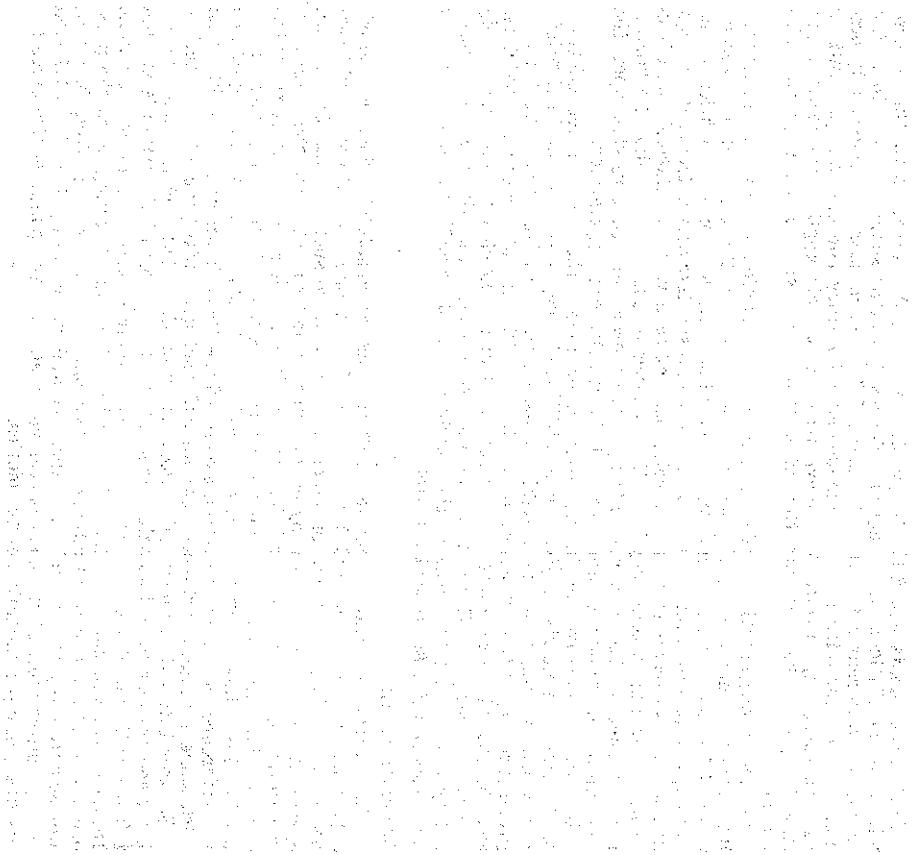
For more conjectural hazards, such as the introduction of antibiotic resistance into common, non-pathogenic species, the high security requirements recommended by the committee may be an inordinate burden for laboratories (who, in fact, will pay for them?) in relation to the prospective gains. The best strategy in such a case seems to be the development of safe vectors: plasmids and bacteria engineered so that they have little chance of surviving outside the laboratory. In fact, in the long run this is a safer procedure that relying upon the uncertainty of human compliance with fixed rules and regulations.

Remaining controversies in this area center upon rather complicated analyses of the most remote risks. Given some additional time, most research institutions will work out their own reasonable plans, based on the national guidelines. A prematnre imposition of external regulation will not only frustrate nseful research, but will also hinder that research which is needed to more accurately assess the dangers. Those who consider themselves guardians of the public safety must count the costs to the public health of *impeding* research, as well as the speculative *hazards* of research.

Society's consent

This partly volnntary approach will not assure absolutely that no foolish experiment is ever attempted. Bnt the history of human institutions should suffice to show that *no* system of sanctions can achieve such a goal. The human species is inevitably attended by contaminating and parastic microbes—the person suffering from an enteric infection who fails to wash his hands or the influenza victim who insists on going to work is behaving nnethically and to the peril of his fellows. But we wonld scarcely invoke serious regulatory sanctions in preference to public education, except where there is an unusual public risk with some attendant evidence that an enforced quarantine would be effective.

Senator Edward Kennedy (D-Mass.) has remarked that society must give its informed consent to technological innovation. The power of the purse is enough to enforce that doctrine; nor can there be any quarrel with it on ethical grounds. Informed consent surely includes knowing the hazards of saying no to the prospects of significant medical advances. DNA splicing research, far from being an idle scientific toy or the basis for expensive and specialized aid to the privileged few, promises some of the most pervasive benefits for the public health since the discovery and promulgation of antibiotics.



APPENDIX 12

(Reproduced from: The Sciences, September/October 1976 by permission of the publisher, the New York Academy of Sciences and Dr. George Wald, Harvard University.)

THE CASE AGAINST GENETIC ENGINEERING

(By George Wald)

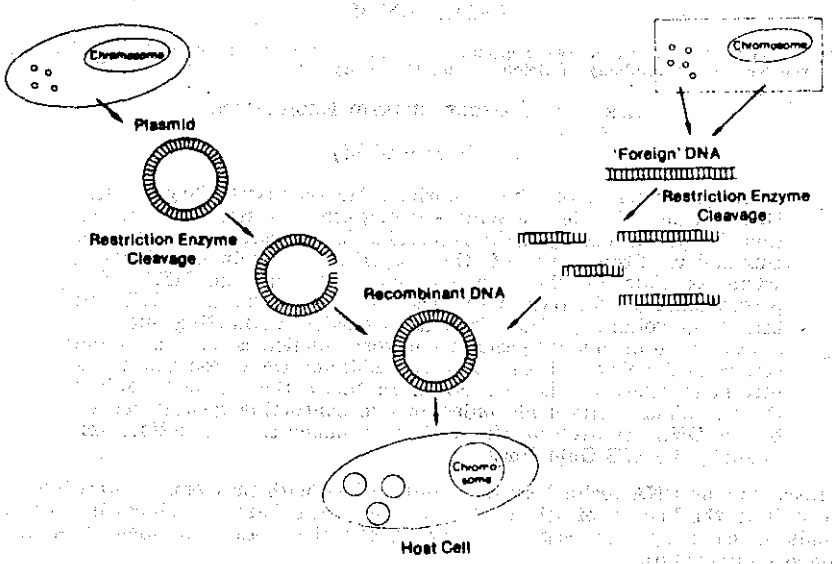
During hearings before the Cambridge, Massachusetts City Council, Harvard biologist George Wald—among others—testified in opposition to performing genetic recombination research at Harvard University. Proponents of the experiments included Harvard scientists Matthew Meselson and Mark Ptashne and MIT Nobel prize-winner David Baltimore. Despite the fact that the NIH had issued its voluntary Guidelines days earlier, permitting such research to go on under special laboratory conditions, on July 7 the city council voted a three-month recombinant DNA research moratorium to study the issue further. In this article, Nobel laureate George Wald outlines his objections to continuing genetic recombinant DNA research at Harvard, even under the restrictions imposed by the NIH Guidelines.

Recombinant DNA technology faces our society with problems unprecedented not only in the history of science, but of life on the Earth. It places in human hands the capacity to redesign living organisms, the products of some three billion years of evolution.

Such intervention must not be confused with previous intrusions upon the natural order of living organisms: animal and plant breeding, for example; or the artificial induction of mutations, as with X-rays. All such earlier procedures worked within single or closely related species. The nub of the new technology is to move genes back and forth, not only across species lines, but across any boundaries that now divide living organisms, particularly the most fundamental such boundary, that which divides prokaryotes (bacteria and bluegreen algae) from eukaryotes (those cells with a distinct nucleus in higher plants and animals). The results will be essentially new organisms, self-perpetuating and hence permanent. Once created, they cannot be recalled.

This is the transcendent issue, so basic, so vast in its implications and possible consequences, that no one is as yet ready to deal with it. We can't deal with it until we know a lot more; and to learn those things we would have to venture out into this no man's land. It is nothing like making new transuranic elements. New elements only add to the simple series of integral atomic numbers that underlie the Periodic System. Their numbers are limited and their properties highly predictable. Not so new organisms. They can be as boundless and unpredictable as life itself.

Technologically Redesigning Living Organisms



Recombinant DNA technology was launched in 1973 and 1974, largely through researches carried out in the laboratories of Stanley Cohen at Stanford University and Herbert Boyer at the University of California in San Francisco. A rapidly growing number of available restriction enzymes can be used to cut short specific segments of DNA usually containing several genes out of the chromosomes of any type of cell. These segments are then spliced with the help of the same and other enzymes, ligases, into viruses or the naturally-occurring small circular extra-chromosomal particles of DNA called plasmids. The plasmids can then be taken up by bacteria or animal or plant cells in which they reproduce, either in phase with the host cell or sometimes independently and many times faster. On occasion, the new genetic material fuses with the host chromosomes and reacts thereafter as a normal component of the host's genetic apparatus. In effect, such cells that have received foreign genes are new organisms, permanent hybrids of the host cells and whatever organism donated the transplanted genes. Their properties and capacities may differ profoundly from either host or donor.

—GEORGE WALD.

Up to now living organisms have evolved very slowly, and new forms have had plenty of time to settle in. In has taken from four to 20 million years for a single mutation, for example the change of one amino acid in the sequence of hemoglobin or cytochrome c, to establish itself as the species norm. Now whole proteins will be transposed overnight into wholly new associations, with consequences no oue can foretell, either for the host organisms or their neighbors.

It is all too big, and is happening too fast. So this, the central problem, remains almost unconsidered. It presents probably the largest ethical problem that science has ever had to face. Our morality up to now has been to go ahead without restriction to learn all that we can about nature. Restructuring nature was not part of the hargain; nor was telling scientists not to venture further in certain directions. That comes hard. With some relief, most biologists turn away from so vast and uncomfortable an issue and take refuge in the still knotty but infinitely easier technical questions: not *whether* to proceed, but *how*. For going ahead in this direction may be not only unwise but dangerous. Potentially, it could breed new animal and plant diseases, new sources of cancer, novel epidemics.

We must never forget that the first intimation of these potential hazards came from workers in this field. All honor to them. Faced with unique problems, as they alone then realized, they did unprecedented things. They brought about a voluntary moratorium on certain, more clearly dangerous kinds of experiments. And now, after three years of debate, consultation and negotiation, the National Institutes of Health issued its Guidelines on June 23.

THE NOBEL LETTERS

During the hearings a number of leading biologists wrote letters to Cambridge Mayor Alfred E. Vellucci defending genetic recombinant research at Harvard. Below are excerpts from letters by three Nobel laureates.

In my view these Guidelines are far more stringent than is reasonably necessary for the protection of public health. In every case where reasonable doubt could be entertained, it has been resolved in a way that imposes the most serious and conservative protective requirements. Most of the risks in question are purely conjectural and no substantive basis can be found for the dire prediction that the public health could be endangered by recombinant organisms. Nevertheless, the Guidelines in their present form have accepted every such speculation as if it were accepted reality. In summary, even the most cautious view of the NIH Guidelines should give citizens ample assurance that they go far beyond what is necessary to protect their health.

Elsewhere I have commented that the very act of setting up such elaborate precautions would frighten people because they go so far beyond what we do in other spheres of life. This seems to have happened in the present case—it is the very security precautions having been doubled and redoubled that has generated an unjustifiable fear. On the other side of the coin. I take the opportunity to indicate that research in this area has the potential for the most extraordinary contributions to medical advance and I would hope that Cambridge, Massachusetts would be proud to be the seat of major accomplishments in this direction.

JOSHUA LEDERBERG,
Stanford University Medical School.

In terms of our present knowledge, I feel that there are no real accidental dangers involved in research on animal virus and vertebrate cell DNAs under the NIH Guidelines. The specific dangers that have beeu suggested involve combinations of events that are either known not to occur or occur only at very low probabilities. Therefore, the likelihood of the occurrence of any specific danger is so low that it can be considered zero. In fact, I consider that the Guidelines are probably too restrictive in terms of our present knowledge of animal virus and vertebrate DNAs.

Furthermore, I consider it ineffectual to regulate on a local level research involving possible infectious entities. Unless there is national, and preferably international, regulation, local regulation would not serve to protect the inhabitants of that locale.

In addition, I have found the members of the Department of Biochemistry and Molecular Biology, Harvard University, conservative in respect to possible safety hazards from research with animal viruses and vertebrate cells.

As taxpayers and governmental officials, you have a responsibility to insure public health and safety, but you also have a responsibility to promote the public welfare. It is conceivable that the technique of recombiuant DNA may lead to major benefits in terms of public health and welfare. Therefore, a balance must be made between the "zero" likelihood of harm and the possibility of beneficial results.

HOWARD M. TEMIN,
University of Wisconsin Medical Center.

I implore you to encourage the progress of the planned facilities for genetic research at Harvard and to do your utmost to foster a spirit which advances this exceedingly important direction in medical science.

The new NIH Guidelines to which these Harvard facilities and investigators will adhere go far beyond reasonable needs for personal and public safety. I

assure you that the current hazards in many chemical, bacteriological, biological and physical laboratories in Cambridge, public and private, are far greater than those anticipated in recombinant DNA research.

I realize you have heard a different point of view from some Harvard and MIT scientists who have testified before you. I believe their views are not based on sound scientific evidence and are highly exaggerated. In my estimation, they represent a tiny fraction of the scientific community.

I implore you again not to suppress the serious and responsible search for new knowledge. If scientific inquiry is stifled in Cambridge, it will be done in Waltham, Palo Alto or Moscow. In 1976, please do not squander your most precious human resources.

ARTHUR KORNBERG,
Stanford University Medical Center.

"And God blessed Noah and his sons, and said to them, 'Be fruitful and multiply, and fill the Earth. The fear of you and the dread of you shall be upon every beast of the Earth, and upon every bird of the air, upon everything that creeps on the ground and all the fish of the sea; into your hand they are delivered. Every moving thing that lives shall be food for you; and as I gave you the green plants, I give you everything.'"

—GENESIS.

One can hardly read the Guidelines, or the careful and sensitive statement by Donald Frederickson, the Director of NIH, on releasing them, and not be impressed with the goodwill and concern that animate them. Yet there is much in this enterprise and in the Guidelines themselves that troubles me greatly.

First and foremost: the very existence of the Guidelines begs the central question, whether this kind of research should proceed at all. The experiments are quite simple and straightforward. Can they be stopped? Perhaps they can. If one could neither publish the results nor exploit them commercially there would be little incentive to do them.

As for the Guidelines themselves, the first thing to understand is the context of utter ignorance of what to expect in which they had to be formulated. The Guidelines begin by saying: 'At present the hazards may be guessed at, speculated about, or voted upon, but they cannot be known absolutely in the absence of firm experimental data—and, unfortunately, the needed data were, more often than not, unavailable."

Physical containment.—The purpose here is to keep the recombinants from escaping the laboratory. The Guidelines list four levels of containment labeled P1 to P4; but in effect there are only two levels, a lesser—P3—and a greater—P4. This classification is itself deceptive, for it makes the prevalent P3 facility sound better than it is, three quarters of the way to the top, whereas in fact it is the lowest level of containment. P1 is just a laboratory, P2 the same laboratory with a warning sign on the door. A young woman demonstrating a P2 experiment at an open hearing before the Cambridge City Council made a point of putting on the prescribed laboratory coat; but she had long, loose, abundant hair that could have carried more bacteria or viruses than a dozen lab coats.

A P3 facility such as has just been authorized at Harvard employs various devices intended to minimize the escape of recombinants. Yet the reason proponents of the facility at Harvard gave for building it within our Biological Laboratories, close to the laboratories of prospective nsers—though the building is half a century old and infested with ants and cockroaches—was that workers in the facility would be the principal means of spreading contamination, aud hence should have to move as short distances as possible. I think it is probably correct that the laboratory personnel will be the principal means of spreading any potential infection. But in that case, whereiu lies the containment? Why the elaborate and costly precautions within the facility?—the small unit at Harvard is estimated to cost more than $800,000. And what matter whether distances between the labs are short or long? All these workers move freely throughout the building and the city; they meet with us, eat with us, and—most importantly—they teacb classes of young students. I see no reason to believe that P3 containment, even if conscientiously enforced, can effectively contain.

Biological containment.—One of the most unsettling aspects of present recombinant DNA research is that the host organism that receives the plasmids that

carry foreign genetic material is almost always the colon bacillus, *Escherichia coli*, a constant inhabitant of the human bowel. To do potentially hazardous experiments, why pick an organism that lives in us? The reason is that we know more about *E. coli* than about any other living organism. Yet what is to keep some hybridized *E. coli* turned pathogenic from infecting its conventional human hosts? Or transferring those plasmids to human cells?

Hence the stress on the assurance that all recombinant experiments with *E. coli* will use the K12 strain, which, we are told, can exist only under special laboratory conditions and neither survives nor reproduces in the human gut. The use of this strain is the "biological containment."

In this connection Stanley Falkow of Seattle, Washington, submitted to the NIH Recombinant DNA Advisory Committee a highly informative report on the ecology of *E. coli*. According to Falkow, almost innumerable serologically distinct strains of *E. coli* inhabit the human colon from time to time, the population constantly changing. The more persistent (resident) strains last several months, other (transient) strains only a few days. The statement that the K12 strain does not survive in the human bowel rests primarily on observations by E. S. Anderson and H. Williams Smith that this strain "is a poor colonizer of the human alimentary tract." Smith found a mean survival time of about three days, Anderson about six days. Anderson also found that it "multiplied to some extent in two of eight subjects." Hardly an impressive statistic! Furthermore he could detect plasmid transmission from K12 to other enteric flora when it was fed "in substantially high numbers."

Falkow confirms these observations, and adds another that is singularly important: Working with calves, he found that introducing certain plasmids into K12 increased its survival and multiplication in the gut many times over. He concludes that "it may not be too farfetched to suggest that some DNA recombinant molecules could profoundly affect the ability of this *E. coli* strain to survive and multiply in the gastrointestinal tract".

These are oddly inadequate data to carry such weight. We would like to know much more. How does K12 get along in persons whose colons are relatively empty of bacteria and hence offer it little competition?—such as newborn infants, or persons who have just been treated with sulfa drugs or antibiotics? So-called biological containment seems to me as problematical as P3 physical containment.

Enforcement.—The Guidelines are just that, hence wholly voluntary. The only penalty now available for simply disregarding them is the possible withholding of federal research support. Obviously this applies only to research dependent on federal funds. It leaves out completely the rapidly growing industrial exploitation of recombinant DNA technology.

Benefits and risks.—I have up to now said almost nothing of the potential benefits of this technology. I think that the most certain benefits to come out of it would be scientific: increased understanding of important biological phenomena, such as the mechanisms that turn specific gene activities on and off, that trigger cell multiplication and differentiation, that regulate cell metabolism. We are also offered the prospect of large practical benefits: teaching cereal plants to fix their own nitrogen from the air, new bacterial syntheses of drugs and hormones, the hope that increased understanding of cancer may lead to its cure. I cannot think of a single instance of such developments, scientific or practical, that does not also involve large potential risks.

Consider cancer. If indeed it turns out that recombinant DNA research will improve our understanding of cancer, that would still be far from showing us how to cure it. In spite of many statements, as vague as they are optimistic, that the cure of cancer lies in this direction, it is hard to see how that is to happen. Any such hope must be balanced against the real possibility that recombinant DNA experiments may induce new cancers. If right now I had to weigh the probabilities of either event I would guess that recombinant DNA research carries more and earlier risks of causing cancers than hope of curing them.

Add that about 80 percent of cancer in this country is now believed to be of environmental origin. The largest single cause of lung cancer is smoking, but one is free to smoke or not. About 40 percent of those environmental cancers happen in the work places, through involuntary exposure to a rapidly increasing variety of toxic materials in industrial use. If one were really concerned about cancer, there is the obvious place to attack it, with sure and immediate results.

Or consider a frankly industrial development. General Electric is reportedly

trying to patent a newly assembled strain of *Pseudomonas* bacteria that can wholly digest crude oil. It was developed there by Ananda Chakrabarty by transferring plasmids from several strains, each of which could digest oil partially, into a single strain that can do the whole job. It is pointed out that this organism could be very useful for cleaning up oil spills. Very true; but how about oil tbat has not spilled?—oil still in the ground, or on the way, or stored? Can this organism be contained, kept from destroying oil we want to use? Or will we need to begin to pasteurize oil?

The corporate connection.—As early as February 1974 *Fortune* magazine hailed the coming importance of genetic recombination in industrial developments. "The best microbes are freaks," it said and "many scientists see an important industrial role ahead for the powerful new metbods of transferring genetic material from one cell to another." It named a number of them, including a few who are already directing corporate activities.

The industrial exploitation of recombination technology raises special problems, for in that, as any other business enterprise, the major goal is to maximize profits and, frequently in the past, public and worker safety and health have been subordinated to that end. Last May representatives of ahout twenty drug and chemical companies met with NIH Director Frederickson to discuss the proposed Guidelines. They expressed "general support," but made tbree points: (1) the fear that voluntary Guidelines might lead to enforceable regulations, (2) for reasons of competition, the companies could not afford to reveal what recombinant DNA experiments they were performing and (3) they found other features of the Guidelines onerous, for example the restrictions on large-volume experiments, which of course are less easily contained, but which they require in testing procedures for commercial feasibility.

The dilemma of the NIH.—The recombinant DNA development faces NIH with an interesting predicament. Anything I say of this is said sympatbetically, for under Donald Frederickson's perceptive leadership it is doing as well as could be hoped. Yet is it possible for the same agency both to promote and regulate? The old Atomic Energy Commission, set up originally to regulate, turned instead to promoting nuclear power, and that eventually destroyed it. It has been replaced by two separate agencies, one for research and development, the otber for nuclear regulation.

NIH, on the contrary, set up to promote scientific and medical research, is now being forced into regulation. Its entire impulse, as that of all other institutions concerned with research, is to avoid regulation, to maintain full freedom of inquiry. Probably that is why it can bring itself only to promulgate voluntary gnidelines. Surely it recognizes the previous history of ineffectuality of voluntary self-regulation in other areas. For the NIH Guidelines to be enforced, academically and particularly industrially, they would have to become regulations, backed by legislation, with adequate provisions for licensing, inspection and supervision. The NIH would like to avoid such measures and so, as a scientist, would I. Yet this situation seems to demand them, and I fear that scientists and science will eventually have to suffer because of them.

What to do.—First, I think it essential to open a wide ranging and broadly representative discussion of the central issue: whether artificial exchanges of genetic material among widely different living organisms should be permitted.

Second, in consideration of the potential hazards and our present state of ignorance, I would confine all recombinant DNA experimentation that transcended species boundaries to one or a few national or regional laboratories where they can be adequately confined and supervised. There, every attempt shonld be made to define the hazards that are now only guessed at. If trouble should arise, I would expect it to involve first the workers in snch laboratories and their families whose health should be carefully monitored. Until such trials have told us better what to expect, this kind of investigation should have no place in crowded cities or edncational institution.

Third, industrial research and development in this area need most of all to be brought nnder control. The usual secrecy that surrounds industrial research is intolerable in a province that can involve such serious consequences and hazards. The need for licensing, inspection and supervision will probably require national legislation. Hearings in the Congress should begin at once to consider these issues.

As I write these words, they trouble me greatly. I fear for the future of science as we have known it, for humankind, for life on the Earth. My feelings are ambivalent, for the new technology excites me for its sheer virtuosity and its

intellectual and practical potentialities; yet the price is high, perhaps too high. We are at the threshold of a great decision with large and permanent consequences. It needs increasing public attention here and worldwide, for it concerns all humankind. That will take time, during which we can try to learn, as safely as that can be managed, more of what to expect, of good and ill. Fortunately there is no real hurry. Let us try, with goodwill and responsibility, to work it out.

THE DEFENSE DOESN'T REST

One of the leading advocates of recombinant DNA research is Mark Ptashne of Harvard's Biological Laboratories. The Sciences gave him an opportunity to reply to George Wald's case against genetic engineering at Harvard.

George Wald's plea that "artificial exchanges of genetic material among widely different organisms" should be banned from "crowded cities or educational institutions" is based on a curious mixture of fantasy, misinformation. and irrelevancies. Consider tbe following:

1. Wald notes that the Guidelines specify four levels of physical containment, P1 to P4. He states that "P1 is just a laboratory, P2 the same laboratory with a warning sign on the door," and "P3 . . . is the lowest level of containment," and "I see no reason to believe tbat P3 containment, even if conscientiously enforced, can effectively contain."

In fact, a P2 lab, as specified in the NIH Guidelines, does have a sign on the door, but in addition, it is subject to the following regulations, among others: all liquid and solid wastes are incinerated or otherwise decontaminated before removal from the lab; reusable equipment (glassware, etc.) is prohibited and biological safety cabinets must be used for all operations that generate aerosols. Hospital laboratories are typical examples of P2 facilities, and they are used for routine culture of agents as dangerous as those which cause anthrax, pneumonia, plague, measles, mumps, influenza, gonorrhea, etc. P3 specifies more sophisticated construction, requiring—among other features—a constant negative air pressure so that air flows from the corridor to the lab when the doors are opened. Wald is apparently unaware of just how well P3 laboratories do contain. For example, W. Emmett Barkley, Director of Research Safety of the National Cancer Institute, points out that experience shows that the most highly infectious biological agents may be used in a properly run P3 laboratory with minimal risk. He estimates that, on average, workers in such laboratories incur about two infections for every 100 person-years of work—and this with the most highly infectious agents known. Moreover, many years of experience with P3 facilities at the Center for Communicable Diseases in Atlanta, and at Fort Dietrick, and the laboratories of the National Institute of Health in Maryland show that, even in those rare cases of infection of a laboratory worker caused by human error, secondary spread to people outside the lab is virtually nonexistent. According to studies conducted in Barkley's office by Barkley, Arnold Weedum and others, there is no evidence that proper handling of even the most dangerous organisms under P3 conditions—or in many cases under less restrictive conditions—endangers the surrounding community. Wald's statement that P3 labs do not contain is simply false. Of course the designations P2, P3, or P4 may be misused, but then even ordinary chemicals present in any biochemical lab can be misused, and in the latter case with immediate and serious danger. It must not be forgotten that recombinant DNA experiments which present some plausible scenario for significant danger have been banned altogether by the Guidelines, and many experiments are confined to the extraordinarily protective environment defined as P4.

2. Wald objects to the use of *E. coli* in recombinant DNA experiments. He notes that *E. coli* is a "constant inhabitant of the human bowel." and he asks ". . . what is to keep some hybridized *E. coli* turned pathogenic from infecting its human host?" Wald implies tbat *E. coli* turns pathogenic at the drop of a gene. He is apparently unaware of the years of sophisticated experimentation that have been devoted to analyzing the factors required to render *E. coli* pathogenic. In fact, despite these efforts, no one yet has managed to transfer any gene or set of genes from a pathogenic *E. coli* to *E. coli* K12, the commonly used laboratory strain, aud render that strain pathogenic. This is not surprising: pathogenic organisms are highly evolved to occupy a very specialized niche, and it is no easy matter to confer those multiple properties required for pathogenicity on a non-

pathogenic bacterium grown for many generations in the laboratory. The scenario behind Wald's query involves a series of extremely improbable events. We would have to imagine a segment of foreign DNA—representing about .1 percent of the DNA of the bacterium into which it is placed—rendering K12 pathogenic. The K12 would then have to colonize the human gut (which K12 does not ordinarily do), and survive excretion and a sojourn outside the gut *en route* to infect another person or animal. As far as we know, the probabilities of any one of these events occuring is extremely small, and the aggregate probability, vanishingly small. Wald formulates his questions in such a way as to simply ignore these issues.

3. Wald questions the concept of "biological containment." He asserts that ". . . all recombinant experiments with *E. coli* will use the K12 strain . . ." and he goes on to cite, among other reports, the fact that when K12 is fed to human beings in large numbers it can be detected for some days in the intestinal tract. He points out that plasmid transmission has been detected from one bacterium to another *in vivo* when bacteria carrying these plasmids are ingested in large numbers. Wald's characterization of biological containment is rudimentary. In fact, most experiments done in P3 and P4 laboratories would require use of specially enfeebled strains—for example, mutants of K12—that have been demonstrated to survive only 10^{-2} times as well as ordinary K12 in laboratory conditions. Tests performed with candidates for such strains have revealed no survival in the animal gut, even when ingested in extremely high numbers. Moreover, the plasmids used in these experiments are of the non-transmissible type. The transfer resulted quoted by Wald were obtained with plasmids that transfer at very high frequency. In fact, no one, to my knowledge, has demonstrated transfer of a non-transmissible plasmid *in vivo* from K12, even under strong selective conditions.

4. Wald believes that "recombinant DNA technology faces our society with . . . [unprecedented] . . . problems," because "the nub of the new technology is to move genes back and forth, not only across species lines, but across any boundaries that now divide prokaryotes from eukaryotes." The dogmatic statement of fact that so moves Wald is highly debatable. It is well known that among prokaryotes *in vivo*, DNA passes not only across species lines but also across genus and family lines, and it is far from obvious that DNA does not frequently pass the "prokaryotic-eukaryotic" barrier as well. Many biologists believe that some of the organelles of higher cells, such as mitochondria and chloroplasts, are descendents of bacteria that existed as symbiotes of eukaryotic cells. It has been estimated that the Earth's human populatiou alone excretes some 10^{22} bacteria per day. *E. coli* absorbs foreign DNA in the laboratory quite readily when treated with calcium iou, aud the frequency with which such couditions arise in the gut, or with which mutants arise that are competent to accept foreign DNA under ordinary conditions, is probably not much below 10^{-6}. Other common strains of bacteria, such as *Haemophilus*, are competent, under almost all conditions, to absorb foreign DNA. Of course, host restriction will decrease the efficiency of any initial transfer, but once DNA is past this barrier it is no longer recognizable as foreign. Wald's scenario for disaster assumes that the addition of a bit of foreign DNA would render a bacterium a strongly virulent pathogen. It is likely that such a recombinant, with a marked selective advantage over ordinary bacteria, would not have been produced and selected for by nature? Wald apparently does not take his "barrier" argument very seriously, because he freely asks, "Yet what is to keep some hybridized *E. coli* from transferring those plasmids to human cells?"

5. Wald states: "I think that the most certain benefits to come out of [this technology] would be scientific: increased understanding of important biological phenomena, such as the mechanisms that turn specific gene activities on and off, that trigger cell multiplication and differentiation, that regulate cell metabolism." He adds: "I would guess that recombinant DNA research carries more and earlier risk of causing cancers than hope of curing them." Wald presents not a shred of argument or evidence that would make plausible the extraordinary statement that recombinant experiments might cause cancers. The first part of Wald's statement is in fact a good summary of what most workers in the field believe to be the likely benefits of this work.

6. Wald has added a series of diversions, the relevancy of which I cannot determine. It is no news to most of us that smoking causes lung cancer, but what are we to make of the admonition: "If one were really concerned about

cancer, there is the obvious place to attack it [i.e., smoking], with sure and immediate results." Does Wald mean to imply that those who work on DNA are not "really concerned about cancer?" What about those who work on vision? In fact we know very little about how carcinogens cause cancer—in particular we know almost nothing about the mysterious process of promotion wherein the potency of carcinogens is vastly increased by the presence of otherwise apparently harmless substances. A real understanding of the relations between carcinogenesis and the environment may depend on our understanding of these processes which in turn requires basic research.

What are we to make of Wald's cry that the NIH Guidelines do not now apply to industry, and that there is some ominous "corporate connection" between something and something else? Does this mean that the Guidelines themselves are inadequate? At this moment Senators Kennedy and Javits are attempting to make the Guidelines into federal law. Perhaps making the Guidelines into federal, enforceable law will satisfy Wald, or perhaps not. What Wald may be after is outlawing these experiments altogether by statute.

Finally, it is perhaps worth noting that so far as I am able to ascertain, the overwhelming majority of informed scientists, most especially experts on infectious diseases, regard the Guidelines as providing more than sufficient levels of safety for the recombinant DNA experiments.



APPENDIX 13

Recombinant DNA—On Our Own

The recombinant DNA issue will not go away. It is but the churning edge of a turbulent sea of concerns to come, as mankind again extends its dominion, this time to redirect the course of biological evolution. And as we find our course in this changed world, we should not expect that the ways of science will remain unchanged.

It is the success of science that has ended its pleasant isolation from the strident conflict of interests and the often passionate clash of values. The great discoveries in molecular and cellular biology—in particular the elucidation of the structure and functions of the nucleic acids—have provided us with a definitive understanding of the nature of life. Earlier in this century splendid discoveries in physics and chemistry provided us with a definitive understanding of the nature of matter. From that understanding has come the technology to reshape the inanimate world to human purpose. And many are less than pleased with the consequences. Now the description of life in molecular terms provides the beginning of a technology to reshape the living world to human purpose, to reconstruct our fellow life forms—each, as are we, the product of three billion years of evolution—into projections of the human will. And many are profoundly troubled by the prospect.

With the advent of synthetic biology we leave the security of that web of natural evolution that, blindly and strangely, bore us and all of our fellow creatures. With each step we will be increasingly on our own. The invention and introduction of new self-reproducing, living forms may well be irreversible. How do we prevent grievous missteps, inherently unretraceable? Can we in truth foresee the consequences, near- and long-term, of our interventions? By our wits mankind has become the master of the extant living world. Will short-sighted ingenuity now spawn new competitors to bedevil us?

The apparent significance of the potential hazard of recombinant DNA depends markedly on the perspective in which the issue is seen. Viewed narrowly the potential hazard seems slight. Most of the novel microorganisms will likely be innocuous. A few, by careful design and selection, will be of value for human purpose. A few might inadvertently be perilous. The chance of release of these organisms is statistically small although it can hardly be null. The chance of a series of events necessary to produce a plague seems slim, in any one experiment.

Viewed broadly, however—over long years, in numerous environs, with countless experiments—a far larger penumbra of hazard appears. Nature has developed strong barriers against genetic interchange between species. What do we know of the consequence of breaching these barriers? In particular and specifically, what may in time ensue if we introduced genetic intercourse between ourselves (and our biological relations) and the ubiquitous microorganisms with which we live so intimately?

We can have no assurance that science will not bring us into a more dangerous world. The search for knowledge has often been hazardous; many explorers have faced great perils. Now the hazards can encompass the planet, and we may not continue to rely upon the resilience of nature to protect us from our follies.

New circumstances bring new perspectives. As scientists we have had the rare luxury to pursue truth, unhampered by conflicts of compassion. Caution has been an unfamiliar virtue while boldness and curiosity have been hallowed. As we cut free the strands of our inheritance, a different blend of virtues may be in order and other traditions may be helpful.

We should not underestimate these stakes, now and in time to come. We will need to establish in each time a sense of limits commensurate with our finite vision and shaped by our sense of the moral—limits within which we believe we can explore without fear and with decency, and beyond which we should

tread most gingerly. These limits will change continually as knowledge grows. In their definition and redefinition we should involve all who can help and respect all of those affected.

As scientists who seek to understand nature, we should not unthinkingly and irreversibly perturb it. As human beings we have a responsibility always to be concerned for our fellows and our fellow creatures and the future generations.

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NOTE.—Sinsheimer, Robert L. Recombinant DNA—On Our Own. Bioscience, v. 26, October 1976: 599. Reproduced from: Bioscience, v. 26, October 1976, by permission of the publisher, the American Institute of Biological Sciences.

APPENDIX 14

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PUBLIC LECTURE, HARVARD SCIENCE CENTER: DARWIN, PASTEUR, AND THE ANDROMEDA STRAIN

Recent developments in molecular genetics have made it easy to insert small fragments of genetic material (DNA) from any organism, including man, into tiny self-replicating units of DNA from bacteria, called plasmids. These can be reintroduced into bacteria, such as the common *E. coli* of the human gut, which can then be used to manufacture large quantities of the inserted DNA in pure form. The possibilities opened up by this technique have aroused enormous interest among biologists but have also generated wide public concern, focused on two potential risks. The first is the immediate risk of harm from some of the novel organisms produced. It is useful to consider this risk in terms of three probabilities: the probability of producing a pathogenic organism, the probability of its infecting an exposed laboratory worker, and the probability of its spread in the community. The second risk is a more conjectural, long-term one: that our interference with evolution, by recombining DNA from distant sources, will eventually create unforeseeable disasters.

Both these issues raise ethical questions, on which a public consensus is the ultimate arbiter. But a rational decision requires an informed public—and despite claims that we are entirely in the dark in this novel territory, we actually possess a good deal of relevant information. It is this knowledge—in microbiology, epidemiology, and evolutionary theory—that I wish to review. For the extensive public discussions of the hazards have been built largely on the assumption that any novel organism we may produce is likely to survive and spread, and this assumption ignores what was Darwin's great discovery: the dominating role of natural selection in determining what survives, multiplies, and evolves. Working with the invisible organisms that were not part of Darwin's world, Pasteur made essentially the same discovery, though it was expressed in different terms: he showed that bacteria do not arise by spontaneous generation but are ubiquitous, and the kinds that grow out in any medium are the ones that are selected by that medium. For example, the same mixture of contaminants from the air grows out one kind of organism in grape juice, producing an alcoholic fermentation, and another kind in milk, producing a lactic acid fermentation.

Unfortunately, the evolutionary considerations that I shall invoke cannot provide the hard data that we have become accustomed to in modern experimental biology, and a skeptic might dismiss the arguments as mere handwaving. But then nearly all of Darwin's arguments, based on inferences about the past and not on verifiable experiments, could be similarly dismissed. And I would remind you that Darwin's theory remains the most profound generalization in biology—unifying the field, enormously supported today by the evidence for continuous evolutionary progression in DNA sequences, and rich in implications for our understanding of man.

Let us start by reviewing some principles from evolution and microbiology that seem pertinent to the problem of estimating the risks of recombinant DNA research.

I. BACKGROUND

A. *Microbiological and evolutionary principles*

(1) *The meaning of species.*—As evolution proceeded from prokaryotes (i.e., bacteria with a single chromosome) to eukaryotes (i.e., higher organisms, with a more complex genetic apparatus), it created the process of sexual reproduction, which reassorts genes and thus provides vastly increased genetic diversity for natural selection to act on. But while diversity is necessary for evolution, un-

limited combinations from the pool of genetic material in the living world would not be useful, for a succesful organism must have a reasonably balanced set of genes. Hence the development of sexual reproduction was accompanied by the development of species: groups of organisms that reproduce only by mating with other members of the same group, and not with members of other species. Some closely related species produce hybrid offspring, such as a mule, that are viable but not fertile, while more distant crosses produce no offspring at all. The evolutionary value of such fertility barriers is clearly to avoid useless production of grossly unfit, non-viable progeny.

Unlike eukaryotes, prokaryotes ordinarily reproduce by the asexual process of cell division, which means that the genetic properties of a strain remain constant for generation after generation, except for rare mutations or rare gene transfers. The gene transfers, which are usually mediated by plasmids or viruses, do not show a sharp species boundary: they simply become less efficient the greater the evolutionary separation between the donor and the recipient. Prokaryotes therefore have no true species: they have an almost continuous spectrum of genetic patterns, and the borders between what we call bacterial species are arbitrary and often controversial. *E. coli*, for example, is not a homogeneous species. It is the name given to a range of strains with certain common features and also with a variety of differences—in surface molecules, nutrition, growth rate, sensitivity to inhibitors, etc. These differences determine the relative Darwinian fitness of various strains for various environments.

(2) *Bacterial ecology.*—Every living species is adapted to a given range of habitats. Tbe set of bacterial strains called *E. coli* thrive only in the vertebrate gut. They survive temporarily in water but quickly die out. (Indeed, for that reason the *E. coli* count of a pond or a well is a reliable index of its continuing fecal contamination.) In the gut there is intense Darwinian competition between strains, depending on such variables as growth rate, nutritional requirements, ability to scavenge limited food supplies, adherence to the gut lining, and resistance to antimicrobial factors in the host. Hence most novel strains are quickly extinguished. It is the kind of selection by competition envisaged by Darwin for higher organisms, bnt it happens in days rather than in eons, becanse the generation time of many bacteria is only 20 minutes and the selection pressures are often intense.

This effect of the environment in the gut (i.e., type of food and physiological state) on the normal flora is readily recognized. For example, when breast feeding is replaced by solid food the character of the stool changes dramatically, as lactic acid bacteria, which produce sweet-smelling products, are replaced by *E. coli* and other foul organisms. And in an experimental example, early in this century, Mechnikov romantically hoped to promote longevity by reversing the process, by supplying a large number of lactic acid bacteria, in the form of yogurt, to displace the presumably toxic foul organisms. The experiments were a dismal failure, but perhaps a commercial success. In a third, more recent example we frequently see the normal bacterial population of the gut disturbed by administration of antibiotics, and it has not proved possible, despite commercial interest, to accelerate recovery by administering desired strains. It is clear that in the gut the environment plays a dominating role in determining what strains persist.

(3) *Pathogenesis.*—Only an incredibly small fraction of all bacterial species can cause disease. The rest play essential roles in the cycle of nature, in which CO_2 from the air is fixed in plants or bacteria by photosynthesis, the plants are eaten by animals, the animals and plants return to the soil after death, and there microorganisms digest the dead organic matter and return tbe carbon to the atmosphere as CO_2.

Infections bacteria differ from each other in several distinct respects: infectivity (i.e., the infectious dose, ranging from a few cells of the tularemia bacillus to around 10^9 cells of the cholera vibrio); specific distribution of the organism in the body; virulence (i.e., the severity of the disease once the infection has overcome natural resistance); and communicability from one individual host to another (including length of survival in nature). As with any complex property, these attributes depend on the coordinate, balanced activity of many genes, which are capable of independent variation. It is especially important to distinguish the ability to produce a serious disease from the ability to spread. For example, the tetanus bacillus is a normal, non-invasive inhabitant of the gut, but it can cause fatal illness when trauma gives it access to a susceptible tissue.

(4) *Stabilizing and diversifying selection.*—When an organism grows continuously in a relatively constant environment natural selection has a stabilizing effect, weeding out the variants that deviate too far in any direction from the well adapted norm. But when the environment is changed the same basic process of natural selection has another, diversifying effect: the new circumstances select for the preferential survival and reproduction of variants with increased fitness for those circumstances. This Darwinian process explains the fluctuation in the properties of bacterial cultures that confused the early workers. For example, when pathogenic bacterial strains from infected hosts are isolated in the laboratory and then repeatedly transferred in artificial culture media they face an abrupt change of environment, and they rapidly develop improved adaptation to the new environment at the expense of decreased adaptation to the old one (i.e., they lose virulence).

The mechanism is now clear, and it does not involve any directive effect of the environment on the shifting bacterial population. Instead, rare mutants of all kinds are constantly appearing in the successive generations—in fact, as much as 10% of the cells in each generation may have a change in one of the several million bases of the cell's DNA, though most of these mutations are not recognized because their effects are either too small to be seen or too large to yield a viable cell. Among the viable progeny those that are better adapted to the new culture medium (i.e., that can grow slightly faster, or can grow slightly longer with a limited food supply) outgrow the original strain.

We can see a similar effect of the wide use of antibiotics in man and in domesticated animals, except that the environment being changed is now the animal host rather than a laboratory culture medium: the result has been increased prevalence of drug resistance among some of the microbes that normally inhabit or that occasionally infect those hosts. The key is again selection: unless the drug is present in the environment to exert a selection pressure the introduction of even large numbers of a variant with specific drug resistance will not lead to its spread unless the variant is as well adapted as its competitors.

It is clear that natural selection plays an overwhelming role in evolution. With bacteria its role was long unrecognized: the population shifts seemed too rapid for an undirected process, and the existence of genes and mutations in bacteria were not recognized until the 1940's. But by now selection has become the foundation of bacterial ecology.

B. Benefits of recombinant DNA research

Before going on to analyze the hazards we should take a brief look at some of the benefits, which must also be considered in any decision.

Synthesis of recombinant DNA in vitro is not just a toy to satisfy the curiosity of investigators. It is an extraordinarily powerful and simple tool for studying the structure and function of mammalian DNA, and it has rapidly become as indispensable as radioactive isotopes or the electron microscope. In particular, we do not understand the regulation of mammalian genes nearly as well as that of bacterial genes: the cells are harder to work with in many ways, and they contain DNA equivalent to several million genes, or about a thousand times the amount in a bacterium. The recombinant DNA technique, which can purify fragments containing a single gene and its regulatory elements, provides an enormous simplification of the system and thus promotes its analysis. In addition, the value of such purification has recently been enormously enhanced by the development, by W. Gilbert and by F. Sanger, of an extremely simple technique for determining the sequence of short fragments of DNA, up to two hundred bases long. In two days one can now completely determine such a sequence, which previously took two years; hence we can anticipate rapid progress in determining the chemical structure of innumerable mammalian genes.

While no one can foresee all the consequences of a basic discovery, the history of molecular biology assures that these new developments in handling DNA will lead to great advances in our understanding of mammalian gene regulation, the key to normal development and differentiation and also to the defective regulation of cell growth in cancer. One can also safely predict the use of such bacteria for producing medically valuable human cell products, such as insulin and other protein hormones, specific antibodies to replace deficiences, specific antigens for immunization against tumors, and the specific genes or their products that may ultimately be used to treat hereditary enzyme deficiencies.

In addition to these practical benefits, I need hardly emphasize for this audience the enormous cultural importance of encouraging free inquiry, and the potential loss to society from a precedent of curtailing such inquiry. At the same time, it has always been clear that the right to freedom of inquiry has limits, just like the right to freedom of expression. One such limit is cruelty: a medical experimenter must recognize that he is dealing with human subjects and not with objects. Another limit is unacceptable hazard, whether to individuals, to the population, or to the environment: hence the acceptance of regulations and licening requirements for research with radioactive materials. In turning now to the question of hazard I would suggest that we canuot pretend to compare risks and benefits as closely as we can compare costs and benefits: we must rather ask whether a particular set of risks is acceptable, in terms of the increment that it may add to the risks that we already live with. For a demand for absolute freedom from risk would be a prescription for paralysis.

II. HAZARDS

In trying to estimate the immediate hazards we must consider, as I mentioned earlier, three probabilities: that experiments with a given kind of DNA will produce a dangerous organism, that that organism will infect a laboratory worker, and that the organism will escape and spread in the community or the environment. For it is easy to draw up a scary hypothetical scenario, if one's imagination need not be limited by considerations of probability. But any realistic discussion must consider probabilities.

A. DANGER OF PRODUCING A HARMFUL ORGANISM

If one deliberately transfers into *E. coli* a bacterial gene for toxin production the probability of its having the expected phenotypic effect on the cell, and producing its toxin as long as it survives in the new host, is very high. If one introduces the total genome of a tumor virus the hazard will be less, for it would require release of the viral DNA and its infection of host cells; but while that probability may be very low, we cannot assume that it is negligible. Both these kinds of experiments are appropriately prohibited in the NIH Guidelines today.

I would like to concentrate on a kind of experiment that is allowed but is causing great concern and is restricted to quite special facilities: the so-called "shotgun" experiment, in which one transfers random fragments of DNA from mammalian cells. Here it is clear that the probability of isolating a strain with a gene for a toxic product, or with the genes of a tumor virus, is exceedingly low.

Evolutionary considerations provide an additional and independent approach to the question whether shotgun experiments are likely to create novel and harmful microbes. In my opinion it is exceedingly doubtful that our new-found ability to introduce mammalian DNA into bacteria in the laboratory will create a truly novel class of organisms, for evolution had an earlier crack at the problem. It is known that bacteria can take up naked DNA from solution and, in fact, two different strains of pneumococcus have been shown to be able to produce a third, recombinant strain in an animal body, by release of DNA from a lysed cell of one strain and its uptake by an intact cell of the other. Moreover, bacteria in the gut are constantly exposed to fragments of host DNA that are released as the cells lining the gut die; while bacteria growing in carcasses have a veritable feast.

The efficiency of such uptake of mammalian DNA by bacteria is undoubtedly very low. However, because of the extraordinarily large scale of the exposure in nature, recombinants of this general class must have been formed innumerable times over millions of years and thus have been tested in the crucible of natural selection. Moreover, such organisms are undoubtedly also being formed in nature today. If they had high survival value we would be recognizing short stretches of mammalian DNA in *E. coli*. We do not. In addition, if the naturally occurring recombinants included serious pathogens, as is feared from artificial recombinants, we would be seeing epidemics of serious disease due to *E. coli*. We do not. If, on the other hand, naturally occurring recombinants are appearing and even causing disease, but are escaping our attention, we would have to ask how much our laboratories could add, since nature experiments with about 10^{25} bacterial cells produced in the human species per day.

As an additional danger, it has been suggested that terrorists might deliberately create harmful recombinant bacteria, as a powerful new tool. But it is hard to see why a terrorist would be interested in an *E. coli* strain containing, say, a gene for botulinus toxin, when that gene is already housed in the naturally occurring *Clostridium botulinum*, a well adapted organism with proven survival value. With that organism the terrorist could manufacture, at the cost of a few dollars, enough botulinus toxin to poison all the inhabitants of a large city.

B. Danger of laboratory infection

In moving now from the probability of inadvertently producing a harmful organism to the probability of its causing a laboratory infection, let us assume the worst case: an *E. coli* strain producing a potent toxin absorbable from the gut, such as botulinus toxin. (This experiment is at present prohibited). Such a strain would present a real danger of laboratory infection. But there are a number of reasons to expect it to be less, with even this worst hypothetical recombinant pathogen, that with the pathogens that are handled every day in diagnostic and research laboratories.

(*a*) The known laboratory infections (about 6,000 recorded in the history of microbiology) have been largely due to organisms that cause respiratory infections, spread by droplets (mostly before safety cabinets were introduced in the 1940's). Because enteric infections occur through swallowing of contaminated food or other material, even the most virulent enteric pathogens are relatively safe to handle in the laboratory with simple precautions, such as not putting food or a cigarette on the laboratory bench.

(*b*) Strain K12 of *E. coli*, used in almost all genetic work, has been transferred for at least 30 years in the laboratory, during which it has become much better adapted to artificial media than to the human gut. In fact, recent tests showed that after a large dose in man (much larger than what one would expect from a laboratory accident) this strain disappeared from the stools within a few days. Its problems of survival are analogous to those of a delicate hothouse plant thrown out to compete with the weeds in a field.

(*c*) The addition of a block of foreign DNA to an enteric organism will ordinarily decrease its adaptation to survival in the gut and hence its probability of spreading. For at the least, replication of useless DNA exacts a metabolic price for an organism; while if the DNA is active its products are likely further to disturb the metabolic balance.

(*d*) A very large safety factor is added by the provision for biological containment in the present Guidelines. All work with mammalian DNA must be carried out only in a strain derived from *E. coli* K12 (the class called EK2) that has a drastically impaired ability to multiply, or to transfer its plasmid, except under very special conditions provided in the laboratory. For example, in the presently certified EK2 strain the defects include loss of the ability to synthesize an essential wall component. The strain is maintained in the laboratory by supplying the missing component, which is not found in the gut. Cells growing without that component quickly burst, because they grow without forming more wall; hence survival is less than 10^{-8} in 24 hours.

We thus see that with a strain known to have added the gene for a potent toxin in a serious laboratory infection requires the compounding of four low probabilities. With strains from shotgun experiments we have a fifth, very low probability, already mentioned: that an apparently harmless mammalian tissue will yield a dangerous product.

I conclude that in the kinds of experiments now permitted (which exclude the introduction of a known gene for a potent toxin or a known tumor virus) the danger of a significant laboratory infection is vanishingly small compared with the dangers encountered every day by medical microbiologists working with virulent pathogens. And such dangers must ultimately be balanced against the potential benefits, both practical and cultural. In the United States, up to 1961, of the 2400 recorded cases of laboratory infections 107 were fatal—over half of these from diagnostic laboratories. On the other side, millions of lives were saved by bacteriological research and diagnosis.

But even if the risks in recombinant DNA research are much smaller than the public has been led to believe, it is important to keep all the probabilities low. In particular, even if a toxin-producing strain would survive only very briefly in the gut, a large enough dose might meanwhile produce enough toxin to cause disease. Hence it is important for molecular biologists working in this area to

learn, and to use, the standard techniques of medical microbiology, at least until we have acquired much more experience with the organisms. Indeed, the enforcement of such practices could be a major benefit from the current discussion.

C. Danger of spread

I now come to the most important point of all, with respect to protecting the public interest. The difference between the danger of causing a laboratory infection and the further danger of unleashing an epidemic is enormous. In our government's bacteriological warfare laboratories at Camp Detrick, working for 25 years on the most communicable and virulent pathogens known, 423 laboratory infections were seen. Moreover, most of these infections were picked up by the respiratory route. Yet despite our very imperfect control of respiratory transmission there was not a single case of secondary spread to a member of the family or to any person outside the laboratory. Similarly, in the Communicable Disease Center of the U.S. Public Health Service 150 laboratory infections were recorded, with one case of transmission to a spouse. Elsewhere in the world there have been about two dozen laboratory-based microepidemics recorded, each involving a few outsiders.

With enteric pathogens the danger of secondary cases is minimal, for with this class of agents modern sanitation provides infinitely better control than we can provide for respiratory infection: in contrast to influenza, the appearance of a case of typhoid in a home does not lead to an epidemic. Enteric epidemics appear only when sanitation is poor or has broken down, or when a symptom-free carrier with filthy personal habits serves as a food handler; and such epidemics are always small (except when sewage freely enters the water supply).

There is no doubt that this epidemiological information is pertinent to the recombinants that we are discussing. For despite widespread apprehension about the presumed biparental chimeras with totally unknown properties, the fact is that these recombinants are genetically 99.9 percent *E. coli*, with about 0.1 percent foreign DNA added. It is exceedingly improbable that such an organism could have a radically expanded habitat, no longer confined to the gut. It is even harder to see that the organism would be more communicable, or more virulent, than our worst enteric pathogens, which cause typhoid and dysentery. The Andromeda Strain remains entertaining science fiction.

I conclude that if by remote chance a recombinant strain should be pathogenic, and if it should cause a laboratory infection, that infection would give an early warning, which would decrease the chance of spread.

Moreover, if a case should appear outside the laboratory the enteric habitat of *E. coli* provides powerfnl protection, in a country with modern sanitation, against the chain of transmission required for an epidemic.

We must therefore ask whether the problem merits deep concern by the general public, any more than the problem of how laboratories performing diagnostic work or research on known pathogens should be operated. To produce a serious epidemic by introducing fragments of mammalian DNA into *E. coli* would reqnire the componnding of five low probabilities. By any reasonable analysis the risk seems very much less than that from pathogens that are being cultivated in laboratories all the time.

D. Tumor viruses

Tumor viruses present a special problem. Unlike other viruses, whose entry in an adequate dose regularly canses disease in a susceptible host, tumor viruses do not cause a tumor regularly after infection but require special circumstances. Indeed, their frequent presence in apparently normal animal tissues is the main source of the fear of shotgun experiments. Moreover, whether they make any contribution to human cancer is still qnite unknown. Nevertheless, if they should do so it wonld be after a latent period of years. Hence any conceivable infection by a hacterium containing a tumor virus genome would lack the early warning of the toxin producers.

However, all other aspects of the problem remain the same. And this loss of one protective feature is balanced by the fact that these viruses, by definition, have their own means of spread. Indeed, in general the natural spread of viruses is even more effective than that of bacteria, each infected animal cell producing thousands of infectious virus particles. Moreover, since viral DNA in a bacterium would have to get out of its host cell and enter human cells throngh an extremely inefficient process, it is hard to imagine that that naked DNA would be more hazardous than the same DNA in its own infectious, viral coat, adapted by

evolution for entering animal cells. In addition, if we fear the danger of such indirect uptake of unrecognized tumor virus DNA from normal mammalian tissue, one must ask whether the direct ingestion of such tissue, e.g., in steak cooked rare, may not present at least as great a danger.

It therefore seems fair to ask whether there is greater danger if we use the recombinant DNA technology to help us to understand tumor viruses, or if we presumably play safe and inhibit that research. For if we choose the latter we meanwhile allow the tumor viruses to spread as they presently do in nature, under circumstances where we really do not understand their relation to human tumors at all. If I may engage in a bit of speculation, I would suggest that ordinary blood transfusions probably have a much higher risk of exposing us to tumor-producing agents. For since tumors are not detected until they reach a substautial size, the probability that the average transfusion has come from an early tumor patient is not negligible: it may be as high as 0.1 or 1 percent. The choice between cancer cells injected into one's bloodstream, and tumor virus genes in bacteria in one's gut, would not seem difficult.

E. The NIH guidelines

Though extensive discussion preceded formulation of the Guidelines I believe it did not include nearly enough input from experts in infectious disease and in evolution, who could have debated the real hazards rather than unlimited hypothetical scenarios. And in the light of what I see as the technical realities I would regard the present Guidelines as excessively conservative. On the other hand, I wonld also regard them as a reasonable response to the level of public anxiety that has been raised, though they make the research substantially more expensive. And in the face of the alleged dangers that have been described I cannot blame the public for having a high level of anxiety. But I do blame the New York Times for publishing in their Sunday Magazine last August a one-sided presentation by a molecular biologist who displayed extraordinarily little understanding of either microbiology or evolution. In speaking of *E. coli* as though it were a standard, uniformly distributed organism, which would carry with it through the world any additional genes that we insert, he ignored the most important factor of all: natural selection. He also made the remarkable statement that the insertion of tumor viruses into bacteria may make them infectious. And his scary scenarios concluded with the suggestion that scientists working in this field may produce yet another Andromeda strain—as though the first strain existed in fact rather than in fancy.

Given the present level of public anxiety, scientists in this field seem quite willing to accept the Guidelines. But I hope it will not be too long before these rules are modified in the light of further experience. For since the technique is potentially useful for a large number of investigators, the requirement for elaborate facilities will add up to a very large expense. There must be some limits to the laudable principle of erring on the side of caution.

III. INTERVENTION IN EVOLUTION

The hazard that we have been discussing—that of creating novel, dangerous organisms—is a legitimate cause for public concern; there is no question about society's right to limit hazardous activities. However, when we move to the question whether our increasing power to manipulate genetic material creates long-term evolutionary dangers we are moving into quite a differnet area, involving the concept of dangerous knowledge rather than dangerous actions. The most prominent exponent of this view is Robert Sinsheimer, of Caltech. Perhaps we can clarify the issue by trying to translate into more specific terms some of the general sources of apprehension that he has expressed in various publications.

1. Dr. Sinsheimer questions our moral right to breach the barrier between prokaryotes and eukaryotes, since we simply cannot foresee the consequences. This argument seems to turn voluntary principles through 180 degrees. Evolution is concrned with selection for fitness, in the Darwinian sense, and the barriers that it has established between species are designed to avoid wasteful matings, i.e., matings whose products would be monstrosities, in the sense of being unable to survive, rather than monsters, in the sense of taking over. Since survival of an organism depends upon a balanced genome, evolution proceeds in small steps, no one of which will excessively unbalance the genome in one respect while improving its adaptation in another. And since crosses between even closely related species are excluded in nature on these grounds, it is exceedingly unlikely that arti-

ficial transfers of genes between eukaryotes and prokaryotes would pass the test of Darwinian fitness.

2. "This is the beginning of synthetic biology." I wonder whether this statement can really be defended, considering that man has been domesticating animals and plants by selective breeding since neolithic times, and has also been cloning vegetables by grafting.

3. "The power to change the evolutionary process is as significant as cracking the atom." But atoms are not subject to extinction by Darwinian selection. Stores of nuclear weapons are likely to be more permanent than any dangerous organism that might reach the world from a laboratory working with recombinant DNA. Similarly, the statement by George Wald that "a living organism is forever" is dramatic, but it disregards two powerful evolutionary predictions: first, that natural selection will rapidly extinguish all evolutionary departures except for the infinitesimal fraction that have improved their adaptive fitness; and second, that the recombination of genes from distant sources has an exceedingly small probability of improving fituess.

4. "We no longer have the absolute right of free inquiry." But we never had: as I noted above, dangerous procedures have always been subject to limitations. But to invoke dimly foreseen, undefined dangers seems to be starting on the slippery slope of excluding dangerous ideas.

5. Power over nucleic acids, as over the atomic nucleus, "might drive us too swiftly toward some unseen chasm. . . . We should not thrust inquiry too far beyond our perception of its consequences." While we have become increasingly aware of the costs and dangers of technology, and should increase our alertness to these problems as they become visible, I would paraphrase this statement and suggest that we should not thrust our limitations on research too far beyond our perceptions of its hazards. Some claim that scientists are arrogant and wish to steam ahead regardless of the consequences. But considering the history of the benefits of science, and the sad history of Italy's elimination from the race by Pope Urban VIII after its head start under Galileo, perhaps it is more arrogant for a handful of opposed scientists, having presented their arguments, to try desperately to place severe restrictions on recombiuant DNA research.

6. Finally, Sinsheimer suggests that this is the beginning of a genetic engineering that will ultimately extend to man. In contrast to the vagueuess of the preceding propositions, this one is concrete, and one can wrestle with it. Moreover, I suspect that it lies at the heart of his anxiety, and that of much of the audience.

This is too large a topic to consider in detail here. In 1970 it received extensive discussion, which then subsided but has been reactivated by the very different question of genetic engineering in bacteria. I would only point out briefly that the medical aim of genetic engineering in man is gene therapy for diseases due to single defective genes, with a well defined chemistry. I believe we are still a long way from being able to introduce DNA in the reliable, controlled way that would he required. But eveu if this guess is wrong, it is clear that success in such therapy would still leave us very far from being able to manipulate in any useful way the large number of genes, all still undefined, that affect the structure and function of the brain. Moreover, in an already developed organism no conceivable manipulation of DNA could prescribe the wiring diagram of the already formed brain. Hence the possibility that a tyrant could use genetic engineering to manipulate personalities seems still too remote to justify present concern. In addition, I would question whether the technological imperative would uecessarily (or even likely) lead us to use genetic technology to manipulate human personalities if we could. If the simple but effective techniques of selective breeding and artificial insemiuation are not used to influence the gene pool, one must question what motivation would lead society to use the much more elaborate techniques that might emerge from current research.

Philosophical questions about the effect of science and technology on man's fate do not start with recombiuant DNA but go back to Galileo. We cauuot uulearn the scientific method, and if we restrict it in one place it will turn up in another. In a world that has only recently come to realize how large (aud often unexpected) a price we are paying for various aspects of technology, it is only too easy to take the benefits of science and technology for granted, and to object to the new problems that they are raising. But in the long run it is difficult to see how we can plot a more prudent course than to try to recognize the hazards of

specific possible applications as they arise, to seek a reasonable balance between the demand for freedom of action and the demand for protection from excessive risks, and to seek orderly and responsible methods for involving the public in matters that so deeply affect its interests.

I share Sinsheimer's concern for the future, and his passionate advocacy of vigilance. But the vigilance must be directed at specific definable applications. Vigilance concerning new knowledge that might someday be misused is, to me, a threat to freedom of inquiry, and I believe a threat to human welfare. If we are entering dangerous territory in exploring recombinant DNA, we may enter even more dangerous territory if we start to limit inquiry on the basis of our incapacity to foresee its consequences.

BERNARD D. DAVIS,
Bacterial Physiology Unit,
Harvard Medical School.

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90TH CONGRESS
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TO PROMOTE THE PROGRESS OF USEFUL ARTS

REPORT OF THE PRESIDENT'S COMMISSION ON THE PATENT SYSTEM

COMMITTEE ON THE JUDICIARY
UNITED STATES SENATE
SUBCOMMITTEE ON PATENTS, TRADEMARKS, AND COPYRIGHTS

FEBRUARY 2, 1967.—Ordered to be printed, with illustrations

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SENATE RESOLUTION 52

Submitted by Mr. McClellan of Arkansas

IN THE SENATE OF THE UNITED STATES,
Agreed to February 2, 1967.

Resolved, That the report of the President's Commission on the Patent System, entitled "To Promote the Progress of Useful Arts", be printed with illustrations as a Senate document.

SEC. 2. There shall be printed three thousand additional copies of such document for the use of the Committee on the Judiciary.

Attest:

FRANCIS R. VALEO,
Secretary.

FOREWORD

The Senate Subcommittee on Patents, Trademarks, and Copyrights has been engaged in recent years in a review of our patent system. As part of this undertaking the subcommittee published a series of 30 studies exploring the scientific, economic, and legal aspects of the patent system. To assist in evaluating various proposals to institute changes, the subcommittee obtained the views of industry, inventors, economists, and the patent bar.

On the basis of its study the subcommittee concluded in 1965 that while "the objectives of the patent system are as valid today as at its inception," there "has not been adequate adjustment of our patent laws and procedures to reflect changing conditions and to respond to the critical problems confronting the Patent Office." I, therefore, welcomed President Johnson's decision to establish the President's Commission on the Patent System. This Commission, composed of distinguished public and Government members, has rendered a significant service. It has undertaken a comprehensive survey of our patent laws and procedures and addressed itself to the critical problems which demand solutions. Its unanimous conclusion that the patent system continues to provide an essential incentive for the conduct of research and the investment of capital is in accord with the findings of the subcommittee. Its concern with the long pendency of patent applications and the great uncertainty and considerable expense involved in the enforcement of patents is shared by the subcommittee.

The 35 recommendations of the Commission deserve the careful consideration of the Congress, the Patent Office, and all Americans who desire to see a stronger patent system. In order to provide for a wider dissemination of the report, it has been published as a Senate document. Of course, the views expressed are solely those of the Commission and do not necessarily reflect the opinions of the Subcommittee on Patents, Trademarks, and Copyrights. Its publication, however, does testify to my belief that it represents a valuable contribution toward the improvement of the U.S. patent system.

JOHN L. MCCLELLAN,
Chairman, Subcommittee on Patents, Trademarks, and Copyrights.

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"To Promote The Progress of ...Useful Arts"

In An Age of Exploding Technology

REPORT OF
THE PRESIDENT'S COMMISSION
ON THE PATENT SYSTEM

WASHINGTON, D.C.
1966

November 17, 1966.

The PRESIDENT,
The White House,
Washington, D.C.

DEAR MR. PRESIDENT:

We have the honor to present the report of the President's Commission on the Patent System.

Your Commission was established by Executive Order No. 11215, on April 8, 1965, and the membership was announced on July 23, 1965. The Commission has held thirteen meetings, beginning August 15, 1965, each meeting lasting from one to four days, for a total of thirty-one days.

The recommendations conveyed in this report have been developed through study and discussion by the members of the Commission and, as a whole, represent their combined judgment and general agreement. The recommendations, in all of their details, however, do not necessarily bear the endorsement of every member.

Background material prepared by the staff and the Commission, reflecting more extensively the considerations taken into account in the development of these recommendations, is being completed and will be transmitted as a supplement to the report.

The principal objectives of the Commission's study are set forth in the Introduction. To the extent that the Commission's recommendations promote the attainment of these objectives, they will assist in furthering the mission of the United States patent system—to promote the progress of useful arts, advance the standard of living everywhere, and contribute toward world peace and tranquillity.

One point, Mr. President, merits emphasis. The accompanying recommendations should not be regarded as a catalogue of

discrete remedies. The report considers the patent system as a whole and contemplates revision by means of a coordinated plan of interrelated recommendations.

The recommended changes taken together, we respectfully suggest, will strengthen the patent system, and thus will assist in the attainment of the Nation's domestic and international goals in today's rapidly changing environment.

Members of the Commission deeply appreciate the responsibility assigned to them and offer their continued cooperation.

Respectfully yours,

HARRY HUNTT RANSOM
SIMON H. RIFKIND
Cochairmen.

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CONTENTS

		Page
Letter of Transmittal		ii
Names of Commission Members and Observers		iv
Names of Staff Members		v
Acknowledgements		vi
INTRODUCTION		1
RECOMMENDATIONS		5
	Patentability of Inventions	
I	Prior Art	5
II	Preliminary Application	8
III	Exceptions to Prior Art	9
IV	Patentable Subject Matter	12
	Application Filing and Examination	
V	Assignee Filing and Joinder of Inventors	14
VI	Claim for Priority Date	16
VII	Publication	16
VIII	Continuing Applications	17
IX	Standby Optional Deferred Examination	19
X	Burden of Persuasion	22
XI	Citation Period	23
XII	Quality Control	24
	Direct Review of Patent Office Decisions	
XIII	Presumption of Correctness	26
XIV	Review by Court of Appeals	26
	Procedure for Amending and Cancelling Patents	
XV	Cancellation	29
XVI	Reissue	30
	Liability and Enforcement	
XVII	Interim Liability	32
XVIII	Term of Patent	33
XIX	Secrecy Order	34
XX	Terminal Disclaimer	35
XXI	Importation	35

		Page
XXII	Patent Right Transferability	36
XXIII	*In Rem* Invalidity	38
XXIV	Civil Commissioners	39
XXV	Expedited Procedure for Limited Claims	41
XXVI	**Statutory Advisory Council**	43
	Patent Office Operations	
XXVII	Patent Office Financing	45
XXVIII	Propriety of Final Rejection	47
XXIX	Classification and Information Retrieval	48
XXX	Information Dissemination	50
XXXI	**Transition**	52
XXXII	**Government Patent Policy**	53
	International Action	
XXXIII	Inventor's Certificates	54
XXXIV	Term Measurement	54
XXXV	Universal Patent System	55
CHARTS		58

INTRODUCTION

The United States patent system is an institution as old as the Nation itself. Stemming from a Constitutional mandate, patent acts were passed in 1790, 1793, and 1836. The Act of 1836 established the pattern for our present system by providing statutory criteria for the issuance of patents and requiring the Patent Office to examine applications for conformance thereto. Although the law has been amended on numerous occasions—and even rewritten twice since 1836—no basic changes have been made in its general character in the succeeding one hundred and thirty years.

However, during this period of few statutory changes, major developments have occurred in the social and economic character of the country. The United States has undergone a dramatic transformation, creating and utilizing an enormously complex technology, to emerge as the world's most productive industrial community.

In the agricultural economy of 1836, individuals who engaged in inventive activity usually did so alone, and on their own initiative. Such activity still continues. The lone, independent inventor, even in this day of sophisticated technology, still contributes most importantly to the useful arts. But the field is no longer his alone. Organized research is carrying a steadily increasing share of the task of exploration.

Research and development are now commanding a scale of expenditure which is possible only because of the application of the resources of government, private industry and institutions of learning.

Scientific and technical information is being generated and made available to the public in an ever growing torrent. What

the patent fraternity calls prior art is growing so fast that it is becoming almost unmanageable by conventional means of storage and retrieval. Disclosures are becoming increasingly complex, and many are in foreign languages.

The trend in the number of patent applications is clearly upward and their subject matter is increasing in sophistication and complexity. The current backlog of pending applications is over 200,000, the average period of pendency being two and one-half years from filing to final disposition. However, a substantial number of applications have a period of pendency of five to ten years or more.

All of these factors have cooperated to make it exceedingly difficult for the patent examiner to screen what is truly novel and what is truly inventive.

Agreeing that the patent system has in the past performed well its Constitutional mandate "to promote the progress of . . . useful arts," the Commission asked itself: What is the basic worth of a patent system in the context of present day conditions? The members of the Commission unanimously agreed that a patent system today is capable of continuing to provide an incentive to research, development, and innovation. They have discovered no practical substitute for the unique service it renders.

First, a patent system provides an incentive to invent by offering the possibility of reward to the inventor and to those who support him. This prospect encourages the expenditure of time and private risk capital in research and development efforts.

Second, and complementary to the first, a patent system stimulates the investment of additional capital needed for the further development and marketing of the invention. In return, the patent owner is given the right, for a limited period, to exclude others from making, using, or selling the invented product or process.

Third, by affording protection, a patent system encourages early public disclosure of technological information, some of which might otherwise be kept secret. Early disclosure reduces the likelihood of duplication of effort by others and provides a basis for further advances in the technology involved.

Fourth, a patent system promotes the beneficial exchange of products, services, and technological information across national boundaries by providing protection for industrial property of foreign nationals.

Having satisfied itself as to the worth of a patent system, the Commission then undertook an extensive analysis of the many studies of U.S. and foreign patent systems. The Commission also sought and received additional views, criticisms and suggestions from numerous sources, including business and trade associations, individual patent practitioners, patent law associations, groups and individuals within the Patent Office, educators, inventors, scientists, businessmen, and other interested parties. From these sources the Commission identified numerous broad areas of concern.

Recognizing that it could not consider adequately all the matters of potential concern in the limited period of its existence, the Commission selected a number of areas with which it felt it could deal most effectively. In making this choice, the Commission took into account several factors, including its own membership, present investigations by other executive and legislative groups, and the potential contribution the Commission could make in any given area.

Within the boundaries thus defined, the Commission identified the following objectives:

1. To raise the quality and reliability of the U.S. patent.
2. To shorten the period of pendency of a patent application from filing to final disposition by the Patent Office.

3. To accelerate the public disclosure of technological advances.
4. To reduce the expense of obtaining and litigating a patent.
5. To make U.S. patent practice more compatible with that of other major countries, wherever consistent with the objectives of the U.S. patent system.
6. To prepare the patent system to cope with the exploding technology foreseeable in the decades ahead.

Many of the problems related to these objectives are intertwined. An attempt to solve or reduce a problem at one point of the system can expose or create a dislocation at another. Separate and uncoordinated solutions to individual problems would yield a gerrymandered patent system full of internal contradictions and less efficient than the one we now have. It is this circumstance—not any claim to superior wisdom—which led the Commission to propose the following changes, all as part of one interrelated and coherent plan.

RECOMMENDATIONS

Patentability of Inventions

I

The following recommendation would result in several significant changes in present practice: (a) when two or more persons separately apply for a patent on the same invention, the patent would issue to the one who is FIRST TO FILE his application; (b) there would be no grace period; (c) foreign knowledge, use and sale would be included as prior art; and (d) there would be revised criteria for the form of prior art.

Prior art shall comprise any information, known to the public, or made available to the public by means of disclosure in tangible form or by use or placing on sale, anywhere in the world, prior to the effective filing date of the application.

A disclosure in a U.S. patent or published complete application shall constitute prior art as of its effective (United States or foreign) filing date.

(a) In a first to file system, the respective dates of "conception" and "reduction to practice" of the invention, presently of great importance in resolving contested priority for an invention claimed in two or more pending applications or patents, no longer would be considered. Instead, the earliest effective filing date would determine the question of priority. This necessarily follows from the provision that the disclosure in a patent or published complete application shall constitute prior art as of its effective filing date. Interference proceedings thus would be abolished.

74-184 O - 67 - 3

Important considerations dictate this departure from our present practice. A first to file system will: encourage prompt disclosure of newly discovered technology; substitute for the delays and expense of interference proceedings a fair and inexpensive means by which an inventor can establish priority; and bring U.S. practice into harmony with that prevailing in almost all other industrial nations.

The Commission believes it is as equitable to grant a patent to the first to file as to the one who wins an interference. Many circumstances may determine the winner in either case. But the first to file is more apt to be the inventor who first appreciated the worth of the invention and promptly acted to make the invention available to the public.

(b) Regardless of the time the invention was made, any relevant information, known or made available to the public, antedating the effective filing date of the first application containing the subject matter on which the claim to such invention is based, would constitute prior art as to such claim. Even the applicant's own earlier disclosure would bar the grant of a patent if made public before the earliest effective filing date to which the particular claim was entitled. As a result, there would be no grace period, and the question of whether the invention is obvious would be considered as of the filing date, rather than as of the time the invention was made.

This change would speed the examination procedure in the Patent Office by eliminating the time-consuming consideration of affidavits presently submitted to establish an earlier date of invention and thus overcome *prima facie* prior art. Also, the applicant no longer would need to maintain extensive records now required to corroborate such affidavits, or thereafter, to prove his actual date of invention in an infringement suit.

Greater international uniformity would also be achieved, since the present grace period has no counterpart in most foreign systems. Further, inventors no longer would forfeit their foreign patent rights through disclosures made in reliance on the U.S. grace period.

(c) Foreign knowledge, use and sale would be included as prior art. Present arbitrary geographical distinctions would be eliminated. The same high standard of proof now required for showing domestic public knowledge, use or sale would also be applied to such foreign prior art.

The anomaly of excluding, from prior art, public knowledge, use or sale in a border town of Mexico or Canada, and including the same kind of disclosure in Alaska or Hawaii, would be eliminated.

This change would prevent the granting of valid U.S. patents on inventions which would be unpatentable abroad, because of long use or sale there. It would be another step toward conformity with European patent laws and would promote acceptance of a common definition of universal prior art. Additionally, it would promote the establishment of international scientific data banks, thus eliminating one of the barriers to the useful exchange of search results among patent offices of various countries.

(d) "Printing," presently a technical requirement in certain circumstances, would no longer be necessary for a publication to constitute prior art. Instead, any information made available to the public in a tangible (non-oral) form, prior to the effective filing date, could so serve.

Such a change would establish as a logical and modern standard of the form of prior art: that either publicly known or made available to the public in a preservable form. It should end present disputes and avoid future controversy, by accepting as prior art typewritten copy, microfilm, computer print-out, or any other tangible expression of technological data, made available to the public.

(e) The disclosure in a patent or published complete application would have, as its effective filing date, the date of its earliest filing in the United States or a foreign country. This would resolve present uncertainty caused by conflicting court decisions.

This also would avoid an anomaly whereby two applications, with the same effective filing date, would have different dates for the purpose of constituting prior art where one is based upon a foreign application. Further, it would appear to be a necessary adjunct of a first to file system, to prevent two patents from issuing on the same invention.

II

To substitute for the present grace period, a first to file system should include some technique for allowing the inventor to seek support or test his invention in the marketplace. It also should encourage the free discussion of new discoveries in the academic and scientific communities. To meet these needs, a preliminary application, an "instant" form of disclosure to the Patent Office free from the delays and expense of a formal application, is proposed.

A preliminary application may be used to secure a filing date for all features of an invention disclosed therein, if the disclosure subsequently appears in a complete application. Requirements as to form shall be minimal and claims need not be included.

One or more preliminary applications may be consolidated into one complete application filed within twelve months of the earliest preliminary or foreign application relied on.

Under this recommendation an applicant would file a written description of his invention in a preliminary application, a document with minimal requirements as to form and needing no claims. This would permit early filing of an application, since it could be prepared by someone having little knowledge of patent law and procedure. Applicants should be made aware, however, that the protection afforded by a preliminary application will depend greatly upon the adequacy of the disclosure contained therein.

Additional preliminary applications could be filed to cover aspects of the invention developed subsequent to filing of the first

preliminary application. Records an inventor now must keep could be replaced by disclosures submitted to the Patent Office, where they automatically would be certified as to date. One or more preliminary applications also could be used to supplement the disclosure first presented in a foreign application.

Information contained in these applications could be disclosed to the public without risk, through publication or market testing, for example, as long as a complete application was filed within twelve months of the earliest preliminary or foreign application relied on. By a complete application is meant one which complies with present requirements for an application. Accordingly, many of the advantages of a grace period could be obtained without the associated problems.

Each claim in the complete application would be entitled, for the purpose of overcoming prior art, to the date on which its supporting disclosure was first fully presented in a validly asserted foreign, preliminary or earlier complete application. Also, disclosure in a complete application, if published, would constitute prior art as of its first presentation date.

The preliminary application technique would create no significant burden for the Patent Office. Preliminary applications need only be stamped with their date of receipt and stored pending the filing of a complete application, and even then would only be considered if the effective date of the complete application was brought into question.

III

Prior art shall not include, as to the inventor concerned, disclosures of an invention resulting from:

1. **A display in an official or officially recognized international exhibition; or**
2. **An unauthorized public divulgation of information derived from the inventor;**

As provided below.

1. Two international treaties define and regulate "official and officially recognized international exhibitions." The Paris Convention for the Protection of Industrial Property requires that "temporary protection" be granted with respect to inventions exhibited at such exhibitions.

The United States has had no need for a special provision with respect to exhibitions because the present grace period protects against the adverse effect of disclosures occurring within one year before the filing date of an application. Since the Commission now suggests elimination of the grace period, a method to safeguard patent rights under these circumstances must be provided to conform to the Paris Convention.

It would appear that the preliminary application (Recommendation No. II) complies both with the spirit and the letter of the Paris Convention in providing temporary protection for inventions shown at international exhibitions. However, if a preliminary application proves not to satisfy the Convention, it is recommended that:

> A display at an official or officially recognized international exhibition by an inventor, or assignee, shall not constitute prior art against his complete application to the extent that the information disclosed by the display appears in a notice having the format of a preliminary application; provided: that the notice is filed in the Patent Office no later than the public opening of the display and the complete application is filed within six months after filing of the notice.

2. With respect to unauthorized public disclosures, it is recommended that:

> An unauthorized public disclosure of information derived from the inventor or his assignee shall not constitute prior art against him, if, within six months after said disclosure, a complete application for the invention is filed by the inventor or assignee.

> Any allegation, that a disclosure should not constitute prior art because it was unauthorized, shall be considered by the Patent Office only if it is verified, sets forth details establishing a *prima facie* case, and is accompanied by proof that notice has been served on the party accused of making the disclosure.
>
> If the party accused promptly contests the allegation, the application shall not issue as a patent until the matter is finally judicially determined in favor of the applicant.

Currently, under certain circumstances, a disclosure will not bar the issuance of a patent if such disclosure was made within the grace period.

In the absence of this recommendation, an inventor or his assignee would lose his patent rights if an unauthorized public disclosure of the invention in any form (including patent applications or patents) was made prior to his filing an application. This recommendation furnishes a procedure to nullify the effect of such disclosure upon the inventor. It would allow the Patent Office to ignore alleged unauthorized disclosures as prior art in those instances where the allegation is not contested by the accused party. At the same time, it is designed to discourage an unsupported assertion that a disclosure should not be used to bar a patent. In a subsequent litigation, failure on the part of an accused party to contest the assertion in the Patent Office would not preclude reliance on such a disclosure to invalidate the patent.

The application would not receive the benefit of the date of the unauthorized disclosure for purposes of priority. Rather, any intervening untainted disclosure, occurring between the date of the unauthorized public disclosure and the application filing date, would constitute prior art as to the applicant. The unauthorized public disclosure also would constitute prior art as regards all other applicants.

IV

The classes of patentable subject matter shall continue as at present, except:

1. **All provisions in the patent statute for design patents shall be deleted, and another form of protection provided.**
2. **All provisions in the patent statute for plant patents shall be deleted, and another form of protection provided.**
3. **A series of instructions which control or condition the operation of a data processing machine, generally referred to as a "program," shall not be considered patentable regardless of whether the program is claimed as: (a) an article, (b) a process described in terms of the operations performed by a machine pursuant to a program, or (c) one or more machine configurations established by a program.**

This recommendation would end the practice of granting patents on designs and plants. It also would eliminate whatever possibility exists under the present statute, if any, for directly or indirectly obtaining a patent covering a program or a patent covering the operation of a data processing machine pursuant to a program.

The Commission believes strongly that all inventions should meet the statutory provisions for novelty, utility and unobviousness and that the above subject matter cannot readily be examined for adherence to these criteria.

1. Designs: A patent now may be granted on any new, original and ornamental design for an article of manufacture. Despite the statutory requirement of unobviousness, patents on designs are now granted, in effect, solely on the basis of novelty. Courts often find these patents invalid on the ground that the design is obvious.

The Commission is aware of legislative proposals to protect ornamental designs against copying. Nevertheless, it believes

that some means *outside* the patent system should be developed for the protection of new and original ornamental designs.

2. Plants: A patent may be granted today on any new and distinct variety of specified types of asexually reproduced plants. The statute imposes the requirement of unobviousness for patentability. In practice, however, patents are granted if the Department of Agriculture notifies the Patent Office that, as far as it can determine, the plant variety is new, and the examiner finds no art indicating the contrary.

While the Commission acknowledges the valuable contribution of plant and seed breeders, it does not consider the patent system the proper vehicle for the protection of such subject matter, regardless of whether the plants reproduce sexually or asexually. It urges further study to determine the most appropriate means of protection.

3. Programs: Uncertainty now exists as to whether the statute permits a valid patent to be granted on programs. Direct attempts to patent programs have been rejected on the ground of nonstatutory subject matter. Indirect attempts to obtain patents and avoid the rejection, by drafting claims as a process, or a machine or components thereof programmed in a given manner, rather than as a program itself, have confused the issue further and should not be permitted.

The Patent Office now cannot examine applications for programs because of the lack of a classification technique and the requisite search files. Even if these were available, reliable searches would not be feasible or economic because of the tremendous volume of prior art being generated. Without this search, the patenting of programs would be tantamount to mere registration and the presumption of validity would be all but nonexistent.

It is noted that the creation of programs has undergone substantial and satisfactory growth in the absence of patent protection and that copyright protection for programs is presently available.

74-184 O - 67 - 4

V

To prevent delay, which may be detrimental to the owner of an invention, while retaining safeguards to protect the rights of the inventor, it is recommended that:

1. **Either the inventor or assignee may file and sign both the preliminary and complete applications.**

   Any application filed by the assignee shall include a declaration of ownership at the time of filing and, prior to publication of the application, shall include a declaration of originality by the inventor and evidence of a recorded specific assignment.

2. **Every application shall include, at the time of filing, the name of each person believed to have made an inventive contribution.**

3. **Omission of an inventor's name or inclusion of the name of a person not an inventor, without deceptive intent, shall not affect validity, and can be corrected at any time.**

1. The present patent act requires (with specified exceptions) that the inventor, at the time of filing, must sign the application and make an oath or declaration that he made the invention. Occasionally, inventors are unavailable or unwilling to sign an application immediately after it is prepared. Moreover, it is sometimes difficult to determine the identity of an inventor at the time the application is prepared. Delay in complying with the requirements has resulted in loss of rights to the application owner. Such delay would be more serious when the effective filing date is treated as the date of invention.

The intent of this recommendation is to simplify the formalities for filing an application by allowing the owner of the patent rights to sign and file the necessary papers. Many detrimental delays thus would be avoided.

Before publication of the application, however, the assignee must provide both a declaration of originality and a specific assignment from the inventor to safeguard the interests of the inventor and the public. The present statutory exceptions which allow an interested party to file an application when the inventor is deceased, is incapacitated, cannot be found or refuses to cooperate, would be continued to prevent forfeiture of rights.

2. At present, it is often difficult to determine who should be named as the inventor in any given application. A contributing factor is court rulings that for a valid patent to be granted to joint inventors, each person named must have been a joint inventor with respect to each claim in the patent.

Many complex inventions result from the combined efforts of persons working separately, often at different times and in different sections of an organization. In such cases, adequate protection may be impossible because all of the claims required for protection cannot be presented properly in a single application, and the individual contributions cannot properly be made the subject matter of separate patents.

This recommendation would simplify the initial determination of who should be named as inventors in a given application and render it unnecessary for each person named to be the joint inventor of the invention asserted in each claim in a patent.

3. Today, a patent in which a sole inventor is incorrectly named will be held invalid. In the case of joint inventors, the omission or improper inclusion of a name will not necessarily invalidate a patent; however, correction procedures may be burdensome and the issue of whether correction is required can become an item of costly litigation.

This recommendation is intended to avoid a holding of invalidity, as above mentioned, as well as to facilitate correction of applications and patents.

VI

Claim for a priority date must be made when a complete application is filed.

This recommendation would require that any claim for a priority date based on an earlier U.S. or foreign application must be made at the time a complete application is filed. Present practice allows a claim for priority to be delayed until the final fee is paid.

Early knowledge of the priority date on which an applicant intends to rely would become more important with the adoption of a first to file system. Such knowledge would be necessary for proper determination, without wasted effort on the part of the Patent Office, of what references may be used as prior art against an application.

VII

Publication of a pending application shall occur eighteen to twenty-four months after its earliest effective filing date, or promptly after allowance or appeal, whichever comes first.

An applicant, for any reason, may request earlier publication of his pending complete application.

An application shall be "republished" promptly after allowance or appeal subsequent to initial publication, and again upon issuance as a patent, to the extent needed to update the initially published application and give notice of its status.

The only printed publication now made by the Patent Office of an application is that which occurs upon the issuance of a patent. Today, such publication can be delayed significantly beyond two years from the effective filing date of an application.

This recommendation sets an outside limit on the time for publication. An application, unless abandoned and kept secret,

would be made available to all concerned within a reasonably short time. Early publication could prevent needless duplication of the disclosed work, promote additional technological advances based on the information disclosed, and apprise entrepreneurs of their potential liability.

An applicant would be permitted to abandon his application prior to the time for publication and retain the invention in secrecy. Alternatively, an applicant could have his application published promptly after filing, with or without abandonment, which would make his disclosure available earlier for prior art or interim liability purposes. However, the Commissioner could refuse such publication where the subject matter is nonstatutory, immoral, or the like.

In the case of an application which is given a notice of allowance, or in which an appeal is filed to the Board of Appeals, within the eighteen to twenty-four months after its earliest effective filing date, immediate publication would permit citation of prior art by the public (Recommendation No. XI).

Republication after a notice of allowance or the filing of an appeal would be required if amendments to the claims or specification are made after the first publication. Printing costs should not be increased substantially since republication could consist merely of a notice, published in the Official Gazette, with copies of the allowed claims prepared and made available to the public. When considered appropriate by the Commissioner, integrated copies of the specification and drawings could be prepared and made available.

VIII

This recommendation is intended to prevent the repetitive filing of dependent applications. It is designed to eliminate undue postponement of the publication of the scope of protection granted, bring the United States into accord with international practice, and permit more efficient Patent Office examination.

Unless a later filed application is:

1. **A continuation application and is filed before the occurrence of any of the following events: (a) the abandonment of, (b) the allowance of all pending claims in, or (c) the filing of an appeal to the Board of Appeals as to any claim in, the original parent application; or**
2. **A continuation-in-part application and is filed before the publication of any of its parent applications; or**
3. **A divisional application filed (a) on one of the inventions indicated to be divisible in a restriction requirement and is filed during the pendency of the application in which the restriction was first required, or (b) during the pendency of the original parent application;**

The later filed application shall not be entitled to the effective filing date of a parent application for matter disclosed in the parent, and the parent, if published, shall constitute prior art against the later filed application.

At present, an applicant may serially file continuing applications for an unlimited period of time and maintain his invention in secrecy. Such practice makes effective examination in the Patent Office more difficult and expensive, and indefinitely prolongs the time before the issuance of a patent and the resultant publication of the scope of protection granted.

Permitting an applicant to file a continuation application during the indicated portion of the pendency of his original parent application would provide some latitude for one who felt that inadequate opportunity existed in the parent case to reach a clear issue. At the same time, it would avoid needless effort in preparing examiner's responses to appeal briefs, as well as unduly prolonged prosecution of the same invention.

Requiring that a continuation-in-part application be filed before publication of the parent application, as would appear to

be required if the provisions of the present Council of Europe Treaty and proposed Common Market Patent System were observed, normally would allow both the parent and continuation-in-part applications to be examined contemporaneously, possibly by the same examiner. Further, the public would learn sooner of the scope of patent protection that ultimately might be obtained based on the invention disclosed in the parent application.

Providing that all divisional applications must be presented during the pendency of the original parent application, or the application in which restriction first was required, would shorten the period of public uncertainty as to the scope of patent protection that eventually may be granted on the subject matter disclosed in the parent application. On the other hand, the applicant would have ample opportunity to perfect an appeal or to file a petition that may affect the propriety of a restriction requirement.

IX

The Commission clearly favors a high quality immediate examination system if it can be maintained without a constantly increasing backlog. Nevertheless, it is recommended that:

Standby statutory authority should be provided for optional deferred examination.

Although this recommendation reflects the consensus of the Commission, a split exists among the members as to when and how such authority should be exercised.

One view favors optional deferred examination going into effect, on a pilot basis, as soon as appropriate legislation can be enacted. Proponents of this view feel that early experience with optional deferred examination is desirable, and that it can be obtained effectively only by instituting a pilot program as early as possible. For example, the pilot program could apply to applications filed within a given period of time or to applications concerned with some given subject matter.

The other view favors the institution of optional deferred examination, whether on a pilot basis or in whole, only if the Statutory Advisory Council (Recommendation No. XXVI) should find that a high quality immediate examination system no longer could be maintained.

Justifications for an optional deferred examination system are that not all applications for patents are of the same value, that it is not good economic practice for the Patent Office to devote substantial effort to applications having little value, and that the applicant and his competitors are in the best position to select out such applications.

Such a system should reduce the number of applications requiring prompt examination. It is probable that a number of applicants, such as those who had not yet determined the value of their inventions, would prefer to have examination of their applications deferred. To the extent that applications are deferred, the remainder should be reached for examination sooner. In some cases, examination might never be requested, and the applications would become abandoned.

An optional deferred examination system shall include the following provisions:

1. **The examination shall be deferred at the option of the applicant, exercised by his election not to accompany the complete application with an examination fee.**

   Request for examination, accompanied by payment of an examination fee, may be made anytime within five years from the effective filing date of the application.

2. **A deferred application shall be promptly inspected for formal matters and then published.**

3. **Any party, without being required to disclose his identity, may provoke an examination upon request and payment of the fee.**

4. **Unless made special upon the request of any party, an application initially deferred shall**

be inserted in the queue of applications set for examination in an order based on the date of payment of the examination fee.

5. Examination of pending parent or continuing applications shall not be deferred beyond the time when examination is requested of any of the parent or continuing applications.

1. A five year period should balance the interests of the public, the applicants and the Patent Office. The public should learn, within a reasonable time, about any patent protection. Applicants should have adequate time to ascertain the commercial value of their inventions before investing in an examination fee and prosecution costs. The Patent Office should benefit from the abandonment of a number of applications prior to examination.

2. A complete application which is not accompanied by an examination fee would be inspected for formal matters immediately upon filing. The application would be classified under the Patent Office classification system and published at the earliest possible date. No prior art search would be made before a full examination is requested, since otherwise the saving of examiner's time would be minimal.

3. By requesting an examination, a potential infringer or other interested party could receive a relatively prompt determination of the invention's patentability.

A third party could initiate the examination without identifying himself to the Patent Office. As a result, the applicant would not be given any additional advantage when drafting his claims, nor would the third party be inviting suit for infringement after issuance of the patent.

4. The provision as to order of examination is intended to assure fair treatment to those who initially paid for an immediate examination.

74-184 O - 67 - 5

5. Concurrent examination and prosecution of the entire family of pending parent and continuing applications would be required in those cases where examination of one of such applications has been requested. If a third party requests and pays the fee for examination of an application, the applicant would be required to pay the examination fee promptly for all other parent or continuing applications.

This contemporaneous examination would provide earlier determination of the scope of the composite monopoly to be granted.

X

To reinforce the statutory presumption of validity, and to assist in the prevention of the issuance of invalid patents:

The applicant shall have the burden of persuading the Patent Office that a claim is patentable.

Until recently, the Patent Office has followed a policy of (a) instructing the examiner to resolve all reasonable doubts in favor of the applicant, and (b) prohibiting the examiner from indicating that he is allowing a claim despite his doubt as to its patentability. The Commissioner has instructed the examiners to abandon this policy in obedience to the views expressed this year by the Supreme Court. Present experience is insufficient to reveal how the courts directly reviewing Patent Office practice will treat this change.

Many have long recognized that resolving doubt in favor of the applicant is inconsistent with giving a patent a strong presumption of validity. Little justification exists for giving weight to a decision made by the Patent Office when it resolves doubt in this manner, since it is passing the question of patentability on to the courts instead of exercising its judgment. Inasmuch as the examiner does not indicate when he has applied the rule of doubt, all patents may be questioned in this regard.

This recommendation would require the applicant, in all cases, to persuade the Patent Office by a preponderance of proof

that his claims are allowable. By eliminating doubt as an element favoring patentability, the overall standards of patentability applied by the Office should be raised.

XI

To increase the likelihood that all pertinent prior art is considered before issuance of a patent, the following technique is provided.

The Patent Office shall consider all patents or publications, the pertinency of which is explained in writing, cited against an application anytime until six months after the publication which gives notice that the application has been allowed or appealed to the Board of Appeals. If the Patent Office, after the citation period, determines that a claim should not be, or have been, allowed, the applicant shall be notified and given an opportunity *ex parte* both to rebut the determination and to narrow the scope of the claim. The identity of the party citing references shall be maintained in confidence.

Public use proceedings, as at present, may be instituted during the citation period.

Presently, anyone who has reason to believe that an application is pending may seek an *inter partes* proceeding to to determine whether alleged public use or sale should bar issuance of a patent. Also, publications or patents may be submitted for *ex parte* consideration by the Patent Office.

This recommendation would provide a citation period of at least six months in which the public, informed by publication of the content of an application, could submit patents or publications, together with an explanation of their pertinency. Such references would be evaluated and, to the extent found applicable, used to reject claims even if such claims previously were allowed or under appeal.

Little delay in the issuance of patents would result from this procedure. The applicability of newly cited art would be

determined immediately after expiration of the six month period following the publication which gives notice of allowance or of the filing of an appeal. Moreover, the applicant need not suffer from such delay since, under certain circumstances, damages could be recovered for infringement during the period following publication (Recommendation No. XVII).

The recommended procedure could benefit both the applicant and the public. The applicant could gain by the opportunity to narrow his claims, when possible, to avoid prior art, rather than having the claims later held invalid. Inasmuch as the procedure will be an *ex parte* one, as distinguished from a full scale adversary procedure, the additional cost of the citation practice to an applicant would not be great. The public should benefit by the opportunity to cite prior art inexpensively to the Patent Office rather than through costly litigation. Under this procedure, both would benefit from the greater reliance that could be placed upon the validity of patents in general.

Citing, or failing to cite, prior art during this period would not preclude a later challenge on that art.

XII

Indispensable to the improvement of the quality and the acceptability of patents being issued is the establishment of an objective technique for measuring the quality of the work product of the examining corps. The Commission therefore recommends that:

The Patent Office shall develop and maintain an effective control program to evaluate, on a continuing basis, the quality of the patents being issued by the examining groups and art units therein, and to furnish information for the publication of an annual rating of the overall quality of the patents issued each year.

The Patent Office is presently in the process of putting into effect a quality control program.

This recommendation is intended to encourage and expand this effort so that an effective quality measurement can be made, on an objective basis, of the patents being issued by each of the examining groups and art units within the Patent Office.

Development of an effective patent quality measurement technique should be followed by the publication of a rating reflecting the quality of patents issued during a given period. For example, if effective quality measurement is achieved during 1968, the quality rating for that year could be used as a base of comparison and set at 100. Each year thereafter, a quality rating could be determined with this technique and the trend in the quality of patents being issued observed.

Such ratings should prove helpful to the Patent Office, the public, the courts, and the Congress in making required judgments concerning the patent system.

The continual review by a Statutory Advisory Council (Recommendation No. XXVI) of the quality of patents being issued and the effectiveness of any quality control program in operation should result in greater acceptability of the quality rating and the control program by all concerned.

XIII

A Patent Office decision refusing a claim shall be given a presumption of correctness, and shall not be reversed unless clearly erroneous.

Currently, the weight given on appeal to a Patent Office decision denying a patent depends upon which court reviews the decision. The Patent Office's decision is presumed correct in the District Court for the District of Columbia and the Court of Appeals for the District of Columbia Circuit, but not in the Court of Customs and Patent Appeals.

The Patent Office should be recognized as having technical and legal expertise, important in deciding questions of patentability. While a reviewing court certainly will have legal expertise, and perhaps general technical knowledge, it seldom will possess the particular technical skill in the art with which a Patent Office examiner is equipped. Further, it is only after both the examiner and the Board of Appeals have concurred in the refusal of a claim that the matter comes before a reviewing court. Such concurrence should not be rejected by the court unless the action is, in its judgment, clearly erroneous.

This recommendation should settle the conflict over "scope of review," by defining the court's responsibility to be *review* of the Patent Office decision, rather than substitution of its own judgment. The court would determine only whether the Patent Office had reasonable basis for its decision, not whether a different decision logically could have been reached on the same record. The burden of persuasion would be on the applicant, and the Patent Office decision should not be reversed unless, in view of all of the evidence, the court has a thorough conviction that there was no reasonable basis for the decision.

XIV

Either the applicant or the Patent Office may appeal from a decision of the Court of Customs

and Patent Appeals to the United States Court of Appeals for the District of Columbia Circuit, and from a decision of the latter court either may petition the Supreme Court for a writ of certiorari.

An applicant presently may seek review by two alternative routes from a decision by the Board of Appeals of the Patent Office. He may appeal to the Court of Customs and Patent Appeals (C.C.P.A.) on the record made in the Patent Office; or, he may proceed in the United States District Court for the District of Columbia where he may offer evidence and issues not considered by the Patent Office. Only a decision of the District Court may be appealed, by either party, to the United States Court of Appeals for the District of Columbia Circuit.

When the Court of Appeals and the C.C.P.A. render conflicting decisions reflecting a disagreement on a point of substantive law, the Patent Office must choose one of the decisions to follow, for the sake of uniformity within the Office. In practice, the Patent Office generally adopts the guidelines in the decision most favorable to the applicant, since it is the applicant who selects the reviewing court.

The present procedure also has caused inconsistency in the application of the law. As recently observed by the Supreme Court [*Graham* v. *John Deere Co.*], there is "a notorious difference between the standards applied by the Patent Office and by the courts." This difference results not only from the fact that proceedings in the Patent Office are *ex parte*, but also because the C.C.P.A., which to a large extent determines the standards applied in the Patent Office, is a court which has neither general jurisdiction nor jurisdiction in infringement cases.

Under the recommendation, all immediate direct review of the Patent Office would be subject to further review by the United States Court of Appeals for the District of Columbia Circuit. Thus, a single court of general jurisdiction ordinarily would be the final reviewing authority. This should produce decisions wherein interpretation and application of substantive

law is more akin to that in infringement suits in the several judicial circuits. Thus, the public reasonably could expect that the law relating to patentability as applied in the Patent Office would conform more nearly to that applied in the infringement courts.

Procedure for Amending and Cancelling Patents

XV

This recommendation provides an *ex parte* administrative procedure in the Patent Office for cancellation of claims, which should be faster and less costly than court proceedings.

> **The Patent Office, upon receipt of a relatively high fee, shall consider prior art of which it is apprised by a third party, when such prior art is cited and its pertinency explained in writing within a three year period after issuance of the patent. If the Patent Office then determines that a claim should not have been allowed, the patent owner shall be notified and given an opportunity *ex parte* both to rebut the determination and to narrow the scope of the claim. Failure to seek review, or the affirmance of the Patent Office holding, shall result in cancellation of the claim.**
>
> **When the validity of a claim is in issue before both the Patent Office and a court, the tribunal where the issue was first presented shall proceed while the other shall suspend consideration, unless the court decides otherwise for good cause.**
>
> **Anyone unsuccessfully seeking Patent Office cancellation of claims shall be required to pay the patent owner's reasonable cost of defending such claims, including attorney's fees. The Commissioner shall require an appropriate deposit or bond for this purpose at the start of the action.**

Presently, there is no provision for the Patent Office administratively to cancel any claim in an issued patent. Even where a claim appears to be clearly unpatentable in view of newly discovered prior art, only a court can declare the claim invalid. As a result, the patent owner can continue to assert such a claim because no one is willing or able to expend the resources necessary to obtain a court decision.

To discourage harassment and to promote the citation of references prior to issuance (Recommendation No. XI), a relatively high fee would be required. Further, the patentee's defense costs would be assessed against any party who unsuccessfully sought cancellation. To insure payment, anyone initiating such action would be required immediately to post a deposit or bond in accordance with a schedule fixed by the Commissioner.

In some instances, the cancellation proceeding would benefit even the patent owner, since he still would have an opportunity to narrow any claims found to have been erroneously allowed.

If a party were successful in seeking cancellation, after citing only prior art which he previously presented during the opposition period, the cancellation fee should be refunded.

A three year limit on the time within which a cancellation procedure could be instituted should be sufficient for most prior art to become readily accessible.

It would be desirable for the Statutory Advisory Council (Recommendation No. XXVI) to review this procedure after sufficient time has elapsed to determine its effectiveness, and to recommend any appropriate changes.

XVI

A claim shall not be broadened in a reissue application.

Presently, there are few statutory restrictions against broadening the scope of the invention claimed during prosecution before the Patent Office. Because of this, the potential value of early publication (Recommendation No. VII) cannot be fully realized, since unclaimed disclosure in a published application could not be used by the public free from the possibility that it might be protected by broader claims in the subsequently issued patent. The public would have no guide, other than the entire disclosure, to determine the limits of final patent protec-

tion. Possible claim scope could be divined only after the interested party conducted his own examination of the prior art.

Hence, it is desirable that claims never be broadened after publication, whether presented in the published application or a related continuing or reissue application. However, an all-inclusive prohibition to this effect might be impossible to enforce. Accordingly, this recommendation is directed solely to reissue applications, where broadening of claims can be prohibited effectively.

Liability and Enforcement

XVII

In view of the recommended publication of applications by the Patent Office before a patent issues (Recommendation No. VII), some protection for the patent owner for the period from publication to patenting should be made available. Therefore, it is recommended that:

> **For infringement of a claim which appears in both an application as initially published and in the issued patent, damages may be obtained for an interim period prior to issuance. Such period shall be measured from after the occurrence of all of the following events: (1) the initial publication, (2) a Patent Office holding that the claim is allowable, and (3) a transmittal to the alleged infringer of actual notice reasonably indicating how his particular acts are considered to infringe the claim.**
>
> **The applicant's election to create such interim liability, by his transmittal of notice, shall constitute the granting of a reasonable royalty, nonexclusive license, (1) extending only until the issuance of the patent for any infringement involving a process, and (2) extending to and beyond issuance for any infringement involving a machine, manufacture or composition of matter, which is made prior to the issuance of the patent.**
>
> **In exceptional cases, damages for interim infringement up to treble reasonable royalties may be assessed.**

Under the present statute, liability for infringement begins on the date a patent is issued.

With a requirement of pre-issuance publication of an application, absent this recommendation, anyone could copy the invention and make, use or sell it until a patent is issued, possibly even exhausting its commercial value.

By this recommendation, a patentee whose claims are "infringed" before the patent issues, would have some degree of protection, while at the same time the public would be provided with a clear indication of its possible liability.

The provision that a claim will not be held infringed unless it appears both in the application as first published and in the resulting patent should encourage the applicant, before publication, to present claims he considers patentable. The further requirements of an allowable published claim and actual notice would reduce public uncertainty as to possible interim liability. Also, an infringer would be provided with an opportunity to cease and desist before damages accrue.

In exchange for the right to recover damages during this interim period, an applicant would have to give up any right to an injunction as to things made prior to issuance, and could recover no more than a reasonable royalty for any infringing acts occurring prior to the issuance of the patent. Under any circumstances, suit could not be brought before issuance of a patent.

If an applicant should elect not to pursue an infringer for interim liability, by withholding the required notice, present remedies available after the patent issues would remain undisturbed.

XVIII

The term of a patent shall expire twenty years after its earliest effective U.S. filing date.

The term of a U.S. patent now extends for a period of seventeen years from the date of issuance. Measuring the patent term from this point encourages deliberate delays in the prosecution of applications, particularly those filed primarily for speculative reasons and those having little immediate value. Another effect can be the filing of continuing applications solely to delay the start of a patent term.

The proposed change would induce the applicant to present claims promptly that he believes patentable and to avoid delaying the prosecution of the application. Since the term of a patent stemming from a continuing application would expire on the same day as one issued on its parent application, there would be less incentive to use a continuing application for the purpose of delay.

Measuring the patent term from the earliest domestic filing date will bring U.S. practice into closer conformity with most foreign systems. This would become advantageous if the Paris Convention were to be modified to permit measuring from the earliest foreign filing date asserted (Recommendation No. XXXIV).

XIX

The term of a patent, whose issuance has been delayed by reason of the application being placed under secrecy order, shall be extended for a period equal to the delay in issuance of the patent after notice of allowability.

At present, whenever publication or disclosure of an invention by grant of a patent might be detrimental to national security, the application may be placed under secrecy order by the Commissioner of Patents.

The applicant, provided he receives a notice of allowability, is entitled to compensation for use of the invention by the Government and for damages caused by the secrecy order. In determining this compensation, consideration has been given to the fact that the applicant may benefit by a delayed monopoly, running seventeen years from the date of issuance of the patent.

With the patent expiring twenty years after its earliest effective U.S. filing date (Recommendation No. XVIII), an applicant would receive no such benefit. Accordingly, it is proposed to extend the term of such a patent for a period equal to the delay in issuance of the patent after notice of allowability caused by the secrecy order.

XX

The filing of a terminal disclaimer shall have no effect in overcoming a holding of double patenting.

This recommendation is intended to endorse the interpretation given the present statute, with regard to the filing of a terminal disclaimer to overcome a holding of double patenting, by the Court of Appeals for the District of Columbia Circuit. A contrary decision by the Court of Customs and Patent Appeals has created confusion in this area.

The Commission supports the position that the granting of more than one patent on a single invention, even if there is a common inventor or assignee, would constitute, *inter alia*, an undue "extension of monopoly." While a terminal disclaimer would prevent the extension of monopoly in time, it would not preclude the undue extension of monopoly in scope. In this regard, it would not keep the patentee from "blocking" out a field, by successfully prosecuting applications covering otherwise unpatentable variations of what he already has patented. Further, it would discourage attempts by others to "invent around" the patented invention by developing modifications and improvements.

The granting of more than one patent on obvious variations of a single inventive concept also would minimize advantages to be obtained by the provision for *in rem* invalidity (Recommendation No. XXIII). Otherwise, a patent owner, even after claims in one such patent had been held invalid, still could threaten suit on similar claims in his other patents.

XXI

The importation into the United States of a product made abroad by a process patented in the United States shall constitute an act of infringement.

The unauthorized importation into the United States, or sale or use, of a product made abroad by a process patented in the

United States, does not now constitute infringement. A process patent owner may seek to have the offending product excluded from this country under the Tariff Act of 1930, on the ground that importation will tend to cause substantial injury to an efficiently and economically operated domestic industry. However, because of these requirements, the patent owner has little prospect for success.

This recommendation would make it possible to prevent evasion, of the process patent owner's exclusive rights in the United States, by the practice of his process abroad and the importation of the products so produced into this country.

XXII

The licensable nature of the rights granted by a patent should be clarified by specifically stating in the patent statute that: (1) applications for patents, patents, or any interests therein may be licensed in the whole, or in any specified part, of the field of use to which the subject matter of the claims of the patent are directly applicable, and (2) a patent owner shall not be deemed guilty of patent misuse merely because he agreed to a contractual provision or imposed a condition on a licensee, which has (a) a direct relation to the disclosure and claims of the patent, and (b) the performance of which is reasonable under the circumstances to secure to the patent owner the full benefit of his invention and patent grant. This recommendation is intended to make clear that the "rule of reason" shall constitute the guideline for determining patent misuse.

There is no doubt, in the opinion of the Commission, of the importance to the U.S. economy of both the U.S. patent system and the antitrust laws. Each is essential and each serves its own purpose within the framework of our economic structure. However, conflicts between the two have arisen. But this does not mean that the two systems are mutually exclusive, that a strong patent system is a threat to the antitrust laws, or that the latter

cannot be effectively enforced so long as a patent system grants limited monopolies.

On the contrary, the two systems are fully compatible, one checking and preventing undesirable monopolistic power and the other encouraging and promoting certain limited beneficial monopolies. In this way, each may easily achieve its objectives in a strong economy.

The Commission, therefore, does not favor any proposal which would weaken the enforcement of the antitrust laws or which would curtail in any way the power of the courts to deny relief to a patent owner misusing the patent he seeks to enforce. However, uncertainty exists as to the precise nature of the patent right and there is no clear definition of the patent misuse rule. This has produced confusion in the public mind and a reluctance by patent owners and others to enter into contracts or other arrangements pertaining to patents or related licenses.

No useful purpose would be served by codifying the many decisions dealing with patent misuse into a set of rules or definitions permitting or denying enforceability of patents in given circumstances. The risk of unenforceability is too great and such a codification is wholly unnecessary. All that the Commission believes to be required is explicit statutory language defining, for the purpose of assignments and licenses, the nature of the patent grant heretofore recognized under the patent statute or by decisional law. This is, the right to exclude others from making, using and selling the patented invention.

The mere exercise, conveyance or license of these conferred rights should not in itself constitute misuse of a patent. A patent owner should not be denied relief against infringers because he either refused to grant a license or because he has exercised, transferred or licensed any of the conferred patent rights himself. This should not include immunity of even these conferred patent rights from the antitrust laws when the patent owner becomes involved in a conspiracy to restrain or monopolize com-

merce, or when the patent is itself used as an instrument for unreasonably restraining trade.

There are also a number of conditions and provisions long associated with the transfer or license of rights under patents which must be distinguished from the exclusive right to make, use and sell conferred by the patent grant. Among these are improvement grant-backs, cross licenses, package licenses, patent pools, no contest clauses, and many others which are simply matters of private contract, ancillary to the conveyance or license of a patent right. As such, these conditions and provisions must be judged, along with other purely commercial practices, under the antitrust laws and the patent misuse doctrine. The Commission does not recommend immunization of any of these other provisions or conditions from either the antitrust laws or the application of the misuse rule.

This recommendation also makes it clear that a patent may not be used to control commerce in subject matter beyond the scope of the patent. For example, it could not be considered "reasonably necessary" to secure full benefit to the owner of a machine patent that he attempt to control any of the commerce in an unpatented raw material to be used in the machine. Neither could it be held that such an attempt had a direct relation to the machine claims in his patent. By the same standards, the patent owner could not control commerce in one of the unpatented elements of his combination invention where his claims are to the whole combination.

XXIII

A final federal judicial determination declaring a patent claim invalid shall be *in rem*, and the cancellation of such claim shall be indicated on all patent copies subsequently distributed by the Patent Office.

Under present law, even though one or more claims of a patent have been held invalid in one Federal circuit, the patentee may pursue a different defendant in another circuit for infringement of the same claims.

As a result, a party may be held liable as an infringer or required to pay royalties in one circuit, while his direct competitor is practicing the same invention without restriction in another circuit. Moreover, the mere possession of a patent, even though held invalid in one or more circuits, serves as a potential threat to persons unwilling or unable to defend a suit on the patent.

Under the proposed recommendation, a claim, once held invalid, would be treated as cancelled from the patent. No one thereafter could be required, on the basis of a royalty agreement previously made part of an infringement judgment, to continue royalty payments on the claim. Furthermore, the proposal would preclude a subsequent suit on a patent claim previously held invalid by a Federal court.

A patentee, having been afforded the opportunity to exhaust his remedy of appeal from a holding of invalidity, has had his "day in court" and should not be allowed to harass others on the basis of an invalid claim. There are few, if any, logical grounds for permitting him to clutter crowded court dockets and to subject others to costly litigation.

XXIV

One of the most common grievances called to the Commission's attention, by all branches of the patent-using community, has been the high cost of patent litigation. The following recommendation is directed toward the pretrial period, now the occasion for much expense and vexation.

Offices of "Civil Commissioner" shall be created in those U.S. district courts where justified by the volume of patent litigation. In patent cases, unless otherwise ordered by a district court judge for good cause, a Commissioner shall conduct pretrial hearings, preside at depositions of parties, supervise discovery proceedings upon an accelerated and abbreviated basis, make preliminary rulings upon the admissibility of proofs, and be empowered to vary the

burdens of proof for good cause in secrecy cases.

The wholesome effect of the liberal discovery provisions of the Federal Rules of Civil Procedure (FRCP) is undeniable. Adversaries are compelled to reveal the facts of their cases to each other so that trials are conducted more fully and fairly. Like any other right, however, the right of discovery can be abused and it has been used to harass and oppress litigants. Uncontrolled discovery in patent cases is a prime cause of the enormous expense frequently encountered by the litigants.

One source of this expense is the man-hours required to search for, collect, and assemble for inspection, thousands of documents called for under Rule 34 FRCP. More thousands of documents and other kinds of information may be required to answer interrogatories under Rule 33 FRCP. In the event of a disagreement between the parties about discovery, much more time may be needed for legal research, brief writing and argument before a court. In any event, the general rule in the courts is that the acknowledged burden of a request for discovery is not a valid excuse to avoid producing the information.

Another source of considerable cost comes from taking adverse discovery depositions of parties or of the officers, directors and managing agents of corporate parties. The witnesses may be examined over a wide subject area and for protracted periods of time. Rule 30(b) FRCP provides that a court may limit or terminate an examination if it is being conducted unreasonably or in bad faith. However, this recourse involves still more time and expense.

As a consequence, the high cost of patent litigation results in good and valid patents being defied and going unenforced, invalid patents being kept from court scrutiny, and, finally, compromises, settlements and licensing arrangements, whose only justification is an economic one, i.e., the avoidance of enormous litigation expense.

Adoption of this recommendation should reduce considerably the time and expense to litigants in patent cases. The provision of Civil Commissioners, who would supervise discovery procedures, should help correct abuses and bring about more effective utilization of these procedures.

XXV

The previous recommendation should substantially reduce the cost of litigation. However, even the reduction so accomplished may not be sufficient to insure a "day in court" for the individual or corporation of modest means. The following recommendation is addressed to this problem.

A party to a patent case seeking to reduce his litigation costs, with the consent of the adverse party, may submit his case to the court on a stipulation of facts or on affidavits without the usual pretrial discovery. This procedure may be used where no injunctive relief is asked and only limited damages are sought. Incentives shall be provided to consent to this procedure, as set forth below.

The Commission does not seek to discourage the settlement of patent infringement controversies. On the contrary, public policy strongly favors this method of resolving disputes. However, since there is always a public interest or aspect involved in a patent license, a strong patent system requires that only good and valid patents be the subject of licensing arrangements. Attainment of this desirable objective is presently hampered by the many settlements and patent licenses brought to pass in order to avoid high litigation expenses. But just as it is contrary to the spirit of the patent laws to recognize and pay tribute to an invalid patent, it is also unfair to expect individual or corporate patent owners of limited means to settle, and accept less than their just due, simply because they cannot afford expensive litigation.

The Commission believes that a truly just patent system should provide all patentees fair opportunity for a "day in

court." Similarly, all alleged infringers should have an opportunity to test judicially the validity and scope of patents asserted against them. Neither should be made to suffer or be denied access to the courts because of intolerable litigation expenses.

The expedited procedure recommended should be made applicable to both infringement suits and declaratory judgment actions involving patents.

As an incentive for the alleged infringer to consent to this procedure, any subsequent judgment favoring the patent owner, under this procedure, would omit any injunctive relief and would be confined to a reasonable royalty license for future infringement and reasonable royalties for past infringement. Royalties, both past and future, could not exceed a fixed amount, such as $100,000, unless a higher figure is agreed to by the parties. In addition, if an alleged infringer should refuse to consent to this procedure, and the patent owner, after regular proceedings, is successful, he would be entitled to a mandatory award of all reasonable litigation expenses, including attorney's fees.

Statutory Advisory Council

XXVI

A Statutory Advisory Council, comprised of public members selected to represent the principal areas served by the patent system, and appointed by the Secretary of Commerce, shall be established to advise him, on a continuing basis, of its evaluation of the current health of the patent system, and specifically, of the quality of patents being issued and the effectiveness of any internal patent quality control program then in operation, and whether an optional deferred examination system should been instituted or terminated.

Every fourth year the Council shall publish a report on the condition of the patent system including recommendations for its improvement.

The membership shall consist of not less than twelve nor more than twenty-four. The term of appointment shall be four years, with a maximum tenure of eight years. An executive director, and other support as deemed necessary, shall be provided.

Under this recommendation, a standing advisory body would be created by statute with public members representing the principal areas served by the patent system. This group would meet at regular intervals and would be responsible, on a continuing basis, for effectively analyzing the contemporary condition and needs of the system. The Council would utilize and suggest modern techniques for measurement and evaluation, and regularly report its findings and recommendations to the Secretary of Commerce.

The composition and continuity of the Council should insure objective evaluation of the quality of the patents being issued and enable it to recommend the institution or termination of an optional deferred examination system (Recommendation No.

IX). It also could observe the effectiveness of the recommended cancellation procedure (Recommendation No. XV).

In view of the great pressures on the patent system brought by, for example, the escalating information explosion, the Commission believes that the system's continuing welfare must not be left entirely to those preoccupied with its daily administration, or to examination by a once-in-a-generation Commission. Continuous review of the Nation's changing needs and the capacity of the system to respond is indispensable.

Patent Office Operations

XXVII

Adequate support of the Patent Office is required in order that it properly may perform its mission, now and in the future, irrespective of the nature of the patent examining system utilized. Therefore, it is recommended that:

The Patent Office should be supported adequately to insure first-class staffing, housing and equipment, and

Patent Office financing should be established on the following basis:

1. **The Patent Office should not be required to be entirely self-sustaining.**
2. **The Commissioner of Patents should be authorized to set fees for Patent Office services within broad guidelines established by Congress. Such fees shall be apportioned in accordance with the cost of providing the services.**
3. **The Patent Office should be authorized to establish a "revolving fund" of all its receipts to support its operation.**

The Commission cannot emphasize too strongly that the prime requirement for optimum Patent Office operation is a dedicated corps of career employees possessing a unique combination of scientific and engineering knowledge and the ability to make sound legal judgments. Assembling and retaining such a staff of highly trained professional personnel in a competitive manpower market requires, among other things, an increasing expenditure of resources.

Maximum utilization of the skills of any staff requires a working environment conducive to intellectual output. Supplementing such environment, the best available equipment must be provided for obtaining, storing, and retrieving pertinent prior art and for all other required supporting functions.

1. To recover 100% of Patent Office operating expenses on a sustained basis would require substantial fee increases. This could reduce overall inventive activity which, together with the resultant loss of technological disclosure, could adversely affect our economy. Limited subsidization of the Patent Office has substantial justification. The patent system's incentive to invent, disclose, innovate and market new inventions creates capital, jobs, and tax revenues which more than justify the relatively small expenditure of tax funds required to support Patent Office operations.

2. At present, Congress periodically enacts Patent Office fee legislation which includes a schedule specifically listing the fees that the Patent Office must charge for most of the services it provides. The fees set do not necessarily reflect the actual expense to the Patent Office in rendering particular services. Although Patent Office costs may rise, there is no present provision for a corresponding increase in its service charges. Hence, it is unlikely that any long term fixed relationship between fees received and Office expenditures could be maintained without continuing prompt legislative adjustments. This recommendation would permit the Commissioner of Patents, under guidelines established by Congress, to set fees for types of services and change them as conditions may demand. This would permit recovery of any desired percentage of expenses and provide a more equitable fee structure directly related to the cost of particular services.

3. At the present time, all fees received by the Office must be turned over to the Treasury promptly and the Patent Office must often seek supplemental appropriations because of conditions beyond its control. These include unexpected rises in printing costs and unpredictable increases in demand for services that are furnished below cost. Consequently, it faces periods of uncertainty and delay in carrying out needed programs.

Adoption of the present proposal would establish a fund, consisting of the fees paid for Patent Office services, for

partially financing Patent Office operations. Congressional appropriations could supplement this fund as necessary. The availability of this "revolving fund" would lessen the disruptive effects caused by delayed legislative action on appropriations. It would also enable the Patent Office to offer additional services to the public on a reasonable cost recovery basis.

XXVIII

The applicant should be permitted to amend his case following any new ground of objection or rejection by the Patent Office, except where the new ground of objection or rejection is necessitated by amendment of the application by the applicant.

The Commission believes that the desirable goal of reducing the backlog of patent applications reasonably should be balanced with the opportunity for an inventor to obtain a valid patent of proper scope. Thus, the applicant should be provided a fair opportunity for reshaping his claims to meet new rejections of the Patent Office. On the other hand, it is desirable to avoid prolonged pendency, which can be caused by successive amendments that substantially shift the subject matter area claimed.

Applied to specific problems which most commonly arise in Patent Office prosecution, a practice is envisioned in which: (a) if, prior to final rejection, the applicant should introduce new limitations not found in any of his original claims, the Patent Office could cite a reference in the final rejection to show these new limitations and refuse further amendment to the case; and, conversely, (b) if, following an amendment prior to final rejection, the Patent Office should cite a new reference which is a better anticipation of features previously claimed, the Patent Office could not terminate prosecution of the application.

To accelerate the attainment of a system for the rapid and effective retrieval of pertinent information concerning patents, it is recommended that:

A study group comprising members from industry, technical societies and government should be established to make a comprehensive study of the application of new technology to Patent Office operations and to aid in developing and implementing the specific recommendations which follow.

1. **The United States, with other interested countries, should strive toward the establishment of a unified system of patent classification which would expedite and improve its retrieval of prior art.**

   The United States should expand its present reclassification efforts.

2. **The Patent Office should be encouraged and given resources to continue, and to intensify, its efforts toward the goal of a fully mechanized search system.**

3. **The Patent Office should acquire and store machine-readable scientific and technical information as it becomes available.**

   The Patent Office should encourage voluntary submission by patent applicants of copies of their applications in machine-readable form.

4. **The Patent Office should investigate the desirability of obtaining the services of outside technical organizations for specific, short-term classification and mechanized search projects.**

1. Until the advent of fully automated searching, when all prior art can be retrieved readily, a classification system will continue to be one of the important tools for conducting a prior

art search. The present diversity among national patent laws and classification systems results in a substantial amount of duplicative effort in examining applications on the same invention filed in more than one country. A common classification system would move the world closer to the desired international patent, if principles of patentability are similar (Recommendation No. XXXV), since each country would know what segment of prior art was previously searched by another patent office on an application for the same invention filed in that country. Moreover, it would insure that specialized data banks would be more complete by providing common guidelines as to what information should be included in each of these data banks.

2. As the amount of scientific and technical information continues to grow at a pace which makes the information unmanageable manually, mechanization appears to be the only solution to obtaining reliable, quality searchers of prior art. Hence, it is imperative to utilize fully the existing techniques of mechanized searching and to study new ones as they become available.

The Patent Office should cooperate with other U.S. agencies engaged in the development and implementation of mechanized information retrieval systems, to maximize their value to the Office as well as the other agencies.

The need for cooperative efforts with foreign nations and active participation by the Patent Office in international organizations studying problems of mechanical information retrieval is self-evident and should be pursued.

3. Obtaining as much contemporary information as possible in the form of perforated or magnetic tape, or the like, would permit continuous build-up of a data bank suitable for automated searching. This would avoid the future necessity of transcribing at one time huge amounts of printed information into computer-usable form and permit a speedier and less expensive change-over from a manual to an automated search system.

To insure compatibility of information in machine-readable form with automated data systems envisioned for future Patent Office use, industry, professional societies, government and all others generating data should cooperate in setting up acceptable standards for format and media for machine-readable data.

4. Utilization, on a contract basis, of any knowledge, experience and expertise of outside organizations specializing in mechanized information retrieval technologies could serve as an expeditious and economical means for solving problems which otherwise would require very expensive in-house training, experimentation and delay.

XXX

To facilitate the public dissemination of technological knowledge, and other patent related information, it is recommended that:

The Patent Office should:

1. **Proceed vigorously with the implementing of its plan for microform reproduction of all search files; and**

2. **Cooperate with foreign national patent offices and international patent organizations to develop a worldwide index of patents and published applications for patents.**

1. The Commission recognizes that any visual microform system is intended only as a bridge between the present methods of information storage and retrieval, and future fully automated mechanized search systems (Recommendation No. XXIX). Meanwhile, however, there is the possibility of storing great amounts of information on small quantities of film or cards, which can be readily inspected with semi-automatic reading devices. This not only increases the capability of the searcher to scan more material in a given time but also makes economically feasible the placing of complete copies of classified search files in locales outside the Patent Office. This would permit establish-

ment of satellite public search facilities throughout the United States, resulting in greatly improved dissemination of the technological and legal information contained in patents.

2. On an average, patents now are granted in three different countries for each invention, and an average of 650,000 patent applications are filed each year in eighty different patent offices. These figures lend substantial weight to the desirability of a worldwide patent index. Such an index would provide prompt and reliable means for obtaining information relative to the existence and status of particular patents or applications in any country in the world.

Transition

XXXI

The legislation implementing the proposed recommendations of the Commission should become effective as soon as practical with regard to both patents and pending applications.

Many recommendations, such as the presumption of correctness to be given Patent Office decisions, reasonably could be applied to all pending applications. Others, such as those relating to patent term and prior art, should not apply to pending applications. Specifically, any application filed prior to the effective date of implementing legislation, which is still pending four years after its earliest effective filing date, or two years after the enactment of such legislation, whichever is later, should be published in a manner similar to that of the recommended initial publication (Recommendation No. VII).

Many recommendations, such as those concerning the Civil Commissioner and the expedited procedure for limited claims, could apply to all patents, whenever issued.

It is expected that the legislative draftsmen will determine the time each statutory change proposed may be implemented most effectively.

Government Patent Policy

XXXII

The Commission has noted the increasing participation of the Federal Government in the financing of research, development, testing and engineering, and the many problems related to the ownership of patents resulting from such work.

The Commission decided not to address itself to the question of the distribution of rights in inventions resulting from research and development work financed wholly or in part by the Government. This question is being considered actively elsewhere in the Executive Branch and by Committees of the Congress.

Nevertheless, it is the Commission's hope that any action Congress may take in this regard will promote the purposes of the patent system to encourage invention and innovation and the resulting economic development and benefits.

International Action

XXXIII

To promote more harmonious international relations, particularly with regard to the protection of industrial property:

The United States should take a position in favor of the proposed revision of the Paris Convention whereby a right of priority may be based on an application for an inventor's certificate.

Some member countries of the Paris Convention, in particular the U.S.S.R. and some Eastern European countries, issue inventor's certificates as well as patents. While some Convention countries voluntarily recognize inventor's certificates for priority purposes, there is no obligation under the Convention to do so. At present, the U.S. patent statute prevents the recognition for priority purposes of anything but an application for patent in another Convention country.

The proposal for revision is on the agenda of the Stockholm Conference, which is to be held in 1967. According to the proposal, the date of an application for an inventor's certificate in one Convention country would be recognized for priority purposes in all Convention countries. It is noted that the proposed revision is limited to inventor's certificates from countries in which inventors have the right to apply for either a patent or an inventor's certificate.

XXXIV

Efforts should be made to have the Paris Convention modified to remove any obstacle to measuring the term of a patent from an effective foreign filing date.

The present text of the Paris Convention requires that "Patents obtained with the benefit of priority shall have in the various countries of the Union a duration equal to that which

they would have had if they had been applied for or granted without the benefit of priority."

Since the Convention forbids calculation of the term of a patent from the foreign filing date, it prevents measurement of the term from the effective filing date when foreign priority is claimed. Thus a foreign applicant who can claim a foreign priority date would receive a longer period of protection than an applicant who filed a domestic application on such date. Of course, a corresponding advantage is accorded U.S. inventors filing abroad.

Movement toward a universal patent system (Recommendation No. XXXV) would be promoted if an entire international family of related patents expired at the same time. This requires a common measuring point for the patent term. The effective (foreign or domestic) filing date, unlike the earliest domestic filing date, would constitute such a common measuring point.

XXXV

The Commission believes that the ultimate goal in the protection of inventions should be the establishment of a universal patent, respected throughout the world, issued in the light of, and inventive over, all of the prior art of the world, and obtained quickly and inexpensively on a single application, but only in return for a genuine contribution to the progress of the useful arts.

To this end the Commission specifically recommends the pursuit of: (1) International harmonization of patent practice, (2) the formation of regional patent system groups, and (3) a universal network of mechanized information storage and retrieval systems.

There are great differences today among the patent systems of the various countries. The inventor who desires worldwide or even multi-national patent protection for his discovery must file a multitude of applications, each governed by a separate and distinct system of laws, rules, regulations and procedures.

Even after the patent has been obtained, the inventor is confronted with diverse systems of maintaining patent protection.

These factors increase the cost of securing multi-national patent protection and often cloud the status of an invention in a particular country, thus discouraging foreign investment and marketing.

If change is to be achieved, nations must adopt a single set of long-range goals to guide their intermediate and short-range movements. Any attempt by revolutionary change, to scrap present systems in favor of new ones, in the United States or abroad, is neither feasible nor desirable. It is, however, both possible and advantageous to promote and direct interim steps toward the ultimate goal—a universal patent.

To the extent that harmonization of U.S. practice with prevailing foreign practice can be attained without injury to the quality of the U.S. patent system, such harmonization should be introduced as a first step toward the desired goal. This consideration applies both to the substantive law and to the forms and procedures for implementing it. Other recommendations in this report are responsive to this general objective.

Where, however, U.S. practice appears to be the superior one, it is recommended that appropriate Federal agencies make efforts to secure harmonization compatible with U.S. practice.

As an intermediate step toward attainment of a universal patent, the formation of regional patent system groupings should be encouraged. Within such groupings there will inevitably develop a mutual respect for the search and judgment capabilities of the members. This should lead to cooperative searching and, beyond that, to mutually recognized patents among the members of the group. The avoidance of the duplication of effort, expense and delay is a clearly attainable benefit from such a development.

Finally, as an adjunct to achieving the ultimate goal of a universal patent, the Commission envisages the establishment of a universal network of mechanized information storage and retrieval systems involving all of the patents and other technical literature of the world.

CHARTS

Charts 1 through 5 illustrate a number of recommended changes by providing a graphic representation of procedural steps and effects arising therefrom. Much of the wording used is abbreviated and should be read in the context of the specific recommendation referred to by number.

The flow of events proceeds from top to bottom. Broad arrows pointing *into* the system indicate conditions affecting the system's flow, while broad arrows pointing *outward* from the system, signify results emanating from the system's flow.

CHART 1

Filing An Application

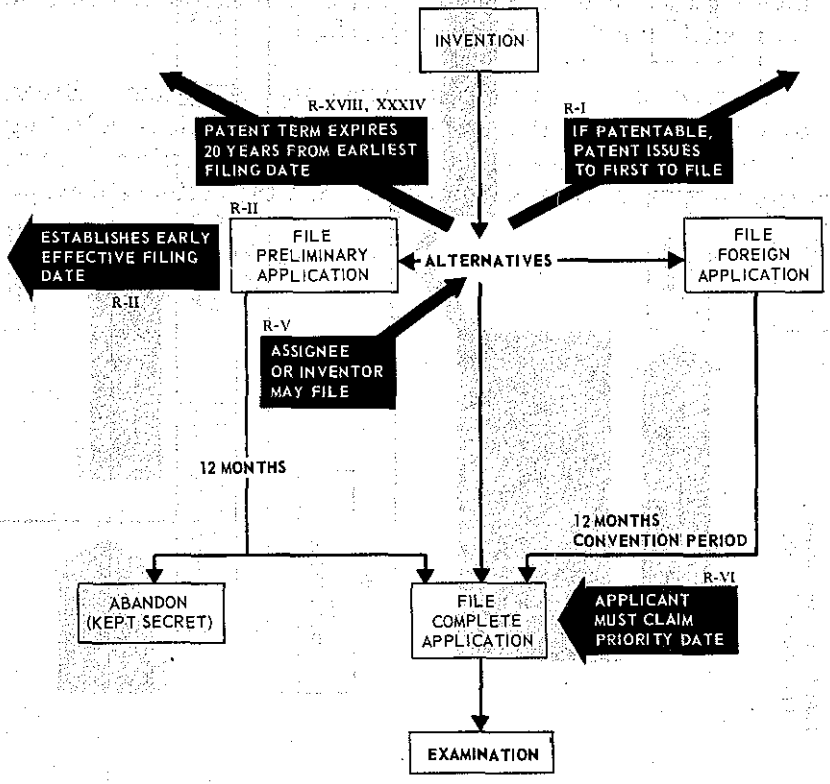
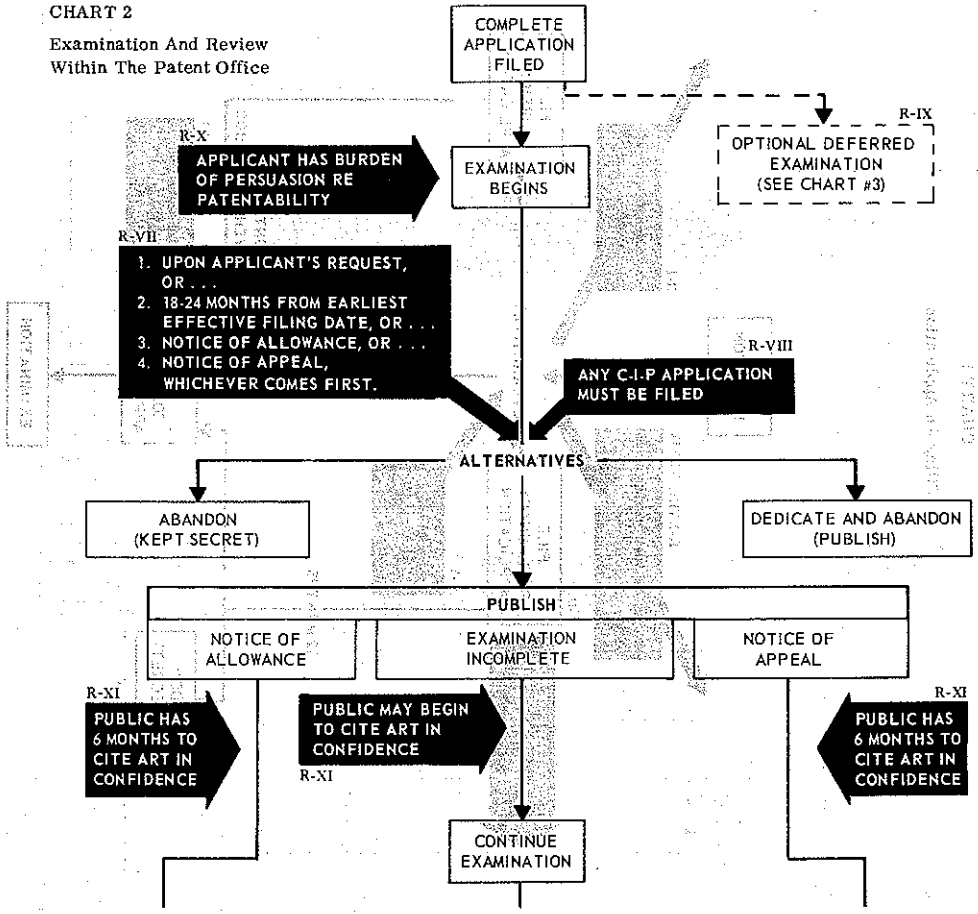


CHART 2

Examination And Review
Within The Patent Office



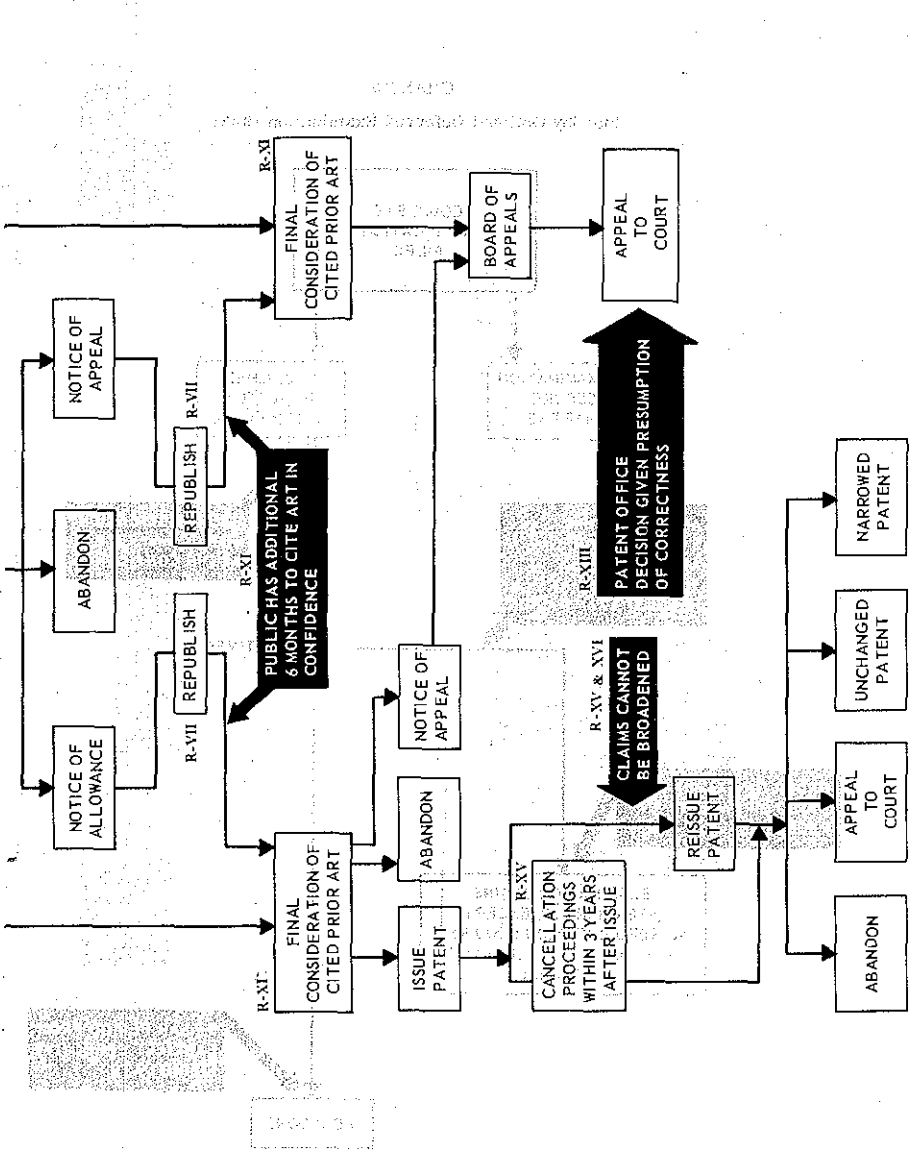

NOTICE OF ALLOWANCE
ABANDON
NOTICE OF APPEAL
R-VII
REPUBLISH
R-XI
FINAL CONSIDERATION OF CITED PRIOR ART
R-XI
PUBLIC HAS ADDITIONAL 6 MONTHS TO CITE ART IN CONFIDENCE
ISSUE PATENT
ABANDON
NOTICE OF APPEAL
BOARD OF APPEALS
APPEAL TO COURT
R-XIII
PATENT OFFICE DECISION GIVEN PRESUMPTION OF CORRECTNESS
R-XV
CANCELLATION PROCEEDINGS WITHIN 3 YEARS AFTER ISSUE
R-XV & XVI
CLAIMS CANNOT BE BROADENED
REISSUE PATENT
ABANDON
APPEAL TO COURT
UNCHANGED PATENT
NARROWED PATENT

CHART 3

Standby Optional Deferred Examination (R-IX)

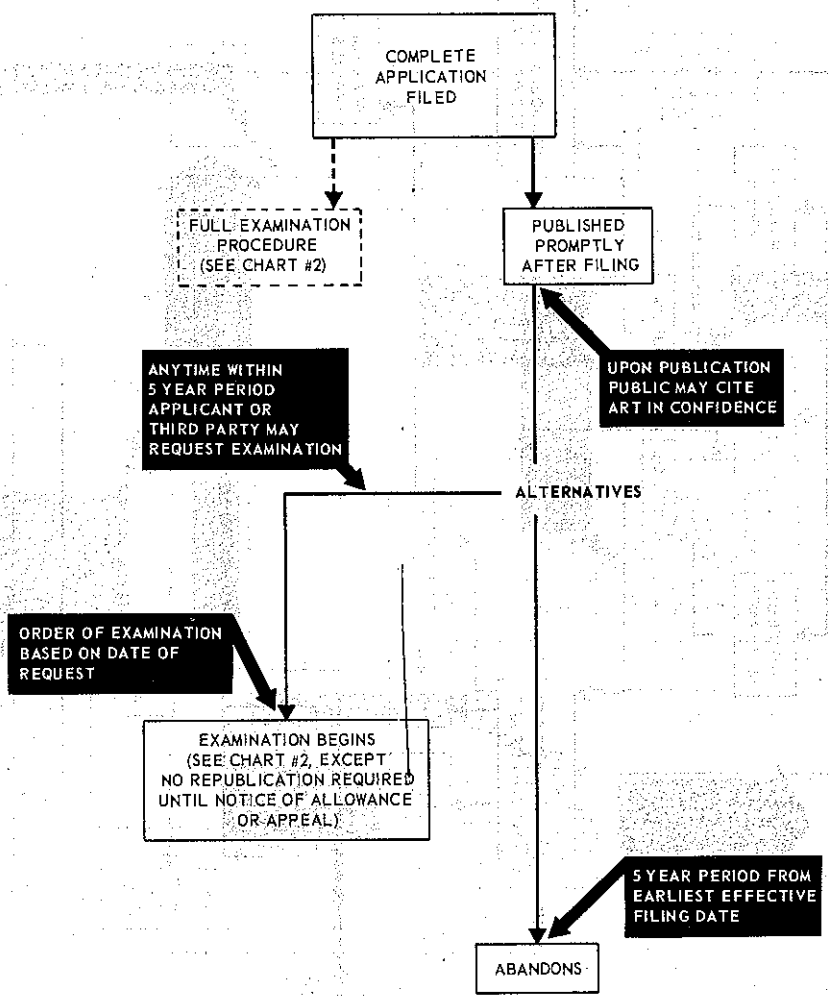


CHART 4

Review Of The Patent Office And Judicial Actions Initiated By A Patent Owner

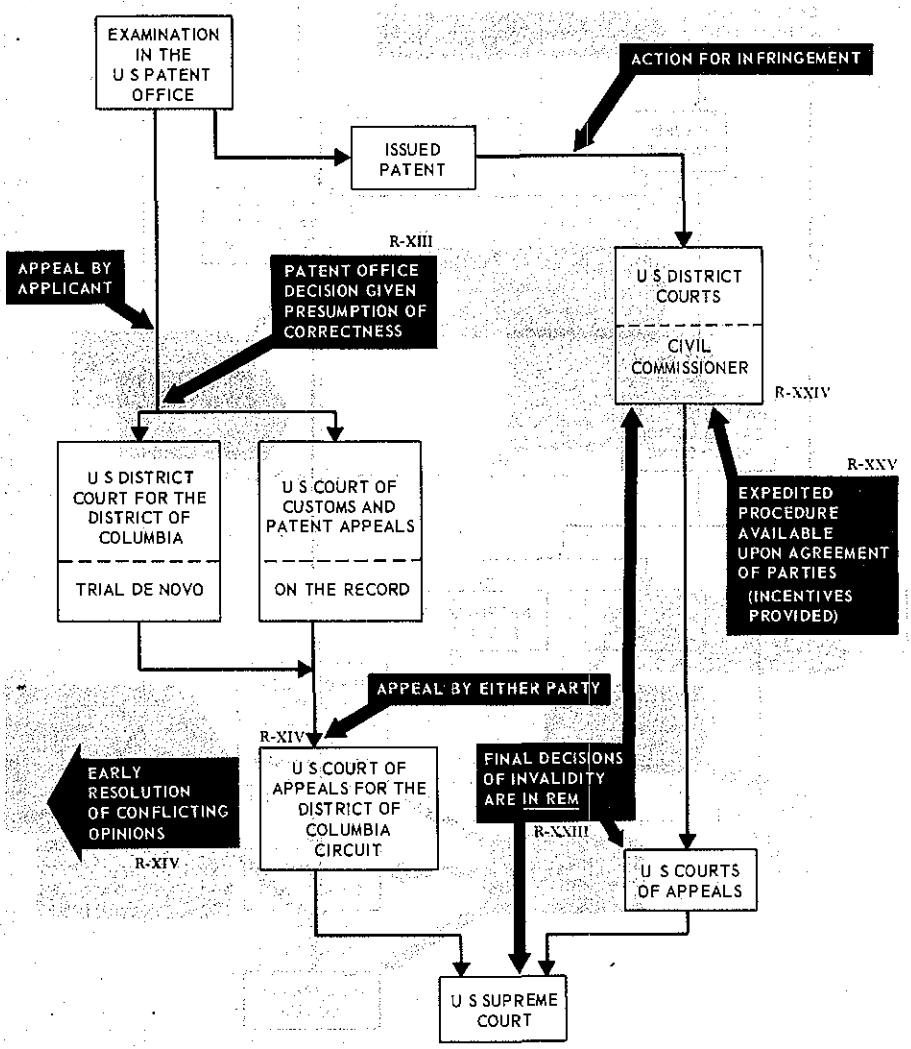


CHART 5

Administrative And Judicial Procedures
Initiated By The Public (Third Parties)

