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Maxine Singer

As a result of the International Conference on Recombinant DNA at Asilomar, California in February 1975, activities were initiated in every country in the world where scientific capability permits the experiments. These activities were, and are, directed toward providing assurance that potentially hazardous organisms, constructed by the new techniques, will not be inadvertently released. It is important to recognize that these activities are proceeding in the absence of any demonstration that hazardous organisms can indeed result from the experiments.

On June 23, 1976, Donald S. Fredrickson, Director, National Institutes of Health, announced publication of guidelines designed to eliminate or minimize any potentially hazardous consequences of recombinant DNA research. The guidelines were subsequently published in the Federal Register (Part II for 7 July 1976).

The promulgated guidelines were the result of a year and a half of intensive work by the NIH Recombinant DNA Molecule Program Advisory Committee as well as consideration by the Director of a variety of views expressed by individuals and private organizations either in writing or at a public hearing in February 1976. A summary of the history of the development of the guidelines as well as the various views expressed by many commentators is available in the Director's Decision Statement, which accompanied publication of the guidelines.

A Draft Environmental Impact Statement was prepared by the NIH, after determining that publication of the Guidelines was a "major federal action" as defined by the National Environmental Policy Act of 1969. The Draft

Statement was published in the Federal Register of September 9 1976, and circulated widely. Comments on the Draft Statement were received and extensively reviewed by the scientific and administrative staffs of the NIH. And the final Environmental Impact Statement is now being prepared.

For the purposes of the guidelines recombinant DNA experiments are defined as those involving 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 reproduce in some host cell, either autonomously or as an integrated part of the host's genome.

In the experiments discussed in the guidelines the host cells are generally single living cells, either microorganisms such as bacteria, or animal or plant cells grown as single cells in tissue culture.

The guidelines start with a statement of general principles and these are consistent with the conclusions published in the report of the Conference at Asilomar. The first principle is that there are certain experiments which, in the light of currently available information, may be judged to present potential hazards of so serious a nature, should they prove to be hazardous that they should not be attempted at this time. Second, a large group of feasible experiments appear to pose lesser or no potential hazard, and can therefore be performed provided that the information to be obtained, or the practical benefits anticipated, cannot be obtained by conventional methods, and provided that appropriate safeguards for containment of potentially hazardous organisms are incorporated into the design and execution of the experiment. Third, that the more serious the nature of any possible hazardous event, the more stringent should be the safeguards against escape

of the potentially hazardous agents. The safeguards should be at least as stringent as those generally used to handle the most hazardous parent of the recombinant. Since the estimation of potential hazards is conjectural and speculative, the levels of containment required for potentially hazardous organisms should be set high initially, and modified only when there is substantial relevant information to advise such modifications. And finally that the guidelines are to be reviewed at least annually in order to account for new information.

Containment Methods: Three approaches to the problem of containing potentially hazardous organisms form the basis of the recommended safeguards. Each of the three set up barriers to the dissemination of potentially hazardous organisms from the laboratory situation, and barriers between the laboratory worker and the organisms. Two of these approaches involve the limitation of the actual physical escape of the organisms, and are referred to as physical containment. The first such approach is the set of standard microbiological practices, that have been developed over a period of many years, and are widely used for handling pathogenic organisms both in research and clinical laboratories. In the hands of well trained personnel, these procedures have proven to be effective in safeguarding both the worker and the environment from the spread of many pathogenic agents. The second approach to physical containment involves the use of special kinds of equipment and facilities - to limit spread of aerosols, - for decontamination and containment of laboratory air and wastes, and for limitating access to laboratories. As with the standard microbiological techniques the type of equipment and facilities are not new, but were developed and have been used for containment

of known pathogenic organisms. There is documented experience on which to judge the efficacy of the various physical barriers in preventing the escape of organisms.

The guidelines go into some detail concerning the practices and facilities required for physical containment: four levels of physical containment are specified. They are termed P1, P2, P3 and P4 in the order of increasing levels of containment. P1, the lowest level, consists of the use of the standard microbiological practices mentioned before. The P2 and the next higher level P3, each require special procedures and facilities (including vertical laminar flow biological safety cabinets and laboratories maintained at lower air pressure than the surrounding building) all designed to limit to increasing extents any possible accidental escape of potentially hazardous organisms. Finally, P4, the maximum level of containment requires sophisticated and isolated facilities designed for maximum containment. Each of the levels, P2, through P4, assumes that the techniques demanded by P1, the standard microbiological practices, will be followed. Furthermore, for each level, relevant training of personnel is mandatory. The training is to include the nature of the potential hazards, the technical manipulations, and instruction in the biology of the organisms and systems. Specific emergency plans, to be used in case of accident, are required and serological monitoring, where appropriate, is to be provided.

The third approach to the problem of containing potentially hazardous organisms within the laboratory is the use of biological barriers. Biological containment is defined as the use of host cells and vectors with limited ability to survive outside of very special and fastidious laboratory conditions.

These conditions are unlikely to be encountered by escaped organisms in natural environments. Biological containment is an integral part of the experimental design, since the host and vector will need to be chosen, in any given experiment, with a view both to the purpose of the experiment and to containment. The guidelines stress that physical and biologic containment procedures are complementary to one another each one serving to control any possible failure in the other. The use of both, in a given experiment affords much higher levels of containment than either one alone. Therefore, the guidelines always recommend both a particular level of physical containment, and a level of biological containment for any given experiment.

The guidelines also recommend that publications describing work on recombinant DNA include a description of the containment procedures used. The guidelines then classify all likely experiments, rank them according to potential hazard and assign the required containment levels. The first class of experiments described in the guidelines are those which are prohibited. These are experiments which are judged to be of potentially very serious hazard, should they in fact be hazardous. The prohibited experiments include the following.

(1) Any experiment in which a portion of the recombinant DNA derives from either a highly pathogenic organism - an organism that causes serious disease in man or agriculturally important plants or animals - and classified as such by the Center for Disease Control, of the United States Public Health Service, or derives from cancer causing viruses classified by the National Cancer Institute as moderate risk. Perhaps I should stress here that these

viruses cause cancer in animals. There is no virus known to cause cancer in humans.

(2) Deliberate formation of recombinants containing the genes for toxins of very high toxicity. Examples of this class are botulinus toxin or diphtheria toxin, and venoms from insects and snakes.

(3) Deliberate creation from plant pathogens of recombinant DNAs that are likely to increase either the virulence of the pathogenic material or the range of species susceptible to the disease.

(4) Certain of the possible beneficial applications of DNA recombinant research involve the creation of organisms with the ability to carry out useful environmental functions. Release of such organisms into the environment may at some point be required to test their efficacy, and certainly to make use of them. Nevertheless, the guidelines state that deliberate release of any organism containing a recombinant DNA molecule is not to be undertaken at present.

(5) Transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.

(6) Finally experiments must be limited in scale to quantities of fluid less than 10 liters with recombinant DNAs known to make harmful products. The guidelines state that the Advisory Committee may make exceptions to this rule for particular experiments deemed to be of direct societal benefit if appropriate equipment is used.

The classification of permissible experiments is first made on the basis

of the host-vector system that will be used. Recognizing the relation between the host-vector system required by the experiment and the design of suitable biological containment, experiments using the same host-vector system are grouped together. Then the guidelines classify permissible experiments according to the best available estimates of potential risk. In the absence of much needed data, these estimates involve informed judgment in many instances. For example, not all recombinant DNA experiments yield novel combinations of DNA....recombination between the DNAs of organisms known to exchange genetic information in nature do not add uniquely man made cells to the biosphere. In these cases, the guidelines follow the principle that the experiments are to be carried out under conditions generally used to handle the most hazardous parent of the recombinant. When DNAs from species not known to exchange genetic material in nature are recombined, more stringent and strictly defined containment is required. Moreover, in many such experiments it is mandatory to use biologically contained agents that have been certified by NIH as unlikely either to propagate outside of rigorously defined laboratory environments or to transfer the recombined DNA to other cells. These agents include certain derivatives of a bacterial species called E. coli Strain K12. Strain K12 has been studied extensively for 30 years and can be readily manipulated for recombinant DNA experiments. This same extensive experience and ease of manipulation permits modification of E. coli K12 and its vectors by classical genetic techniques, for the purpose of establishing biological containment.

The nature and manner of achieving biological containment with this system is described in the guidelines. E. coli K12 appears to be harmless

itself, it does not usually either establish itself or multiply significantly in the normal bowel. These facts suggest that accidental injestion of a small number of bacteria by a laboratory worker would not result in extensive spread of the bacterium outside the laboratory. The normal situation may be altered when people are either taking antibiotics, or have certain abnormal digestive conditions and it is required that such individuals refrain from work for the duration of the abnormal situation. However, while E. coli K12 does not establish itself as a growing strain in normal bowels, it does remain alive during its passage through the tract. Therefore transfer of plasmid or bacteriophage vectors containing foreigh DNA from the original E. coli K12 host to bacteria resident in the intestines or bacteria encountered after excretion must be considered. For any given E. coli K12 host-plasmid combination used in a recombinant DNA experiment it will be necessary to assess the possibilities for transfer of the recombinant DNA in order to evaluate the degree of biological containment. While we are missing some relevant information, the available data suggest that the probability of transfer can be quite low, depending on the particular system.

The use of this bacteria has caused wide concern...and certain facts need to be emphasized. Only one strain of E. coli, called K12, is permitted by the guidelines. Strain K12 is one of a large group of bacterial strains, all of which are called E. coli because they share certain properties in common. But they do not all have identical properties. Some E. coli live normally in the intestines of healthy people and animals: others are pathogens, that is, disease producers. K12, which is rarely found in nature, and does not normally colonize human or animal intestines, is a greatly enfeebled strain

whose principle successful ecological niche is in the laboratories of molecular biologists and geneticists. It is not pathogenic. If it were, you and I would not be here worrying about this research, since all the molecular biologists would long since have disappeared. Pathogenicity is a complex phenomenon....dependent on several properties of the pathogen as well as on the properties of the species being infected. It is very unlikely that alterations in K12 brought about by insertion of recombined DNA will make it into a pathogen....but it is not impossible. It is this remote possibility with which we are all concerned. We are attempting to protect against an unlikely, uncertain, yet unacceptable event. Thirty years of studey of the genetic chemistry of E. coli strain K12 provides confidence that the capacity of these bacteria to escape and spread in the environment can be reduced to immeasurable levels. Thus, should pathogenic organisms arise, it is not likely they would survive to cause disease....nor is it likely that bacteria containing recombined DNA would survive to evolve in unique and fearsome ways. Nevertheless, because of K12s relation to common strains of E. coli, reservations about its use persist. It is important to investigate alternative organisms...and the Guidelines encourage this but it is not at all certain that useful and safer bacteria exist. Predictions about the existence of rare and fastidious organisms, unable to exchange DNA with bacteria inhabiting man or other living things, are highly speculative. Considering then the properties of E. coli K-12, as well as those of the certain known plasmid and bacteriophage vector, the guidelines conclude that, using such host-vector systems, recombinant DNAs are unlikely to be spread by the ingestion or dissemination of the few hundred or thousand bacteria,

such as might be involved in laboratory accidents, given standard microbiological practice. Therefore, these existing systems, are judged to offer a moderate level of biological containment and are defined as EK1, the lowest level of biological containment for experiments with E. coli systems.

As with physical containment levels, increasing numbers specify increasing levels of biological containment for E. coli systems. The next level is called EK2. EK2 host-vector combinations must be demonstrated to provide a high level of biological containment by suitable laboratory tests. They are obtained by genetic modification of either existing E. coli K12 host cells or the relevant vectors or both. More specifically, the guidelines state that in order to qualify as EK2 the modified system composed of derivatives of E. coli K12 combined with a particular vector should not permit survival of the vector in other than specially designed laboratory environments at a frequency greater than 10^{-8} (1 in 100 million). Various examples of the types of necessary modifications are suggested in the guidelines.

One additional level of contained E. coli host-vector systems is defined in the guidelines and is called EK3. EK3 systems are EK2 systems for which the specified containment properties have been demonstrated not only by microbiological and genetic anaylsis but by appropriate tests in animals including humans or primates and other relevant environments.

EK2 and EK3 host vector systems must be recommended for approval by the Recombinant DNA Program Advisory Committee and then certified by the Director, NIH before use. Detailed data on the relevant properties of the system must be submitted for consideration by the Committee. Thus far, several EK2 systems have been approved and certified. No EK3 systems have

been submitted for certification as yet.

Having ranked experiments according to potential hazard, and defined the several levels of physical containment and biological containment the guidelines then assign to each type of experiment both a required physical containment level, that is a P level, and a required biological containment level, that is an EK level and the particular combination of the two reflects the severity of the estimated potential hazard. The Guidelines are organized, for the E. coli systems, according to the source and nature of the foreign DNA. In general it is assumed that potential hazard increases with increasing relatedness of the foreign DNA to man. EXperiments in which the foreign DNA is highly purified, and does not contain harmful genes are assigned lower containment requirements than those in which the foreigh DNA is an unpurified mixture of fragments - the so-called shotgun experiments. Experiments involving such mixtures of DNA fragments are assumed to be of relatively higher potential hazard because of the greater likelihood of dangerous and unknown genes being introduced into a recipient cell compared to experiments with a single, highly purified fragment.

In addition to experiments using E. coli K12, the Guidelines specify containment conditions for certain other host-vector systems. Many recombinant DNA experiments will involve the use of systems in which the host cells are derived from animals or plants but are grown as single cells in the laboratory. Given the current state of technology, DNAs from animal viruses are most likely to be used as vectors in the near future. The cells themselves are fragile and fastidious and there is little or no chance that a living cell could escape from a laboratory in the way that an E. coli

cell might. Therefore containment considerations focus on the viruses since they might escape a laboratory in a viable form. There are two animal viruses whose DNAs are, now, technically useful as vectors; polyoma and simian virus 40 (SV40). In their respective normal hosts, mouse for polyoma, rhesus monkeys for SV40, neither virus causes a known disease. Polyoma does not infect human cells grown as single cells in the lab and also does not appear to infect humans, since humans exposed to polyoma do not produce antibodies. SV40 does infect both human cells grown as single cells in the laboratory, and whole human beings, as evidenced by the active production of antibodies and the reports of isolation of SV40 from humans. This virus contaminated the early Salk polio vaccines and millions of people were inadvertently inoculated with it in the middle 1950s. To date, there is no indication that the recipients of the vaccine suffered any related difficulty. Both polyoma and SV40 cause tumor formation in newborn small laboratory mammals. They are classified as low risk oncogenic viruses by the National Cancer Institute. Because SV40 infects human beings, and also because SV40 and related viruses have been isolated in connection with several human disease states, the proposed guidelines assume that polyoma inherently affords a higher level of biological containment: therefore more stringent physical containment is required for SV40 than for polyoma.

The guidelines require that the viral DNA used for recombination with a foreign DNA must itself be defective - in a manner analogous to the EK2 systems. Physical containment conditions are specified, and these depend, as before, on the nature of the "foreign" DNA fragment to be spliced onto the viral DNA. Experiments with polyoma and SV40 are restricted to P3 and P4 levels only.

The Guidelines also contain recommendations for experiments in which plant cells will serve as hosts for recombinant DNA. The cells might be single plant cells grown under laboratory conditions, or seedlings, plant parts, or small whole plants. This is in fact the only instance where the guidelines address the question of recombinant DNA experiments with whole organisms. Directions are given for modification of the specifications for P1, P2, and P3 physical containment in order to provide conditions appropriate for work with plants.

Implementation of the Guidelines. The guidelines contain a large section defining the roles and responsibilities of individuals and institutions in assuring compliance with required containment levels. The procedures, as described, are primarily directed at grantees of the National Institutes of Health. Similar procedures are in force for work carried within the NIH laboratories themselves, and for work carried out under contract arrangements with the NIH.

The principle investigator is required to assess any potential biohazards, to institute appropriate safeguards and procedures, to minimize effects of possible accidents by planning, to train and inform all personnel, to report any serious or extended illness of a worker or any accidents, and all of these must be carried out on a continuing basis. Thus, the primary responsibility for conducting experiments according to the guidelines is in his hands. Further, in applying for grants to carry out experiments with recombinant DNA, the investigator must include an estimate of the potential biohazards as well as a statement as to the containment procedures that will be used. The application must include certification as to the existence and availability of appropriate facilities, procedures, and training. The

guidelines indicate that institutions in which recombinant DNA experiments are carried out must establish biohazard committees which can serve to examine equipment and facilities and certify their compliance with the requirements. Such committees will also serve as a source of advice and reference on physical containment facilities, on properties of biological containment, and on training of personnel.

According to the proposed guidelines review of the certification and of the investigators judgment concerning the extent of potential hazard and the required containment would be by NIH study sections, during the normal scientific review of the application. After approval, but before work is initiated, the investigator must formalize his commitment to abide by the Guidelines by submitting to the NIH a memorandum of understanding. Both the investigator and a responsible official of his institution must sign this document.

Failure, on the part of an investigator, to comply with the provisions of the guidelines, may result in cancellation of his research grant by the NIH. The NIH has no statutory regulatory authority and is thus limited in the sanctions it can use.

Application of the guidelines to work not supported by the National Institutes of Health. Several agencies of the U.S. government other than the National Institutes of Health provide support for biological and medical research and are currently, or may in the future, sponsor recombinant DNA experiments. All of these agencies have now adopted the NIH Guidelines and will apply them to all research under their sponsorship. These include the National Science Foundation, the Energy Research and Development Administration, the Department of Defense, the Department of Agriculture, and

the National Aeronautics, Space Administration. At present there are no mechanisms except voluntary ones for extending the provisions of the guidelines to work supported by private funds - either for research or commercial purposes or to work supported by state and local funds. Both the executive and legislative branches of the federal government as well as concerned private individuals and organizations have recognized the urgent need to establish such a mechanism.

A federal Interagency committee, chaired by the Director of NIH, and formed at the request of the President is at work. Both research and regulatory agencies are involved. The Committee's charge was to determine whether existing powers within the Federal regulatory agencies, are sufficient to extend control to the non-federal sector....to formulate recommendations as to how this may best be done, and to recommend legislation should that be deemed necessary. The Committee has not yet completed its work, but I can report to you on its progress to date. A careful examination of existing regulatory authorities within the EPA, the OSHA, the Center for Disease Control, and the FDA has been made. It is probable that none of the existing authorities can properly cover the particular requirements of the recombinant DNA situation. The NIH itself has no regulatory authority. Thus the Interagency Committee may conclude that new legislation will be required. In that instance an appropriate legislative framework will need to be devised. The various concerned Federal agencies may be expected to consult with their particular constituencies for advice on ways to proceed - the Department of Commerce with interested industry - the Department of Labor with Unions, the Department of Agriculture with agricultural interests and the NIH with scientific community.

A high level of interest in the Congress is stimulating the Interagency Committee to prompt resolution of this problem: we may therefore expect action to extend the applicability of the guidelines - or some modification of them - to all privately or locally sponsored work with recombinant DNA in this country.