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THE MANAGEMENT AND ASSESSMENT OF RISKS FROM RECOMBINANT ORGANISMS

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Summary

This paper describes the untraditional path followed to develop risk management policies for recombinant DNA research in the U.S.A. It deviated from the usual three stages of research, risk assessment and risk management through which a risk management policy commonly evolves. Almost immediately after the laboratory technique had been developed, restrictions were instituted; this step preceded the research and assessment stages. The paper also describes some of the technical and institutional factors that have influenced this policy.

Introduction

For most hazardous materials, the process leading to a risk management policy progresses through three stages — research, risk assessment and risk management. Elements of each stage are shown in Fig. 1, from a report by the National Academy of Sciences. Through this traditional sequence, a hazard is identified and the risk is characterized based on empirical evidence. These results are then used to support the development of a risk management policy.

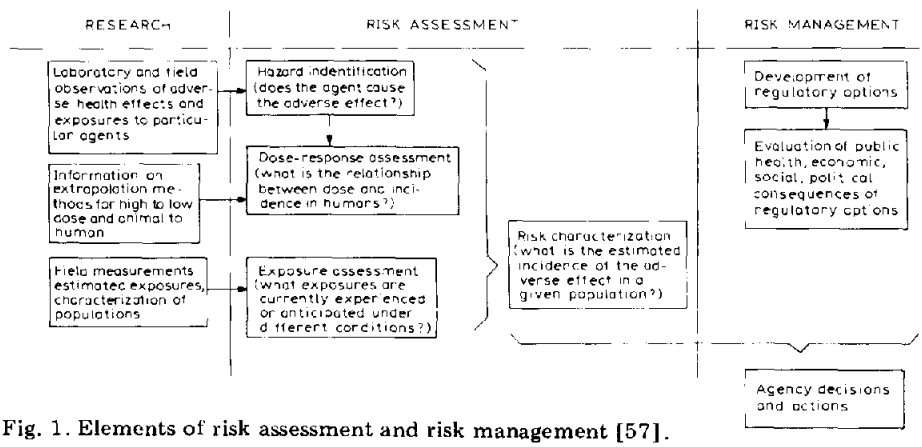

Fig. 1. Elements of risk assessment and risk management [57].

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The process that led to recombinant DNA policy, however, followed an untraditional path. Almost immediately after the development of the recombinant DNA technique, restrictions were introduced to control potential risks. Thus, a risk management policy was formalized before supporting research and risk assessment were performed. This paper recounts recombinant DNA's unusual policy history and examines some of the contributing technical and institutional factors.

DNA and the recombinant DNA technique

Deoxyribonucleic acid, or DNA, is the genetic material in all living cells. It is a macromolecule composed of four aromatic bases attached to a sugar–phosphate backbone. A DNA molecule generally assumes a double-stranded, helical structure. The stability of this configuration is due to hydrogen bonding between each aromatic base and a complementary aromatic base on the other strand.

The genetic information for a cell is encoded in the sequence of nucleotides, or base–sugar–phosphate monomers, in the DNA. The flow of this genetic information is depicted in Fig. 2. As the arrows indicate, DNA serves as the template for its own replication. In addition, DNA serves as the template for the nucleic acid ribonucleic acid, or RNA, which in turn determines what proteins are manufactured. Proteins are the functional and structural components of the cell; their varied roles include catalyzing the chemical reactions of metabolism and effecting the transport of material across the cell boundary. It is the genetic information in the DNA, however, that specifies the properties of each protein.

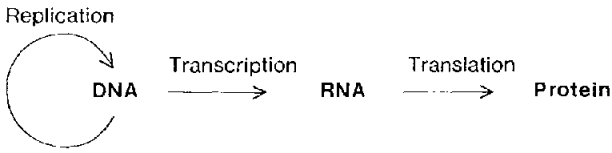

Fig. 2. The flow of genetic information.

Because of the primary importance of DNA in living cells, modifications of the nucleotide sequence in this molecule can lead to substantial changes in the functional or structural characteristics of an organism. The recombinant DNA technique is a powerful tool because it enables microbiologists to modify DNA *in vitro* and re-incorporate this altered genetic material into living cells.

With the recombinant DNA technique, a piece of donor DNA is linked to a piece of vector DNA and of this hybrid molecule is introduced into a host cell. The linking of the donor DNA, which may be a fragment from any organism, and the vector, which can be a plasmid or a bacteriophage, is

accomplished with the aid of enzymes. An example of the recombinant DNA technique is depicted in Fig. 3. Detailed descriptions of recombinant DNA techniques can be found in Refs. [1] and [2].

Bacteria containing recombinant DNA can produce DNA from an unrelated donor organism. Bacterial cells are used as hosts because they multiply much faster than higher organisms, they are inexpensive to maintain, and they occupy very little space.

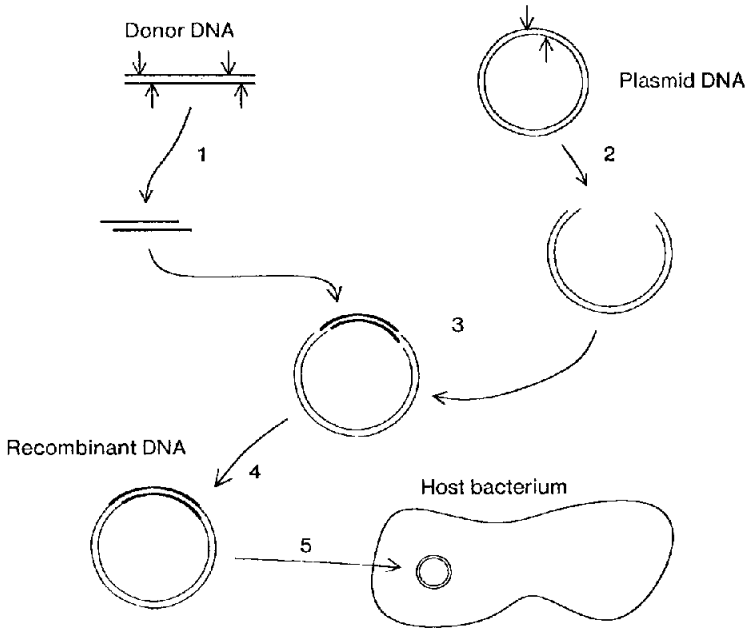

Fig. 3. The recombinant DNA technique.
1. Digestion of donor DNA with a restriction enzyme makes a staggered break at a specific nucleotide sequence and produces DNA fragments with cohesive ends.
2. Digestion of a plasmid vector with the same restriction enzyme produces a DNA vector with cohesive ends.
3. Hydrogen bonding between complementary bases on the cohesive ends leads to coupling between the donor DNA and vector.
4. Nicks in the DNA chain are sealed with the enzyme DNA ligase.
5. The recombinant DNA molecule is inserted into a bacterium host cell where it is replicated.

Recombinant DNA was first developed for basic research, and it has proved to be a powerful tool for probing the structure and function of genes. Recently, however, some commercial applications have become feasible as well. Recombinant DNA techniques provide a way to alter the genetic material in bacteria to include DNA segments that code for valuable protein products. If appropriate DNA vectors are employed, the host cells can be induced to express the foreign DNA. Insulin, interferon, and growth hormone

are examples of pharmaceutical products that can be produced with recombinant DNA technology. Near-term commercial applications are also anticipated for the chemical and foodstuffs industries. In addition, applications of genetically modified organisms in the open environment have been proposed, particularly in agriculture, mining, oil recovery and pollution control. A review of the potential applications of the recombinant DNA technique can be found in Ref. [3].

Potential risks from recombinant organisms

Although subsequent events have focused attention on the potential benefits of recombinant DNA, potential risks dominated discussions of the technique in the mid-1970s. A number of disaster scenarios, which hypothesized damage to human health after an inadvertent release of modified microorganisms, were proposed. A detailed discussion of recombinant DNA disaster scenarios can be found in Ref. [4].

There was particular concern that novel human pathogens might be created because the bacterial cell *Escherichia coli* was chosen as the first host to replicate hybrid genetic material. Although *E. coli* has been a laboratory organism for many years, it was originally isolated from the human intestine. Because of this, it was feared that laboratory-grown *E. coli* containing recombinant DNA might somehow become established in the human intestine. If the modified *E. coli* were harmful, the result could be a new communicable disease. There was also concern that victims of pathogenic recombinant organisms might be more difficult to treat than victims of natural bacterial infections because vectors that impart antibiotic resistance are often used in recombinant DNA experiments.

The first recombinant DNA scenario to be hypothesized concerned an epidemic of contagious cancer. This scenario was suggested in response to a proposed experiment to replicate the animal tumor virus SV40 in *E. coli*. Although the mechanism of oncogeny for animal tumor viruses was not well understood, injections of SV40 into mice and hamsters had been shown to cause cancer. In addition, human cells grown in tissue culture had been transformed into cancerous cells by SV40. Given this background, the following series of conjectures was presented.

- *E. coli* containing SV40 might be inadvertently ingested by laboratory workers.
- The recombinant organisms might become established in the intestines of the human population.
- The natural defense systems that have evolved to combat cancer might be circumvented by the unusual exposure route to the animal tumor virus.
- An epidemic of contagious cancer might ensue.

Because of the uncertainties at each step in this scenario, it was difficult to provide an absolute refutation. The disaster scenario was believed plausible enough to justify postponing the original controversial experiment [4].

Similar scenarios were hypothesized in response to proposed "shotgun" recombinant DNA experiments, which involve bacterial cloning of random DNA pieces from higher animals. It had been determined that viral DNA, such as animal tumor viruses, can insert itself into the animal genome. This raised concern that tumor virus DNA might be closed inadvertently in shotgun experiments. Following the line of conjecture outlined above, this might lead to a cancer epidemic.

The possibility of a new form of contagious cancer, however, was not the only harmful effect from recombinant DNA that was discussed. There were also disaster scenarios concerning DNA that is expressed as biological toxins, such as botulin or snake venom. If:

- DNA coding for the formation of a toxin were inserted in *E. coli*, and
- the modified *E. coli* became established in the human population, and
- the foreign DNA were to be expressed, then a deadly epidemic might result from the modified bacteria.

Disaster scenarios involving bacteria that are intentionally modified to produce valuable human proteins, such as insulin, were also postulated. These scenarios assume as a premise that an organism containing recombinant DNA becomes established in the human population. In one such scenario, metabolic imbalances might result from the unregulated production of a protein that is not recognized by the human immune system. In another scenario, the production of a protein that is similar, but not identical, to a human protein might trigger an immune response against the native form as well as the foreign protein, inducing autoimmune disease.

In a decade of recombinant DNA research activities, none of the hypothetical disaster scenarios has materialized. Literature reviews describing thousands of laboratory-associated infections involving traditional biological hazards [5, 6], however, have been cited to support arguments that any pathogenic recombinant organisms created in the laboratory would pose a threat to human health despite the laboratory safety measures that are typically employed [7]. In general, infections from traditional biological hazards have been attributed to types of accidents and laboratory conditions that could also occur during recombinant DNA operations.

Analyses of laboratory-associated infections involving traditional biological hazards do not, however, provide much support for concern that members of the general population would be likely victims of a pathogenic recombinant DNA laboratory organism. Laboratory workers themselves are the most frequent victims of infections from laboratory agents, followed by family members or visitors who had direct contact with a laboratory worker. While there have been a few reported instances of laboratory-attributed infections in persons not directly associated with either a laboratory or an earlier victim, contact with laboratory laundry or refuse generally can be inferred in these incidents [8].

Although early concerns were largely limited to health issues, the potential hazards of recombinant DNA in the environment have recently received

considerable attention. This shift is in response to some proposed agricultural applications of recombinant DNA that involve the intentional dissemination of organisms [9]. The cases of the gypsy moth, kudzu, chestnut blight, and other incidents where the introduction of a novel organism had spectacular and unpredicted effects are being cited in discussions of potential adverse effects of genetically engineered organisms [10], much as accounts of laboratory-associated infections were raised when attention centered on health effects.

Recombinant DNA policy

The roots of recombinant DNA as a policy issue can be traced to the 1973 Gordon Research Conference on Nucleic Acids where it was disclosed that a group of microbiologists had demonstrated the *in vivo* replication of hybrid DNA plasmids that had been constructed *in vitro* [11]. Some conference participants, troubled by the potential health impacts of this powerful new technique, requested a special conference session to consider these concerns. The outcome was a letter to the National Academy of Sciences that suggested the establishment of "a study committee to consider this problem and to recommend specific action on guidelines, should that seem appropriate" [12].

This letter to the National Academy of Sciences triggered a series of events that led to the codification of Guidelines for Research Involving Recombinant DNA Molecules [13]. These Guidelines are issued by the National Institutes of Health, and have become *de facto* regulations for institutions that are dependent upon NIH or other government funding. Although there are no enforcement provisions beyond the withdrawal of funding, the NIH Guidelines are also the generally accepted standard for privately funded work with recombinant DNA organisms.

TABLE 1

Recombinant DNA policy chronology

1973	Recombinant DNA technique introduced
1974	Voluntary moratorium proposed by NAS committee
1975	Voluntary guidelines adopted at Asilomar
1976	Original NIH Guidelines for research adopted
1977	First local ordinances passed to regulate RDNA research
	U.S. Congress considers RDNA legislation
1978	NIH Guidelines revised and relaxed
1980	NIH Guidelines revised and further relaxed
	NIH issues Recommendations for Large-Scale Work
1981	NIH Guidelines revised and further relaxed
	Several local ordinances passed to regulate RDNA technology
1982	NIH Guidelines revised and further relaxed
1983	NIH Guidelines revised and further relaxed
	EPA considers applicability of TSCA to biotechnology products
1984	Cabinet-level group explores federal role in biotechnology

Table 1 lists key events in the history of recombinant DNA policy in the U.S. Two important trends that are apparent in this outline are:

- the early shift from self-regulation by the scientific community to government oversight, and
- the progressive relaxation of the NIH Guidelines.

The timing of these trends, compared with the development of recombinant DNA technology, make recombinant DNA an unusual science policy case study. Whereas risk management strategies are generally applied to technologies that have demonstrated harm, the initial NIH Recombinant DNA Guidelines were instituted before the technique was widely practiced, to address potential risks. As use of the technique spread, the Guidelines were relaxed.

Ostensibly, the progressive relaxation of the Guidelines since the original 1976 version has been based on additional information about the potential risks posed by recombinant DNA. Although there have been important developments in molecular genetics, a review of risk assessment studies reveals that many of the original concerns remain unresolved. It has been recognized, however, that the remaining concerns are not specific to recombinant DNA. Thus, a third policy trend that can be observed in Table 1 is a broadening of scope from recombinant DNA to biotechnology in general.

Some details of the evolution of recombinant DNA policy are recounted below.

Early recombinant DNA policy

Because of the great uncertainties involved in this work and the perceived potential for catastrophic harm, temporary restrictions on the use of the recombinant DNA technique were proposed by the National Academy of Sciences committee that was established in response to the letter of concern from the Gordon Research Conference. The committee's report called for a voluntary deferment of two types of experiment that were perceived as having the greatest potential for harm. These experiments included:

- the construction of autonomously replicating bacterial plasmids that might provide a bacterial strain with toxin formation or antibiotic resistance powers that it did not naturally possess, and
- the construction of autonomously replicating DNA that would introduce animal viruses into bacteria.

In addition to deferring these two types of experiment, investigators were advised to "carefully weigh" any plans to create hybrids of animal DNA and vectors to transmit them into bacterial cells [14].

The suggested limitations on research activity in the National Academy of Sciences committee report addressed specific activities and concerns of the microbiologists working at that time. In particular, the limitations regarding animal and animal viral DNA reflect the context of research interest in the possible links between animal tumor viruses and cancer. This work had been shadowed by concern that the DNA from such viruses might endanger the health of researchers [15].

In retrospect, one committee member has described the National Academy of Sciences committee report as "a statement of conscience but not of conviction" [16], suggesting that potential risks were overstated. By publicizing the voluntary moratorium in the journals *Science*, *Nature* and *Proceedings of the National Academy of Sciences*, however, the members of the committee aroused general concern about recombinant DNA. Regulators and the public became interested in the outcome of the international conference at Asilomar, California, where microbiologists were to review the voluntary moratorium and decide what actions, if any, might be appropriate to address the potential health hazards posed by recombinant DNA.

The Asilomar conference produced a set of guidelines that replaced the recommendations of the National Academy of Sciences report with a detailed list of recommended levels of physical and biological containment for specific types of experiments involving recombinant DNA [17]. Physical containment conditions included laboratory practices, containment equipment, and special laboratory design features, with the goal of limiting the exposure of workers, the general public, and the environment. The complementary biological containment provisions included vectors and host organisms with an impaired capacity to survive outside the laboratory.

The Asilomar statement was an attempt to match levels of potential risks with levels of containment. Because the risks were unquantified, containment level assignments were based on subjective estimates. For example, the experiments that were believed to present the greatest risk were designated "experiments to be deferred", a category that included the cloning of DNA from highly pathogenic organisms as well as work with more than 10 liters of any recombinant DNA culture. Shotgun experiments with animal DNA that might contain animal viral genomes were designated as "moderate risk" experiments that could be performed only under restrictive physical and biological containment conditions. Low containment conditions were deem-

TABLE 2

NIH physical containment levels for RDNA research

P1	Facility personnel observe good laboratory practices. Biological wastes are decontaminated before disposal. No special equipment or engineering features are required.
P2	Facility personnel wear laboratory coats. Posted biohazards sign. No public access. Autoclave in building. Safety cabinet present for aerosol-producing equipment.
P3	Facility personnel wear gloves. Class I or II safety cabinet required for aerosol-producing organism manipulations. Negative air pressure. Laboratory clothes and gloves decontaminated before disposal.
P4	Facility personnel change clothing before entering or leaving facility. All organism manipulations are performed in glove box. All air decontaminated before exhaust. Autoclave in room. Air locks. Shower room.

ed appropriate for the cloning of DNA for which no adverse effects could be envisioned.

The NIH Guidelines

A contemporary newspaper report quotes an opponent of the Asilomar Final Statement as saying, "Any guidelines passed by the Asilomar conference will be taken as Holy Writ by bureaucrats in Washington" [18]. Government policy makers did observe the conference and the NIH issued similar Guidelines for Recombinant DNA Research in 1976 [13]. Like the Asilomar guidelines, the NIH Guidelines describe hierarchies of both physical and biological containment. Table 2 shows some of the specifications that describe each of the four physical containment levels.

The biological containment levels were originally restricted to *E. coli* systems and were designated as follows:

- EK1, for a host—vector system composed of a weak laboratory strain of *E. coli* and a corresponding vector;
- EK2, for a host—vector system that has been intentionally disabled, so that the survival of organisms containing recombinant DNA released from laboratory conditions would be less than 10^{-8}; and
- EK3, for an EK2 system whose containment properties have been validated in humans or primates.

In revised versions of the NIH Guidelines, biological containment levels with "HV" designations have been added to the original "EK" levels as other "host—vector" systems received certification.

The 1976 Guidelines were based on the premise that all recombinants were potentially harmful, because the possibility of harm could not be properly evaluated [19]. No experiments were considered to be exempt from the Guidelines. This conservative position was adopted with the understanding that the Guidelines would be revised when more information became available.

Since 1976, several revisions of the Guidelines have been made. The changes include:

- the introduction of exemptions to the Guidelines, so that most recombinant DNA research is no longer subject to the NIH rules [20];
- a general lowering of the required containment levels for specific experiments that remain covered by the guidelines;
- the addition of alternative allowable combinations of physical and biological safeguards, which permit an experiment to be performed with containment equipment that is one step lower than specified in the guidelines if the corresponding biological containment level is raised one step; and
- a reduction in the direct role of the NIH and a shift of the primary authority for reviewing recombinant DNA work to local Institutional Biosafety Committees.

As set forth in the 1977 Environmental Impact Statement, the NIH Guidelines were developed "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" [21]. Physical and biological containment specifications were intended to provide this protection directly by limiting the quantity and survival of recombinant organisms released. It was also suggested that the Guidelines would reduce the potential risk from recombinant DNA research by three indirect means:

- Because only a small number of facilities can meet stringent P4 requirements, it was anticipated that the Guidelines would reduce the number of "high-risk" experiments performed.
- By providing laboratory workers with information about safety procedures and potential hazards, it was anticipated that the guidelines would improve general safety performance.
- By requiring that significant illnesses and accidents be reported to the NIH, it was believed the Guidelines would provide a database that could be used to identify unforeseen hazards [21].

With hindsight, however, it appears that these secondary impacts may have been overstated. Demand to use existing P4 facilities has not been high, which suggests that high-risk experimentation has not been significantly inhibited by the limited number of facilities. In reference to expectations of improved safety awareness and safety procedures, there is anecdotal evidence that provisions of the Guidelines may be frequently ignored [22, 23], this lends support to a suggestion that workers who are not convinced that recombinant DNA presents a hazard may not be motivated to learn and follow inconvenient safety procedures [24]. Finally, with respect to the database of significant illnesses and accidents, only a handful of investigators have judged incidents in their own facilities to be of enough significance to report [25].

The original focus of the NIH Guidelines was research; until 1980, activities that involved more than 10 liters of recombinant DNA culture were prohibited. When this prohibition was removed, the NIH Recombinant DNA Advisory Committee (RAC) issued Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules [26]. Although the NIH has no enforcement authority over industrial users, this voluntary safety standard is believed to have received wide acceptance.

Because the NIH Guidelines for Research and Recommendations for Large-Scale Uses were designed to address the risks from inadvertent releases of recombinant DNA, containment is a central theme. Since a 1982 revision of the Guidelines, however, experiments involving the deliberate release of recombinant organisms are no longer prohibited [9]. The NIH has chosen to review proposals for deliberate environmental releases on an individual case basis, rather than develop specific guidelines for managing the risks of this work.

Other policy initiatives

Public pressure for government control of recombinant DNA laboratory activities peaked in 1977. In response to this concern, sixteen bills were introduced in the 95th Congress [27]. The drive to enact federal recombinant DNA legislation subsided without the passage of a bill, however, in response to arguments that the perceived risks of recombinant DNA were unjustified [28].

Although the U.S. Congress failed to pass recombinant DNA legislation, the public concern that motivated the discussions in the 95th Congress led to some action at the state and local levels. Cambridge, MA, Berkeley, CA, Princeton, NJ, and Amherst, MA, as well as the states of New York and Maryland, passed laws that essentially correspond to the NIH Guidelines [29]. In 1981, there was another flurry of legislative activity at the local level when several communities enacted legislation in anticipation of commercial scale recombinant DNA operations. The laws that were enacted in this second wave are based upon the NIH Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules [30].

Special legislation is not necessarily required to regulate recombinant DNA at the federal level. It has been suggested that recombinant organisms, as well as other biotechnology products, could be subject to the regulatory mechanisms by which the EPA regulates conventional chemicals. If this view is upheld, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) could be applied to biotechnology applications related to pesticides, and EPA could use its authority under the Toxic Substance Control Act (TSCA) to consider most other biotechnology products. A cabinet-level Working Group on Biotechnology that has recently been formed will consider the appropriate division of jurisdiction between federal agencies [31].

EPA's approach to recombinant DNA policy would differ from that of NIH in two important respects. First, as a regulatory agency, EPA could require industrial compliance with its decisions. In addition, EPA's broad interest in biotechnology would mean that recombinant organisms would no longer be subject to more stringent risk management considerations than products of other genetic manipulation techniques.

Despite these differences, it is likely that EPA policy for research and industrial applications of biotechnology would utilize risk abatement strategies that are similar to the NIH approach. The tools that have been used by the NIH — prohibiting a specific recombinant DNA activity or requiring combinations of physical and biological containment — are control techniques that can be effectively employed without detailed knowledge of the mechanisms of potential adverse effects.

In a general framework for risk analysis, risks result from combinations of environmental processes, exposure processes and effects processes. With respect to this framework, risk abatement can be approached by modifying the environment, modifying exposure processes, modifying effects pro-

cesses, or compensating for effects that occur [32]. The recombinant DNA risk management strategies described above utilize three of these four approaches.

- Deferring certain types of recombinant DNA experiments *modifies the environment* by entirely eliminating certain types of potential hazard.
- Adopting physical containment strategies *modifies exposure processes* by influencing the release of recombinant organisms to humans of the environment.
- Implementing biological containment *modifies effects processes* by influencing the probability that recombinant organisms will survive and become established.

Only the final general approach to risk abatement — *compensating for effects* — has not been used to address the risks of recombinant DNA. Instead, emphasis has been on preventive strategies.

Potential risks associated with the deliberate release of modified organisms to the environment cannot be controlled, however, with the preventative strategies that have been developed for research and manufacturing activities. Dispersal and survival of organisms in the open environment is necessary for the success of environmental applications of modified organisms; thus, the intermediate levels of risk management that rely on containment options within designated facilities are not appropriate. Without containment options or means of compensating for any potential adverse effects, risk management decisions are essentially reduced to yes/no decisions.

No formal guidelines for making these difficult decisions now exist, although there will likely be an increasing number of proposals for environmental releases of genetically modified organisms. It is apparent that careful risk assessments will be particularly important for these decisions, as the history of novel species introductions includes a number of cases where there have been unpredicted harmful ecological effects [10]. Some research needs to support EPA's activity in this area have been identified [33].

Recombinant DNA risk assessment

When guidelines for recombinant DNA activities were originally developed, there was no history on which to base an assessment of the risks from recombinant organisms. There was also little information available to quantify the effectiveness of the physical and biological containment strategies that could be used to control risks. The research elements that support risk assessment and risk management conclusions in the National Academy of Sciences scheme (Fig. 1) did not exist as empirical studies, but as conjectures.

A formal assessment of the risks from the inadvertent release of recombinant organisms must evaluate each of three factors:

- the potential for recombinant organisms to be released to a laboratory worker or the outside environment,

- the potential for recombinant organisms to survive and become established in environmental niches outside the designated processing facility, and
- the potential for adverse effects from recombinant organisms.

As the initial NIH proposal for a plan to assess the risks of recombinant DNA research notes, "the vast majority of information relevant to recombinant DNA risk analysis has already come from research not primarily designed to provide information on risk" [34]. Specific risk assessment experiments have been undertaken to address only a few concerns. This section reviews studies that have provided some insight into recombinant DNA containment and risk, and identifies some of the issues that remain unresolved.

Recombinant organism release

The initiating event in recombinant DNA disaster scenarios is the release of recombinant organisms to the environment. When recombinant DNA activities were limited to research, the concern was inadvertant release from a designated containment facility.

As part of studies addressing the overall risks of recombinant DNA research, a few attempts have been made to estimate recombinant DNA escape under containment conditions specified in the Guidelines [35—39]. It should be noted that these values, which have been compiled in Table 3, are not based upon actual measurements. The values for the lower levels of containment are presented with little support (e.g., Curtiss labels his values "rough estimates" [36]), while the two estimates for maximum containment facili-

TABLE 3

Estimates of organism releases

Physical containment level	Organisms per investigator per year	Means of release	Ref.
P0[a]	10^4—10^6	Ingestion from mouth pipetting	37
P1	$\sim 10^7$	Contamination of investigator via clothing, ingestion, inhalation; ventilation exhaust, drain disposal, floor sweepings	35
P2	$\sim 10^4$		
P3	$\sim 10^1$		
P4[b]	10^{-1}—10^{2}[c]	Contamination of investigator via clothing, inhalation; waste disposal	39
P4	10^2—10^5[c]	Personnel contact, liquid waste, air ventilation, catastrophe	38

[a]Because mouth pipetting indicates that basic P1 laboratory practices are not observed, the laboratory conditions in this paper have been considered to be P0.
[b]This British study evaluated a facility that is essentially equivalent to P4 containment under the NIH Guidelines.
[c]For purposes of comparison, a "significant release" has been assumed to equal 10^3—10^6 organisms. To put this assumption in perspective, a single milliliter of culture may contain $>10^9$ organisms.

ties resulted from fault-tree analyses based on hypothetical component failure rates.

More recently, estimates of recombinant organism release have been calculated with a model that simulates dispersal, transport and survival processes [40, 41]. Empirical data for initial localized organism dispersals during laboratory operations supports the model [42, 43]. In addition, the effect of the variability of the human operator is incorporated in simulations; this is important because it has been recognized that:

- the laboratory worker has a major influence on the success of biological safety and environmental control programs [44], and
- the physical containment provisions that are specified for recombinant DNA facilities can be subverted through operator carelessness or accidents, particularly at the P1 through P3 levels [24].

Simulation results indicate that technical details of a biotechnology protocol may influence operator and environmental exposure to viable recombinant organisms by as much as several orders of magnitude. In addition, operator quality effects may overwhelm distinctions between nominal containment levels.

Ideally, direct measurements of recombinant organisms should be used to improve estimates of recombinant DNA release. Such measurements could be used to validate the model discussed above. Improved techniques for monitoring and detection could also be used to improve risk assessment for deliberate environmental release experiments, by providing better information on organism dispersal.

Recombinant organism survival and establishment

After organism release, the potential for recombinant organism survival and establishment is the next factor that should be included in a risk assessment. Because certain strains of *E. coli* easily colonize the human intestine, the initial choice of *E. coli* as a recombinant host motivated attempts to quantify the survival and establishment properties of *E. coli* K12, a standard laboratory strain that was designated an EK1 host, and *E. coli* χ1776, the first disabled strain that was engineered to meet the NIH specifications for EK2 biological containment.

Fears that recombinant *E. coli* would become an epidemic pathogen were largely allayed, however, after a 1977 Risk Assessment Workshop at Falmouth, MA [45]. Studies discussed at the conference were cited as support for the following statements:

- *E. coli* K12 appears not to show long term establishment in the human intestine.
- It is not likely that *E. coli* K12 will be able to transfer recombinant DNA to "wild" strains in the human intestine.
- Even if DNA that codes for known virulence factors is inserted into *E. coli* K12, the resulting bacteria do not appear to be pathogenic.

The summary statement containing these conclusions was widely distributed.

Subsequent reports, however, raised some doubt about the strength of each of these conclusions. For example, experiments with a larger number of volunteers than the original study indicated that *E. coli* K12 may survive in the human intestine for many more days than was first estimated [46]. Revisions in estimates of *E. coli* K12 survival time and the identification of additional plasmids with the ability to assist the transfer of recombinant DNA between organisms affected estimates of the probability that a recombinant DNA plasmid will be transferred to a wild host [46]. In addition, some experiments were revealed in which the addition of virulence plasmids to *E. coli* K12 had created a bacterial strain that became sufficiently established to demonstrate pathogenicity in an animal host [4].

Early beliefs that disabled *E. coli* χ1776 could not survive in the human intestine, which were based on experiments with germ-free mice [45], have also been revised in light of additional studies. It has been shown that, although χ1776 alone appears unable to survive in the human intestine, χ1776 may survive after the addition of a plasmid [47].

To supplement the studies of *E. coli* survival and establishment in human and animal intestines, Chatigny et al. [42], at the Naval Biosciences Laboratory, have investigated the survival of *E. coli* dispersed in the air. The bacterial strains tested all lost viability rapidly. A detailed examination of the results, however, reveals some surprises, which illustrate how difficult it is to predict organism survival. For example, while aerosols of the EK1 *E. coli* strain χ1666 showed decreasing viability with decreasing relative humidity, the disabled χ1776 strain showed a minimum of viability at 50% relative humidity, with 30% and 70% relative humidity each providing a better environment for organism survival [42].

In other experiments, Chatigny investigated the survival of *E. coli* that had been deposited on asphalt, cloth, glass, stainless steel or wood surfaces. Viability loss was much slower than for airborne organisms. In addition, for several of the bacterial strains and surface types that were tested, the number of viable organisms appeared to reach a plateau after less than a day [42]. Overall, the survival of *E. coli* χ1776 appeared to be higher than the figure specified for EK2 hosts.

In summary, a number of studies of *E. coli* survival and establishment have been performed to address concerns about the potential risks from recombinant DNA experimentation with this host organism. The results have generally been interpreted as indicating that *E. coli* K12 and *E. coli* χ1776 are unlikely to be transformed into epidemic pathogens, although some experiments have indicated that these *E. coli* strains are hardier than was once believed. Even conclusive results that *E. coli* present no hazard, however, would leave unanswered questions for recombinant DNA risk assessment. Measurements of survival and establishment are also needed for the recombinant host organisms that have become increasingly popular alternatives to *E. coli*, such as *Bacillus subtilis* and yeast [3].

Potential harm from recombinant organisms

It is difficult to establish criteria for assessing potential harm from recombinant DNA. Even if consideration is limited to health hazards from recombinant organisms, a wide variety of disaster scenarios can be envisioned.

In one attempt to place an upper bound on the risks of harmful effects from recombinant organisms, some "worst-case" experiments involving animal tumor virus DNA were performed [48, 49]. The particular tumor virus that was studied was polyoma, which infects mice and causes tumors when injected into hamsters. The results showed that a hybrid DNA molecule containing a single copy of the polyoma DNA did not cause a tumor virus infection in mice when it was injected in purified form or contained in *E. coli*. Although some infection was detected when a recombinant vector containing a head-to-tail dimer of the polyoma DNA was injected directly, even in this form the recombinant molecule was several orders of magnitude less infectious than the polyoma virus itself. The results of the hamster experiments showed, however, that recombinant DNA containing a head-to-tail insert of polyoma DNA induced tumors in the same percentage of animals (19%) as the intact polyoma DNA did when they were each injected directly. Injections of *E. coli* containing the recombinant DNA did not induce tumors in hamsters.

These polyoma virus results have been generally interpreted as affirming the safety of recombinant DNA research involving tumor virus DNA. Although some tumors were induced, the cloned polyoma appeared to be no more virulent than the original virus. When a polyoma recombinant was encapsulated in *E. coli*, it appeared to be considerably less virulent than the original virus. These conclusions are consistent with those of the 1978 "Workshop to Assess Risks for Recombinant DNA Experiments Involving Viral Genomes", which state:

> "... viral genomes or fragments thereof, cloned in *E. coli* K12 using approved plasmid or phage vectors pose no more risk than work with the infectious virus or its nucleic acid and in most, if not all cases, clearly present less risk." [50].

There was some disagreement, however, with the popular interpretations of the polyoma experiments. Among the faults critics found with the experiments were that injection was not the most probable mode of accidental exposure and that this mode of exposure may be biased in favor of negative results [51]. Later experiments, in which polyoma carrying *E. coli* was fed to germ-free mice, addressed some of these concerns [52]. It was also argued, however, that additional viruses should be tested before drawing general conclusions about recombinant DNA safety [3].

Some basic microbiological research that was not designed to investigate risks has reduced concern about one possible mechanism of harmful action from recombinant DNA. The research led to the conclusion that eukaryotic DNA contains segments of DNA that are not expressed, which are interspersed between the pieces of DNA that code for a protein. Because bacteria lack

the splicing capability that enables higher organisms to express these interrupted genes, it appeared unlikely that bacteria would be able to express eukaryotic DNA in shotgun experiments. This allayed fears about a class of disaster scenarios that would be particularly difficult to examine with risk assessment experiments.

The polyoma experiments and the elucidation of the general structure of eukaryotic DNA were specific developments that contributed to a general perception that harmful effects are unlikely to result from recombinant DNA research. Recently, however, it has been shown that certain retroviruses can be present in host organism DNA as "processed" genes that do not contain interrupting DNA sequences. Moreover, one such retrovirus has been shown to have been activated to a tumorigenic state by the process of bacterial cloning [53]. These results have prompted a call to reassess the prevailing relaxed attitude in the microbiological community towards potential harm from recombinants [54].

The discussion above describes risk assessment work concerning potential harmful action from tumor viruses and DNA that might be inadvertently cloned. To address the potential risks from bacteria that have been intentionally modified to produce human proteins, a workshop was held at Pasadena, CA in 1980 [55]. Both the risks from hormone-producing strains of *E. coli* K12 and the possibility of autoimmune disease were discussed.

Calculations presented at the workshop indicated that, even if all the *E. coli* that colonizes the intestine were producing a hormone with great efficiency, the 50 μg of bacterial product would be insignificant compared with natural levels of insulin or human growth hormone. There were some caveats, however, with this result. First, it has been noted that 50 μg of product is above the average lethal dosage for several toxins that could be produced by recombinant *E. coli* [56]. In addition, even for insulin or growth hormone, potentially dangerous quantities could be produced if the recombinant plasmid is transferred from *E. coli* to the anaerobic bacteria that predominate in the intestine. Also, if the recombinant organism produced a hormone that is naturally present in much smaller quantities, such as interferon, the potential yield from *E. coli* alone could affect the body's metabolism.

In order to assess the risks of autoimmune disease induced by proteins from recombinant organisms, participants at the Pasadena Workshop suggested that data be obtained from a test scenario. This project was not pursued, however, after the single response to a 1980 NIH request for proposals in this area was rejected because of "scientific weaknesses" [52]. Although this has not been an area of major concern, the issue is among those that remain unresolved.

Conclusions

The perception that recombinant organisms will produce harmful human health effects has generally decreased since the well-publicized discussion of

potential disaster scenarios in the mid-1970s. Only part of this change, however, can be attributed to formal risk assessment experiments. Few studies have yielded incontrovertible conclusions about the potential for organism release, survival and establishment, or harm. Thus, a decade of genetic engineering experience without detectable biological hazards appears to be primarily responsible for the reduction in concern that has occurred. A reduction in the initially stringent risk management requirements for recombinant DNA research has paralled the changing perception of risk.

The safety record associated with recombinant DNA to date, however, has been achieved under containment conditions in research and manufacturing facilities. Recent proposals to release modified organisms for agricultural applications have raised renewed concerns about potential risks. Questions of ecological balance, which have not previously been addressed for genetically modified organisms, are important considerations for risk assessments of releases to the open environment.

Although risk assessment studies traditionally provide primary support for the development of risk management strategies, recombinant DNA policy has had an unusual history in which formal risk assessment has played a relatively small role. However, potential risks associated with deliberate releases of recombinant organisms to the environment cannot be addressed with the containment strategies developed for research and manufacturing applications. In this context, careful risk assessment is critical to the development of appropriate risk management policy.

References

1 R.W. Old and S.B. Primrose, Principles of Gene Manipulation, University of California Press, Berkeley, CA, 1980.
2 T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.
3 Office of Technology Assessment, Impacts of Applied Genetics: Micro-Organisms, Plants, and Animals, April 1981.
4 S. Krimsky, Genetic Alchemy: The Social History of the Recombinant DNA Controversy, MIT Press, Cambridge, MA, 1981.
5 R.M. Pike, Laboratory-associated infections: Summary and analysis of 3921 cases, Health Lab. Sci., 13(2) (1976) pp. 105—114.
6 S.E. Sulkin and R.M. Pike, Survey of laboratory-acquired infections, Amer. J. Public Health, 41 (7) (1951) 769—781.
7 D. Straton, The genetic engineering debate, Ecologist, 7(10) (1978) 381—388.
8 A.G. Wedum, The Detrick experience as a guide to the probable efficacy of P4 microbiological containment facilities for studies on microbial recombinant DNA facilities, unpublished report to the National Cancer Institute, available from the NIH Office of Recombinant DNA Activities.
9 E. Milewski and B. Talbot, Proposals involving field testing of recombinant DNA containing organisms, Recombinant DNA Tech. Bull. 6(4) (1983) 141—145.
10 F.E. Sharples, Spread of organisms with novel genotypes: thoughts from an ecological perspective, AAAS/EPA Environmental Science and Engineering Fellowship Report, August, 1981, pp. 1—29.

11 S.N. Cohen, A.C.Y. Chang, H.W. Boyer and R.R. Helling, Construction of biologically functional bacterial plasmids *in vitro*, Proc. Natl. Acad. Sci. U.S.A., 70(11) (1973) 3240—3244.
12 M. Singer and D. Soll, Guidelines for DNA hybrid molecules, Science, 181 (1973) 1114.
13 National Institutes of Health, Guidelines for research involving recombinant DNA molecules, Fed. Regist., 41(131) (July 7, 1978) 27902—27943.
14 P. Berg, D. Baltimore, H.W. Boyer, S.N. Cohen, R.W. Davis, D.C. Hogness, D. Nathans, R. Roblin, J.D. Watson, S. Weissman and N.D. Zinder, Letter, Science, 185 (1974) 303.
15 P. Cole, Epidemiological Studies and Surveillance of Human Cancers Among Personnel of Virus Laboratories. Cold Spring Harbor Laboratory, 1973.
16 N.D. Zinder, The Berg Letter: A statement of conscience, not of conviction, Hastings Center Report, October 1980, p. 14.
17 P. Berg, D. Baltimore, S. Brenner, R.O. Roblin III and M.F. Singer, Summary Statement of the Asilomar Conference on Recombinant DNA Molecules, May 1975, Summary of report submitted to Assembly of Life Sciences of National Academy of Sciences.
18 S. Auerbach, And man created risks, Washington Post, 9 March 1975, B1.
19 Working Group for Revision of the Guidelines, Evaluation of the risks associated with recombinant DNA research, NIH Recombinant DNA Advisory Committee memorandum, 21 August 1981.
20 E.A. Milewski, Evolution of the NIH Guidelines, Recombinant DNA Tech. Bull., 4(4) (1981) 160—165.
21 National Institutes of Health, Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules, Department of Health, Education and Welfare, 1977 [DHEW Publ. (NIH) 1489].
22 J.L Hopson, Recombinant lab. for DNA and my 95 days in it, Smithsonian, 8(3) (1977) 55—62.
23 Ad Hoc NIAID Working Group on Risk Assessment, Proceedings of meeting held on August 30, 1979, quoted in Ref. [4].
24 R.P. Novick, Present controls are just a start, Bull. At. Sci., (May 1977) 16—21.
25 W.J. Gartland, Letter, April 9, 1982, A second letter, stating that no additional accidents or violations had been reported, is dated January 26, 1983.
26 National Institutes of Health, Recombinant DNA research; physical containment recommendations for large-scale uses of organisms containing recombinant DNA molecules, Fed. Regist., 45(72) (April 1, 1980) 24968—24971.
27 National Institutes of Health, Review of federal legislation addressed to recombinant DNA activities, Recombinant DNA Res., 2 (1978) 502—811. [NIH Publ. 78-1139.]
28 J. Lear, Recombinant DNA: The Untold Story, Crown Publishers, New York, 1978.
29 S. Krimsky, A comparative view of state and municipal laws regulating the use of recombinant DNA molecules technology, Recombinant DNA Tech. Bull., 2(3) (1979) 121—127.
30 City of Cambridge, Ordinance for the Use of Recombinant DNA Technology in the City of Cambridge, 1981.
31 D.R. Clay, Presentation before the Chemical Manufacturer's Association, Washington, DC, July 17, 1984.
32 M.G. Morgan, Choosing and managing technology-induced risk, IEEE Spectrum, (December 1981) 53—60.
33 J.F. Rissler, Research needs for biotic environmental effects of genetically-engineered microorganisms, AAAS/EPA Science and Engineering Fellows Program, 1983.
34 National Institutes of Health, Proposed plan for a program to assess the risks of recombinant DNA research, Federal Regist., 44(64) (April 2, 1979) 19302—19304.
35 R. Curtiss III, Genetic manipulation of microorganisms: Potential benefits and biohazards, Annu. Rev. Microbiol., 30 (1976) 507—533.

36 R. Curtiss III, J.E. Clark, R. Goldschmidt, J.Ch. Hsu, S.C. Hull, M. Inoue, L.J. Maturin, Sr., R. Moody and D.A. Pereira, Biohazard Assessment of Recombinant DNA Molecule Research, Springer-Verlag, Berlin, 1977, pp. 375—387.
37 R. Holliday, Should genetic engineers be contained? New Sci., 73 (1039) (1977) 399—401.
38 D.R. Lincoln, L.R. Landis and H.A. Gray, Containing recombinant DNA: How to reduce the risk of escape, Technical Report Carnegie-Mellon University, 1979.
39 A. Mottershead, D. Pioli, G. Sharpe and R. Sykes, Hazard study of proposed molecular genetics research programme at the corporate laboratory, Rep. MD 16.927, Imperial Chemical Industries Limited, Mond Division, 21 October, 1975, a summary appears as Ref. [58].
40 E. Fisher, As assessment of the effectiveness of alternative physical containment strategies in recombinant DNA facilities, Ph.D. Thesis, Carnegie-Mellon University, 1984.
41 D.R. Lincoln, E.S. Fisher, D. Lambert and M.A. Chatigny, Release and containment of microorganisms from applied genetics activities, Carnegie-Mellon University Report to the U.S. Environmental Protection Agency, 1983.
42 M.A. Chatigny, M.T. Hatch, T. Adler, D. Besemer, J. Hresko and J. Macher, Studies on the biohazards of laboratory operation involving biological substances used in recombinant DNA research, Final NBL Rep. 55-77-f, Naval Biosciences Laboratory, April 1980.
43 M.A. Chatigny, Appendix III: Reconstruction tests of fermentation process operations, in: Release and containment of microorganisms from applied genetics activities, Carnegie-Mellon University Report to the U.S. Environmental Protection Agency, 1983, pp. 103—120.
44 W.E. Barkley, Facilities and equipment available for virus containment, in: A. Hellman, M.N. Oxman and R. Pollack (Eds.), Biohazards in Biological Research: Proceedings of a Conference held at the Asilomar Conference Center January 22—24, 1973, Cold Spring Harbor Laboratory, 1973.
45 S.L. Gorbach, Letter to D.S. Frederickson, 14 July 1977, printed in: Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA, Part Two, October 1977, p. M-1 [DHEW Pub. (NIH) 1490].
46 R. Curtiss III, Letter to William Gartland, printed in: Recombinant DNA Res., 5 (1980) 260 (NIH Publ. 80-2130).
47 S.B. Levy and B. Marshall, Survival of *E. coli* host — vector systems in the human intestinal tract, Recombinant DNA Tech. Bull., 2(2) (1979) 77—80.
48 H.W. Chan, M.A. Israel, C.F. Garon, W.P. Rowe and M.A. Martin, Molecular cloning of polyoma virus DNA in *Escherichia coli*: Lambda phage vector system, Science, 203 (1979) 887—892.
49 M.A. Israel, H.W. Chan, W.P. Rowe and M.A. Martin, Molecular cloning of polyoma virus DNA in *Escherichia coli*: Plasmid vector system, Science, 203 (1979) 883—887.
50 National Institutes of Health, U.S.—EMBO workshop to assess risks for recombinant DNA experiments involving the genomes of animal, plant, and insect viruses, Fed. Regist., 43(63) (March 31, 1978) 13748—13755. Workshop held at Ascot, England, January 26—28, 1978.
51 B. Rosenberg and L. Simon, Recombinant DNA: have recent experiments assessed all the risks?, Nature, 282 (1979) 773—774.
52 National Institutes of Health, Program to assess the risks of recombinant DNA research; proposed second annual update, Fed. Regist., 47(235) (December 7, 1982) 55104—55109.
53 K. Harbers, A. Schnieke, H. Stuhlmann, D. Jahner and R. Jaenisch, DNA methylation and gene expression: Endogenous retroviral genome becomes infectious after molecular cloning, Proc. Natl. Acad. Sci. U.S.A., 78 (1981) 7609—7613.

54 D. Bartels, H. Naora and A. Sibatani, Oncogenes, processed genes and safety of genetic manipulation, Trends Biochem. Sci., 8(3) (1983) 78—80.
55 National Institute for Allergy and Infectious Diseases, Workshop on recombinant DNA risk assessment, Recombinant DNA Tech. Bull., 3(3) (1980) 111—128.
56 S. Gottesman, Evaluation of the risks associated with recombinant DNA research by the recombinant DNA advisory committee working group on revision of the guidelines, National Institutes of Health, Fed. Regist., 46(233) (December 4, 1981) 59385—59425.
57 National Research Council, Risk Assessment in the Federal Government: Managing the Process, National Academy Press, Washington, DC, 1983.
58 E.C. Dart, Industrial risk analysis, in: R.F. Beers, Jr. and E.G. Bassett (Eds.), Recombinant Molecules: Impact on Science, Raven Press, New York, 1977, pp. 495—496.