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Dr. Donald Fredrickson
Director
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Dear Don,

I have read with interest the interim report of the Federal Interagency Committee on Recombinant DNA Research: Suggested Elements for Legislation. I found the document informative and was partially reassured to find that the suggested elements for legislation were more reasonable than some of the provisions contained in legislative bills previously introduced into the Senate and House. I am, however, extremely concerned that, based on fear, ignorance and misinformation, we are about to embark on over-regulation of an area of science and scientific activities. This letter is written to indicate my assessment of the risks associated with recombinant DNA activities and to suggest what I consider to be reasonable provisions in legislation which may be necessary to regulate research on and use of recombinant DNA. Although I have not included literature citations for the information contained in this letter, I will provide this on any point if that would be helpful.

Three years ago in August, 1974 after reading the Berg et al. letter in Science and Nature, I drafted an open letter to the authors which was also sent to over one thousand scientists here and abroad. In that letter I enumerated various factors that I thought had not been given sufficient attention by the Berg et al. committee and suggested a voluntary cessation of essentially all recombinant DNA research until "potential biohazards can be assessed and means to cope with them established". My beliefs then, later at the Asilomar Conference and as a member of the NIH Recombinant DNA Molecule Program Advisory Committee, have been conservative, which I believe to have been a responsible position until such time as more information was available about the likelihood for manifestation of potential biohazards. Since August, 1974 I have taken four actions, some of which have caused me to become far less apprehensive about recombinant DNA molecule research. First, since I had just initiated attempts to construct recombinant DNA, I decided to cease and have not yet resumed such research. Second, I conceived of possible means for manipulating Escherichia coli K-12 to make it safer for recombinant DNA research, an idea that was put forth in the report written and submitted by Novick, Clowes, Cohen, Falkow and myself at Asilomar, and then following Asilomar undertook, with the help of all of my laboratory colleagues, the design, construction and testing of safer, more useful strains of E. coli K-12 for recombinant DNA research. Third, I initiated an intense but intermittent education of myself with regard to all aspects of recombinant DNA research and all areas of knowledge necessary to assess the potential biohazards of such research. I did this by reading and by talking to

colleagues expert in the areas of sanitary engineering, public health, infectious diseases, gastroenterology, oncology, virology, genetics, etc. Fourth, upon finding that certain information was not available, my colleagues and I have initiated experiments to obtain data that would allow a better assessment of the likelihood for manifestation of potential biohazards.

Much of the criticism and fear of recombinant DNA research has centered around the use of E. coli as a host for recombinant DNA. This species is comprised of thousands of different types, each with unique sets of attributes. Most strains of E. coli are relatively harmless commensals occupying the large intestines of warm-blooded animals. Some strains, however, have the capacity to occupy the small intestine and cause diarrheal disease, whereas others are often associated with infections of the urinary tract. A still smaller number of strains have the capacity, usually in individuals compromised by surgery, organ transplantation or diseases such as cancer, to invade healthy tissues and to multiply in the circulatory system. A still different group of E. coli strains includes those obtained from animal feces or sewage that have been maintained in the laboratory for many years. Many of these latter strains have become rather well adapted to the laboratory environment and have gradually lost the genetic attributes necessary to occupy the intestinal habitat and/or to cause disease. One such E. coli strain, designated K-12, was obtained from a human patient at Stanford University in 1922 and the NIH Guidelines stipulate that it is the only strain of E. coli into which foreign genetic information may be introduced. In considering the likelihood for the manifestation of a biohazardous condition during a recombinant DNA experiment, one must therefore consider the inherent potential of E. coli K-12 to exhibit pathogenicity (herein defined as causing disease or interfering with normal physiological activity) or to transmit recombinant DNA to some other microorganism encountered in nature that could exhibit pathogenicity. Pathogenicity requires that a microorganism colonize a given ecological niche within an "infected" individual and then manifest some virulence trait so as to overcome normal host defenses or interfere with normal physiological function. Sustained exhibition of pathogenicity of an epidemic nature also requires that a microorganism be communicable and survive long enough to be passed from one individual to another.

like a domesticated animal.

It has often been reported that E. coli K-12 is unable to colonize (persist for 7 or more days) in the large intestine when fed in large quantities to healthy, well-nourished mice, rats, chickens, pigs, calves and humans but that some E. coli K-12 cells can survive passage through the intestinal tract. Not much has been mentioned, however, as to why this is so, despite the fact that both published and unpublished information are available to provide a basic understanding of this observation. Normal strains of E. coli that inhabit the large bowel are "smooth" because they produce lipopolysaccharide (LPS) with carbohydrate side chains that are characteristic for any given strain and often have capsular surface antigens (composed of polysaccharides and/or proteins) which may further facilitate colonization. E. coli K-12 is defective in the production of LPS side chains because of defects in at least two genes that are located at widely separated regions of its chromosome; consequently it displays a "rough" phenotype. One of these defects in K-12's LPS genes appears to be a deletion (S. Falkow,

personal communication) and therefore could not revert back to the wild-type state. Attempts to convert K-12 to a smooth phenotype by mating it under optimal laboratory conditions with smooth E. coli, Salmonella and Shigella donor strains have generally been unsuccessful. One exception is the ability to transfer genes to K-12 for the expression of the O8 LPS side chain antigen. These successful experiments, however, required the use of donors termed Hfr that efficiently transfer large segments of chromosome and are constructed in the laboratory; such donor types have never been isolated from nature. E. coli K-12 also does not make any capsular antigen, although under adverse conditions certain strains can produce the capsular polysaccharide colanic acid, which is not known to facilitate colonization by enteric bacterial strains that produce it. Colonization of the small intestine by enteropathogenic strains of E. coli is often facilitated by the presence of plasmids that specify protein surface antigens. Smith and Linggood have observed that the K88 plasmid present in most E. coli strains pathogenic for young pigs does not permit E. coli K-12 to colonize the pig intestine although the E. coli K-12 $K88^+$ strain tends to persist in the intestine somewhat longer than E. coli K-12 $K88^-$ strains. Similar findings have been made by Gyles, Falkow and their colleagues for K-12 strains possessing the K99 plasmid that specifies a protein surface antigen that allows enteropathogenic strains of E. coli to establish in the small intestine of calves. Shipley and Falkow have found that such K-12 derivatives produce large amounts of the K99 surface antigen, but the antigen does not readily adhere to the bacterial cell surface, presumably because of K-12's LPS defects, and is liberated into the culture medium. It thus seems highly unlikely (although not impossible) that one could introduce appropriate genetic information into an E. coli K-12 strain by a recombinant DNA experiment that would permit colonization of healthy, well-nourished individuals when the K-12 strains are already defective because of several mutational defects. For sake of completeness, it should be noted that E. coli K-12 can, of course, colonize gnotobiotic mice that lack a competing intestinal flora but is quickly eliminated when the mice are fed smooth E. coli strains of mouse origin. There is also a recent report that E. coli K-12 can colonize sheep that have been fasted for a day prior to ingesting the E. coli K-12 cells. In general, these studies imply that E. coli K-12 would colonize the intestinal tracts of individuals whose normal intestinal flora had been disturbed due to disease, fasting and/or recent prior antibiotic therapy. Although the NIH Guidelines stipulate that individuals in these categories not engage in recombinant DNA research, there is always the possibility of forgetfulness or an error in judgment that would expose such individuals to E. coli K-12 cells containing recombinant DNA. However, there is another safety feature in the use of EK1 hosts that has been overlooked during the recombinant DNA research debate. Most EK1 hosts currently in use are auxotrophs, having requirements for amino acids and frequently for thymine (or thymidine). Many of the strains are also recombination deficient. H. W. Smith and ourselves have both shown that thymine-requiring K-12 strains survive less well during passage through the intestinal tracts of humans and rats, respectively, than do strains that do not require thymine. We have also shown that recombination-deficient strains survive less well during passage through

the rat intestinal tract. Indeed, rec⁻ mutants are rather sick in that they are inordinately sensitive to sunlight and various chemicals and one out of every ten cells dies during each cell division even in the absence of exposure to these deleterious conditions. Amino acid requirements can also decrease maintenance of E. coli strains in the intestinal tract. We showed some years ago that mutant derivatives of smooth E. coli strains from mice that required amino acids, especially ones that were essential for the mouse, had a decreased potential to successfully compete with wild-type prototrophic E. coli strains and thus soon disappeared from the intestinal flora. These are all important points since most of the feeding experiments with E. coli K-12 strains that are often cited have been done with strains that have few, if any, nutritional requirements. With EK2 hosts like χ1776 that are now required for those experiments deemed most likely to be potentially hazardous, the ability to colonize has been completely lost. χ1776 has six mutations each of which make colonization either unlikely or impossible and which collectively preclude survival during passage through the intestinal tract.

A second aspect of pathogenicity would be a mechanism to somehow overcome host defense mechanisms or interfere with normal physiological function. In this regard, people very often forget about the numerous efficient host defense mechanisms that include the presence of various bacteriocidal activities in tears, saliva, serum, etc. Indeed, in a study we have recently initiated which confirms earlier studies, most E. coli strains isolated from patients with bacteriemias, septicemias, wound infections, etc. are resistant to serum bacteriocidal activity. E. coli K-12 and especially EK2 hosts such as χ1776 are inordinately sensitive to serum bacteriocidal effects and do not seem to be able to mutate to resistance. Other studies have suggested that even compromised patients such as those receiving kidney transplants or those suffering from leukemia or lymphoma still exhibit serum bacteriocidal activity against various microorganisms. We are currently embarking on a study to verify whether this is so, particularly with regard to EK1 and EK2 E. coli hosts. Various species of Shigella and Salmonella possess the ability to invade tissues as part of the disease-causing process. It is known that mutations in LPS genes that result in a smooth to rough conversion cause these organisms to become avirulent. This change usually blocks cell penetration but when it does not, the rough cells fail to grow and multiply in the invaded cells. It is also known that the transfer of LPS genes from E. coli K-12 donor strains to Shigella flexneri 2a leads to partial or complete avirulence of the latter strain. Formal, Gemski and LeBrec have introduced genes from virulent Shigella flexneri 2a into E. coli K-12 so that the E. coli K-12 hybrids express both the group-specific and type-specific surface antigens of Sh. flexneri 2a. These hybrids, which are antigenically identical to the Sh. flexneri 2a parental strain, were not able to cause disease in either animals or humans. It is also relevant to note that the transfer of Sh. flexneri 2a virulence genes into smooth E. coli cells expressing the 08 antigen also does not result in formation of virulent E. coli hybrids. This observation is important in that the genes for the 08 LPS antigen can be transferred to and expressed by E. coli K-12. In further experiments by these workers, a Shigella gene essential for Shigella's ability to penetrate mucosal cells was introduced into a K-12 strain without endowing the E. coli K-12 hybrid with invasiveness. The Shigella virulence gene was present in the K-12 cells as shown by the

K-12 hybrid's ability to transfer the non-functional virulence gene into an appropriate avirulent Shigella mutant and re-endow it with the ability to invade mucosal cells. Furthermore, it is known from studies conducted by H. W. Smith, S. Falkow and colleagues that the introduction of plasmids specifying enterotoxins into strains of E. coli K-12 does not lead to the manifestation of disease even when the K-12 strains also possess another plasmid specifying synthesis of the K88 or K99 surface antigens that permit colonization of the small intestine by enteropathogenic E. coli strains. In view of the requirement for a normal smooth LPS to exhibit virulence, the failures to endow E. coli K-12 strains with virulence and the known and well-established mechanisms of host defense, it is difficult for me to believe that one could cause E. coli K-12 to display virulence or cause physiological harm by the introduction of foreign DNA sequences during a recombinant DNA experiment. This belief is augmented by the well-founded expectation that a display of virulence and/or physiological harm would most likely require that the E. coli K-12 cell be able to colonize some niche in or on humans. Since the ability to colonize is highly unlikely to be acquired in a recombinant DNA experiment in conjunction with introduction of a "virulence" trait, it is evident that even if a gene specifying a potent toxin were introduced into E. coli K-12 that the only individual possibly at risk would be a careless experimentalist that "inadvertently" ingested rather large quantities of the culture.

In terms of communicability of E. coli K-12, we know that enteric diseases caused by enteropathogenic E. coli and various strains of Shigella, Salmonella and Vibrio are transmitted by contaminated food and water and that manifestation of disease symptoms requires consumption of approximately one million bacteria. Such enteric diseases are seldom spread by aerosols. Indeed, it is well known, for example, that cages of mice infected with Salmonella can be housed in the same room with uninfected mice which remain uninfected. The finding that E. coli cells can be recovered from the nasopharynx of approximately five percent of those humans tested might suggest that aerosol spread could occur. Such E. coli cells, however, are only intermittently present in the nasopharynx and are usually found at concentrations too low to initiate an infection even if they were representative of a pathogenic strain. They most likely get into the nasopharynx due to poor personal hygiene. After learning of these observations quite some years ago, I monitored my nostrils and skin for the presence of those E. coli K-12 strains I was working with. I was successful in detecting these strains about ten percent of the time when the monitoring was done at the end of the work day, but never obtained positive results when the monitoring was done the next morning. I should hasten to add that my research with E. coli K-12 at that time involved mouth pipetting and other aerosol-generating procedures on an open lab bench: procedures and conditions which are not permitted by the NIH Guidelines. These results, preliminary as they are, nevertheless suggest that E. coli K-12 does not colonize the nasopharynx. Based on these observations, the fact that E. coli's normal ecological niche is the colon and the fact that transmission of enteric diseases is by ingestion of contaminated water and food, I doubt that E. coli K-12 could be converted to an air-borne "infectious" agent by introduction of recombinant DNA. In

terms of the more usual means for spread of enteric pathogens, it is evident that enteric diseases are very well controlled in the United States by sanitary engineering, even though there have been reports of poor water quality in some parts of the country and higher-than-desired levels of pollution of rivers, streams, etc. There is, however, a concerted effort to improve biological waste water treatment and thus lessen pollution and improve water quality. Even if there were a natural catastrophe such as caused by an earthquake, tornado, hurricane, etc., it is unlikely that E. coli K-12 containing recombinant DNA could initiate or sustain an epidemic in view of K-12's inability to colonize and overcome host defense mechanisms.

Since I believe that it is highly improbable that one could endow E. coli K-12 with pathogenicity and/or alter its means of communicability, it is then necessary to consider the potential of E. coli K-12 cells containing recombinant DNA to transmit that DNA to other microorganisms that might be encountered in nature. In terms of cloning onto the non-conjugative plasmid vectors pMB9 and pSC101, we have conducted a great diversity of experiments under laboratory conditions to measure as many parameters as possible that would affect conjugational transmission in nature. We have thus measured the frequencies of transfer of a diversity of conjugative plasmids to and from E. coli K-12 cells containing pMB9 or pSC101 and the frequency with which these conjugative plasmids mobilize the plasmid vector to another recipient cell. Measurements have also been made to determine the influences of temperature and cell densities on these frequencies. Using these experimentally determined values along with values from the literature on the frequencies of enteric bacteria with conjugative plasmids, the densities of suitable donor and recipient cells in natural environments and the occurrence of restriction as a barrier to inheritance of plasmid DNA, we estimate that the maximum probability for transmission of a non-conjugative plasmid vector from an EK1 host is 10^{-16} per surviving bacterium per day in the intestinal tract of warm-blooded animals. Since we have shown that conjugational transmission is most efficient at 37C and is essentially undetectable at 27C for most conjugative plasmids found in E. coli strains in nature, it is even more unlikely that conjugational transmission of a nonconjugative cloning vector containing recombinant DNA could occur at the ambient temperatures found in sewers, sewage treatment plants, streams and rivers, etc. In giving this less-than-10^{-16} probability per surviving cell per day, I have not taken into consideration the facts that conjugational ability (i) decreases with decreasing metabolic activity of donor and recipient bacteria (E. coli grow at generation times of 40 to 60 min under the optimal laboratory conditions used in our experiments and 5 to 12 hours in the intestinal tract), (ii) is inhibited by volatile fatty acids, bile and other constituents of the intestinal tract, (iii) occurs at suboptimal frequencies at pH 6.1 and at oxidation reduction potentials of -200 mV (the "usual" pH and E_h of the bowel contents) and (iv) can also be diminished by differences in the cell surface between donors and recipients. Although such conjugationally promoted transfer of non-conjugative plasmids has not, and indeed cannot, be measured in vivo, there have been a number of reports from the laboratories of Anderson, Richmond, Smith, Falkow and others which demonstrate that the transfer of a conjugative plasmid from one bacterium to another in vivo is seldom observed. Successful detection of such transfer in these experiments requires use of numerous animals and/or repetitions and very

often depends on the use of mutant plasmids that are transferred at frequencies 100- to 1000-times higher than those conjugative plasmids found in wild-type populations of enteric bacteria. Based on these data, it can be estimated that the frequency of conjugative plasmid transfer in the intestine is about 10^{-8} per donor cell per day. Since transfer of a non-conjugative plasmid requires two such conjugational events and since non-conjugative plasmids are mobilized at frequencies of 10^{-3} to 10^{-4} compared to the frequency of transfer of the conjugative plasmid, the estimated overall probability for transmission of pSC101 or pMB9 from an EK1 host would be between 10^{-19} and 10^{-20} per surviving bacterium per day [10^{-8} x 10^{-8} x (10^{-3} or 10^{-4})]. These values, of course, take into account the contributions of the various environmental factors enumerated above. I should hasten to add, however, that the intestinal environment becomes much more conducive for conjugational plasmid transfer following antibiotic therapy since the pH, E_h and volatile fatty acid concentration change to more favorable values, there is a decrease in drug-sensitive normal flora that permits greater proliferation and titers of a newly introduced strain and a possible increase in drug-resistant flora that possess conjugative plasmids. These facts are, of course, one of the important reasons for stipulating in the NIH Guidelines that individuals not conduct recombinant DNA research during and for seven days after ceasing antibiotic therapy. In terms of the effects of inserting foreign DNA into non-conjugative plasmid vectors on the frequency of plasmid transfer, Crisona and Clark have found that insertion of certain sequences from conjugative plasmids can increase the frequency of pSC101 mobilization whereas Hamer has found either no effect or a decrease in pSC101 mobilization frequency by the insertion of different Drosophila DNA sequences.

In terms of transductional transmission of non-conjugative plasmid vectors containing recombinant DNA, we have only recently initiated our studies. We have found, however, that the frequency of plasmid transduction mediated by phage P1 decreases as the size of the plasmid vector decreases and is essentially undetectable for the plasmid cloning vectors pSC101 and pMB9. The transductional efficiency for these vectors is thus several orders of magnitude lower than for chromosomal markers, even though these plasmids are present in 5 and 40 copies per chromosomal DNA equivalent, respectively. In numerous experiments in which E. coli K-12 strains have been fed to rodents, we have seldom found phage that would infect the fed strain. In those animals with such phage, the titers were generally between 10^2 and 10^3 per gram of feces. Although these observations suggest that titers of potential transducing phages may be very low, we do not have sufficient quantitative data on their titers in various environments (i.e., intestinal contents, sewage, etc.) to make a very accurate estimate of the probability for transductional transmission of plasmid cloning vectors containing recombinant DNA. Based on our preliminary results, the typical concentrations of E. coli cells in the intestine and in sewage and the known properties of E. coli K-12 transducing phages, I believe it is probably as low or lower than the probability for conjugational transmission. It should also be noted that EK2 strains like χ1776 are totally or partially resistant to all known transducing phages of E. coli K-12 and that many EK1 and all EK2 hosts require thymine or thymidine which is needed for productive temperate phage infection.

In terms of transformation, this is not known to naturally occur in enteric bacteria. One can induce it to occur by treating E. coli with calcium chloride at 0C and then rapidly shifting to 42C for a one-minute heat shock; such conditions are unlikely to be encountered in nature. Nevertheless, the potential that recombinant DNA released from E. coli K-12 cells lysing in the intestine might be taken up by cells in the intestinal mucosa or even transform other enteric bacteria has led us to investigate the survival of DNA in rat intestine contents. We have found that a 1:6 dilution of the contents of the small intestine results in the total destruction (i.e., breakdown to acid-soluble material) of 90% of the DNA within the time it takes to add the DNA, mix it and remove a sample. Most likely, the nuclease(s) is introduced into the intestinal tract through the pancreatic duct, although it is also known that cells lining the intestine contain various deoxyribonucleases which might be secreted into the intestine. These results lead me to believe that experiments such as those to be conducted by Rowe and Martin to test whether mice fed E. coli containing polyoma DNA will become infected with polyoma will not give positive results.

In order to obtain additional information on the likelihood of transmission of recombinant DNA, we have also commenced to collect a diversity of E. coli strains obtained from patients with bacteriemias, wound infections and urinary tract infections, from healthy individuals and from sewage. We have been examining these strains for the presence of nonsense suppressor mutations that would allow for the replication and perpetuation of lambda or plasmid vectors that contain nonsense mutations and also for the ability of these strains to be infected by lambda DNA that is tagged by an antibiotic resistance marker. So far, in a test of some 100 strains, we have failed to detect any strain with a nonsense suppressor or that was infectable by lambda. R. Davis, P. Leder and their colleagues have also examined some 2000 E. coli strains for sensitivity to phage lambda and although they found a few strains that appeared partially sensitive to lambda, none would propagate the virus. We intend to test many more strains, but our preliminary results in conjunction with those obtained by Davis and Leder indicate that the use of lambda and plasmid vectors, especially if they possess amber suppressible mutations, provides a greater margin of safety than was previously verifiable by experimental data. In this regard, the three EK2 lambda vectors constructed by F. Blattner, P. Leder, P. Sharp and their colleagues each contain two amber suppressible mutations. Although none of the currently used non-conjugative plasmid vectors have such mutations, work is in progress to isolate these mutant plasmids.

Based on all of the foregoing, I consider that the transmission of recombinant DNA contained on various non-conjugative plasmid and lambda phage vectors to other microorganisms encountered in nature will be a most unlikely event. This conclusion is more certain with use of EK2 host-vectors than with EK1 host-vectors and could only be proven incorrect either by new data that would contradict the substantial body of data already accumulated or by the discovery of a mechanism of gene transfer as yet unknown that would facilitate the transmission of DNA at frequencies higher than those observable by conjugation, transduction and transformation.

The last point to consider in evaluating the likelihood of harm emanating from a recombinant DNA experiment is whether E. coli K-12 will obtain a selective advantage by the introduction of a DNA sequence that codes for some gene product that is foreign to the E. coli K-12 cell. Cameron and Davis have inserted random fragments representing at least 95 percent of the E. coli K-12 and yeast genomes into a lambda vector and then determined the rate with which specific clones gained ascendancy by examining the fragments still present in pooled lambda stocks after increasing numbers of cycles of propagation. They found that one to several unique hybrid phages gained prominence much faster when E. coli DNA was cloned than when yeast DNA was cloned. Thus yeast DNA had a more neutral effect than E. coli DNA which contains more sequences that are disadvantageous to the propagation of the vector. In other less complete studies in which there was no assurance that most of the genome had been cloned onto the lambda vector, these investigators found that DNA from Drosophila had a neutral effect like yeast DNA and that DNA from maize and Dictyostelium was detrimental with most sequences causing the hybrid to be at a distinct disadvantage. Although some highly selected hybrids with E. coli and yeast DNA gave yields of virus per infected cell equal to and in a few cases higher than yields after infection of cells with the original vector, in no case with any DNA insertion from any of the organisms studied was a hybrid found that gave higher yields than or could out compete wild-type lambda. In much more limited studies, S. Cohen and colleagues have found that insertion of foreign DNA onto the pSC101 plasmid vector placed the cells at a selective disadvantage relative to cells with only the pSC101 vector. Based on all these studies, I consider that the probability of a random DNA sequence either being completely neutral or providing a selective advantage to a vector or an E. coli K-12 cell is probably less than 10^{-5}. Although it can be argued that these tests have not been performed in any of E. coli's various natural habitats, there are substantial arguments based on genetic and evolutionary considerations given by F. J. Ayala and B. Davis at the recent NAS Forum to believe that the results of Cameron and Davis and of Cohen would not vary appreciably irrespective of the environment of the test. In view of the already diminished survival potential of EK1 hosts that possess auxotrophic requirements and certainly of EK2 hosts, the ultimate survival and perpetuation of recombinant DNA requires that it be transferred to some other microorganism. Even if this remote possibility did occur, the same arguments would prevail and I would deem it highly unlikely that the foreign DNA would either be neutral or confer a selective advantage on that host.

In view of all the accumulating information discussed above, I have gradually come to the realization that the introduction of foreign DNA sequences into EK1 and EK2 host-vectors offers no danger whatsoever to any human being with the exception already mentioned that an extremely careless worker might under unique situations cause harm to him- or herself. The arrival at this conclusion has been somewhat painful and with reluctance since it is contrary to my past "feelings" about the biohazards of recombinant DNA research. As a means to challenge the above-stated conclusion, I have taken some worst-case scenarios thought up by myself, by my colleagues and by others and subjected them to critical analysis by obtaining information from those scientists most knowledgeable about the genetic control, biosynthesis, mode of action, pro-

duction, etc. of the foreign gene product(s) in question. In no instance have I found evidence that the necessary genetic information could be cloned in one step, would permit E. coli K-12 to colonize the intestinal tract and lead to the production of the product(s) in the intestinal environment that would be harmful to the mammalian host. This is not to say, however, that an individual with considerable skill, knowledge (most of which is currently lacking) and luck could not construct in multiple steps a microorganism that would satisfy all these requirements.

In terms of the likelihood that an escaped E. coli K-12 containing recombinant DNA could cause harm to some non-human organism in the biosphere, there are, of course, less data upon which to base any definitive conclusion. However, the following points are relevant: (i) E. coli strains recovered from sewage, polluted rivers, farmlands, etc. are smooth rather than rough and are prototrophic rather than auxotrophic; (ii) it seems highly improbable that addition of foreign DNA could endow E. coli with the potential to colonize a new ecological niche such as soil or water and also confer ability to cause harm to some other organism; (iii) the probability for transmission of recombinant DNA to some other bacterial species that inhabits soil or water is known to be lower than the values given above for transmission to other strains of E. coli (indeed, plasmid cloning vectors are not likely to be stably maintained in many of these species); and (iv) there is a low probability that the foreign DNA will be either neutral or provide a selective advantage in any microbial host. Based on these considerations, I do not believe that cloning foreign DNA into E. coli K-12 host-vectors poses any threat to non-human organisms in the biosphere. Additional data to substantiate this assessment would, of course, be valuable.

During several recent meetings on recombinant DNA research, I have stated that adherence to the physical and biological containment requirements and practices for any given experiment as described in the NIH Guidelines would preclude manifestation of any potentially biohazardous conditions. I have noted, however, that human error might circumvent the safety afforded by physical and biological containment without really analyzing the degree to which this "feeling" might be true. If the foreign DNA is present in the appropriate EK1 or EK2 host-vector system, then an accident in which a large culture might be spilled would not seem likely to cause any harm even if there were a second error in not implementing an accident plan to disinfect the spill. If there were either gross aerosolization or ingestion of large quantities of such organisms, harm could possibly occur to exposed individuals provided that the recombinant DNA contained in the bacterial host was either itself harmful or specified a harmful product that could be either released or produced in vivo and exhibit its harmful effects in that environment. Even though such an accident might occasionally occur, the likelihood of the other necessary conditions being met seems remote. If, in addition to the above accident, the worker had been receiving antibiotic therapy prior to the day in which the accident occurred the consequences for that worker might be more severe but still would not cause harm to anyone outside the work area. The error of working with recombinant DNA while taking antibiotics would also increase the likelihood of conjugational transmission of the recombinant DNA to some other intestinal microorganism. S. Falkow has found that antibiotic treatment increases the frequency of conjugative plasmid transfer 100-fold. Thus the probability for conjugational trans-

mission of a non-conjugative cloning vector containing recombinant DNA would increase to a maximum value of 10^{-12} per surviving cell per day that reaches the colon, still a very improbable event.

Contamination of cultures during transformation with recombinant DNA has often been mentioned as a likely problem associated with poor technique. Most contaminants encountered in our own lab are Staphylococci shed from the skin (which cannot be transformed with E. coli vectors) and other airborne microorganisms that grow optimally at 20 to 30C and thus grow poorly or not at all at 37C, the temperature customarily used for growth of E. coli. Even if such contaminants could be transformed with recombinant DNA and be recovered as colonies, an individual would have to not recognize the colony as a contaminant and grow up a substantial culture to achieve a sufficient "infective dose" and then have another accident prior to recognizing the initial error. One can also consider a potential worst case in which research with smooth E. coli and other enteric pathogens was being conducted in the same laboratory as recombinant DNA activities (a rather stupid and unlikely situation) such that a wrong culture was chosen as the recipient for transformation with recombinant DNA. First of all, it is known that smooth strains of enteric organisms are very poorly transformable with plasmid DNA and that mutants with defective LPS synthesis that cause a rough phenotype and thus avirulence are much more transformable. Secondly, one has a low probability that a cloned DNA fragment both specifies harmful information and would be neutral or confer a selective advantage for the survival of the host. In addition to these factors, there are a variety of other standard practices in recombinant DNA research that would further minimize the likelihood of either successful transformation of a contaminant or a "wrong" bacterial strain or of failing to recognize such a mishap. For example, the medium used to recover transformants of an EK2 host such as χ1776 precludes growth of many types of contaminating microorganisms, has antibiotics added to it which prevent growth of still more contaminants and contains indicator dyes and a sugar that allow one to visually distinguish χ1776 colonies from those of smooth as well as most rough enteric bacteria. Furthermore, the optimal method for transformation of χ1776 gives 100- to 1000-fold fewer transformants when used for EK1 hosts. In summary, after pondering these and other types of errors, I am convinced, because of the need for a sequence of errors and the improbabilities of constructing a microbe that both has a competitive advantage and displays a harmful trait, that construction and use of E. coli K-12 strains with recombinant DNA poses no threat whatsoever to humans (or other organisms) except for the remote chance that an individual constructing or using such strains as discussed above in the first examples of potential errors might experience some ill effects.

The recombinant DNA research debate has been necessary and valuable. I have become increasingly distressed, however, by the degeneration of the debate. Opinions have often been stated as factual certainty, statements of "fact" have often been put forth that are in conflict with published data and there has often been an unwillingness to adhere to the principles of scientific objectivity. I have also never heard or read any factual information during the debate that would contradict the conclusion about the safety of E. coli K-12 host-vector systems that I have just reached. It is thus my con-

sidered belief that we are about to embark on excessive regulation of an important area of biomedical research based almost solely on fear, ignorance and misinformation.

Although it is my current opinion that legislation to regulate research on and use of recombinant DNA may be unnecessary, there appears to be a concensus that some form of federal legislation is needed. Given that this is so, I would hope that the legislation enacted be kept as simple as possible. The provisions of such an act should, of course, require that the NIH Guidelines (or some slight modification thereof) apply to all individuals using recombinant DNA molecules for whatever purpose, whether they be in the private or public sector. The legislation should require that proof of compliance be mandatory at the time any product or process utilizing recombinant DNA methodologies is submitted for approval, for testing or use to such agencies as the Food and Drug Administration, the Environmental Protection Agency and/or other agencies empowered with the responsibility for the certification of materials to be added to foods, used in treatment of disease, control of insects, etc. This latter provision is designed for the express purpose of ensuring compliance by those concerns which would not be disclosing their recombinant DNA activities to some governmental or funding agency until such time as they had applied for patent protection. The legislative act should contain sections on preemption of state and local laws, registration, imminent hazard, sanctions, employee rights and sunset.

I am not at all in favor of licensure of laboratories and/or scientists, nor am I in favor of inspections, except of P4 facilities. These provisions would be redundant of what is contained in the NIH Guidelines and would preempt and undermine the functions of local or institututional biohazards committees. The NIH Guidelines as now written require institutional biohazards committees to perform a number of very important functions on a regular basis. Members of these committees are hard-working and conscientious. I doubt that they could continue to act in this manner if most of their functions were taken over by federal inspectors and examiners, who would only visit the institution intermittently and could not ever hope to provide the day-by-day advice that is needed by individuals embarking on recombinant DNA activities. It is thus my honest opinion that the establishment of a federal bureaucracy to license and inspect will be less effective than reliance on and trust in institutional biohazards committees. Certainly, a laboratory approved today can be malfunctioning tomorrow; therefore, peer pressure, availability of immediate expert advice and providing for the rights of employees who might object to a given procedure are more likely to lead to safe practices in the conduct of recombinant DNA activities than will dependence on federal licensing and inspections.

I am also opposed to provisions that might require that all P3 and P4 level experiments be conducted at regional national facilities. Such a decision would preclude certain types of experiments until such facilities were operational. This would very much impede basic biomedical research and any resultant improvements in health care delivery, etc., and could also result in some scientists leaving the country. The long-range effect would be for scientists to locate at these facilities and not at universities, thereby making them un-

available for training of graduate, medical and dental students. The worst possible provision would be a stipulation of specific liability. This would act as a de facto prohibition of recombinant DNA activities in this country, the consequences of which would be staggering.

There are in my opinion some subtle but not inconsequential ramifications of enacting and/or implementing excessive regulations for an area of research in which there are no known hazards and an accumulating body of evidence to indicate that there are none. First, it was the scientists most knowledgeable about recombinant DNA research who initially raised the possibility of potential biohazards. They have, of course, spent much time in debating the issues, in adopting stringent guidelines to preclude manifestation of potential biohazards and in gathering data to evaluate the likelihood of such potential biohazards. It therefore follows that if this area of scientific inquiry becomes encumbered with excessive constraints and is subsequently shown to be associated with no hazards, there may develop a degree of contempt for such regulations. Second, if our country embarks on excessive regulation of recombinant DNA activities, it may lead to regulation of other areas of biomedical research in which the biohazards are well known. In view of the commendable safety performance of individuals engaged in research with biohazardous materials and agents, such regulation of their activities is not likely to improve safety. After all, one cannot legislate against human error and I am sure that these individuals, who are well aware of the risks associated with their occupation, do not want to make errors that could result in harm to themselves. Third, it is evident that excessive and/or additional regulation of biomedical research will increase the cost and decrease the productivity of acquiring knowledge necessary to cure diseases and provide for the health care of our citizenry. Certainly, funds spent on enforcing and adhering to such regulations will diminish the funds available for such productive endeavors. Expenditure of funds for such regulation would only be justified if it were necessary to protect the public and therefore ensure the economic well-being of our country. Since recombinant DNA activities, at least with E. coli K-12 host-vectors, pose no known or expected threat, it is my opinion that legislative enactment of regulations, especially if excessive, are not easily justifiable. It would seem more reasonable to utilize available financial resources to train future scientists in the safe use of recombinant DNA technologies, to continue to evaluate potential biohazards under existing guidelines and to begin to reap the substantial benefits afforded by recombinant DNA research.

Sincerely yours,

Roy Curtiss III

RCIII:kb

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Members, NIH Recombinant DNA Molecule Program Advisory Committee
Members, Senate Subcommittee on Health and Scientific Research
Members, House Subcommittee on Health and Environment
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