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Chapter 9

# Mutagens: Regulatory Considerations

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# Mutagens: Regulatory Considerations

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## INTRODUCTION

It is clear from the information gathered in this report that we are some years distant from being able to muster convincing direct evidence of any but very large increases in the rates of heritable mutations in human beings. The ability to predict from experimental data which agents are likely to increase the mutation rate *if* human beings were exposed has not been put to the test. Even for the most likely mutagens, the ability to make quantitative extrapolations is relatively undeveloped. Nonetheless, it is reasonable and prudent to accept that the environment may contain human germ-cell mutagens and that, to the extent possible, human beings should be protected from them at levels that might cause heritable mutations.

Ionizing radiation was recognized as a cause of heritable mutations in fruitflies in the 1920s, and the Federal Government has since made efforts to protect workers and the public from excessive

radiation exposure. Widespread concern about the mutagenic potential of chemicals is more recent, an issue brought into focus by the environmental movement that took shape in the late 1960s. While the potential cancer-causing properties of man-made chemicals have been the driving force behind environmental health laws, two more recent laws specifically mention mutation as an endpoint against which the public should be protected. About a dozen other statutes include language broad enough to charge the Federal Government with the responsibility to protect against heritable mutations. Evidence from current methods for measuring mutation rates suggests that basing regulation of environmental agents on carcinogenicity will likely assure protection against heritable mutations, but new, more sensitive detection technologies, such as those discussed in this report, may necessitate a reexamination of that conclusion.

## FEDERAL INVOLVEMENT IN PROTECTING AGAINST GENETIC RISK

### Radiation Protection

In 1928, the newly created International X-Ray and Radium Protection Commission was charged by the Second International Congress on Radiology with developing recommendations for protection against radiation (156). The following year, the Advisory Committee on X-Ray and Radium Protection was formed to represent the U.S. viewpoint to the international commission. These two bodies were the forerunners of the current International Commission on Radiological Protection (ICRP) and the National Council on Radiation Protection and Measurements (NCRP), the latter chartered by the U.S. Congress in 1964. The ICRP and NCRP have, since their first recommendations in the 1930s based their acceptable radiation exposure limits on both heritable and somatic effects. The limits recommended have

been lowered over the years, reflecting increased knowledge about radiation effects, and particularly about the effects on the population of low levels of radiation.

Neither the ICRP nor NCRP recommendations have the force of law, but by and large, they have formed the basis for the radiation protection limits adopted by U.S. regulatory agencies. The first Federal entity officially charged with providing the agencies with guidance for developing radiation protection standards was the Federal Radiation Council (FRC), established in 1959. In 1960, FRC issued recommendations for both occupational exposure and exposure of members of the public, which drew on ICRP and NCRP work (156). Over the years, the National Academy of Sciences Committee on the Biological Effects of Ionizing Radiation and the United Nations Sci-

entific Committee on the Effects of Atomic Radiation have also been influential in providing analyses that undergird exposure limits.

The National Environmental Protection Act of 1970 transferred the responsibilities of FRC to the new Environmental Protection Agency (EPA). EPA administers several environmental health statutes under which that Agency is responsible for setting standards for radiation exposure in specific conditions. Under its broader responsibilities, EPA has provided guidance for exposure from diagnostic X-rays, which is the regulatory responsibility of the Federal Food and Drug Administration (FDA), and for exposure of uranium miners, who are the responsibility of the Mine Safety and Health Administration (MSHA).

Agencies other than EPA with responsibility for some aspects of radiation protection include: the Nuclear Regulatory Commission, the Department of Energy, the Department of Defense, FDA, MSHA, the Occupational Safety and Health Administration (OSHA), and the Department of Transportation. The States have responsibilities as well. Each entity, depending on its specific charge, is required to protect workers, the public, or both in accordance with the guidance provided by EPA.

The basic occupational and population exposure guidelines have not been revised since 1960. In 1981, EPA proposed new occupational guidelines (153), in line with 1977 ICRP recommendations (47), but these have not been made final, and they do not represent a change in total acceptable dose from the earlier guidelines. They do, however, place less of the emphasis on mutagenesis, and relatively more on somatic effects than do the 1960 guidelines. The ICRP includes in its risk estimates only genetic effects occurring in the first two generations after exposure. That probably accounts for roughly half of the total genetic effect, whatever its size.

The current occupational exposure limit is 5 rem total body dose per year, with not more than 3 rem total body dose from occupational exposure in any one quarter of the year, and a more detailed breakdown for different groups of organs. The 1977 ICRP recommendations abandon the speci-

fications by organ, and use a weighted whole-body dose.

Today, almost all radiation exposures of U.S. workers are well below the regulated limits, though there are exceptions. The quantitative limits set by the ICRP and NCRP and adopted by Federal groups, are accompanied by the "ALARA principle"—that radiation exposures should be "as low as reasonably achievable."

Exposure to the public are to be limited to below 25 millirem (mrem) to the whole body, 75 mrem to the thyroid, and 25 mrem to any other organ. These levels mainly affect the regulation of radionuclides in air and the disposal of radioactive waste. The numbers come from ICRP and NCRP recommendations, and are based on consideration of both genetic and somatic effects.

## Agents Other Than Radiation

Congress formally recognized the need to protect against chemical mutagens in the Toxic Substances Control Act of 1976 (TSCA), and again in 1980 in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, or "Superfund"). These two laws are administered by EPA, as are other statutes that include broad mandates to protect the public from environmental hazards. Other laws designed to protect citizens from external agents under which chemical mutagens could be regulated include the Federal Food, Drug, and Cosmetics Act, administered by FDA; the Occupational Safety and Health Act, administered by OSHA; the Consumer Product Safety Act, administered by the Consumer Product Safety Commission; and the Atomic Energy Act, through which the Nuclear Regulatory Commission is empowered to protect certain workers from radiation hazards.

Although it is almost certain that chemicals that might cause heritable mutations have been regulated, no regulations have been written or standards set for these agents because of that property. In a few cases, the mutagenic potential of chemicals has been considered by regulatory agencies, but carcinogenic properties have driven standard-setting.

OSHA has included thorough reviews of mutagenicity data in notices of regulatory actions for two of the best-publicized chemical hazards of the 1980s: ethylene oxide (EtO) and ethylene dibromide (EDB). Tests for heritable mutagenicity in *Drosophila* and experimental mammals have yielded positive results in at least some systems for both of these chemicals. While results of mutagenicity tests are included in the Federal Register notices for these chemicals, quantitative extrapolations for both the final EtO standard (152), and the proposed rulemaking for EDB (151), are based on protecting against carcinogenicity.

EPA has recognized germ-cell mutagenicity as a class of adverse effects, particularly in its responsibilities under the Federal Insecticide, Fungicide, and Rodenticide Act, under which it, in addition to OSHA, has acted to regulate exposures to EtO and EDB. Unique among the regulatory agencies, EPA's Reproductive Effects Assessment Group (in the Office of Research and Development) has prepared guidelines for mutagenicity testing, which are described later in this chapter.

## REGULATORY ISSUES

Currently there does not appear to be a scientific basis for the specifics of regulatory action against mutagens. The following questions face regulators and the scientific community involved in mutation research:

1. What is an appropriate regulatory definition of a probable human germ-cell mutagen and how does that definition relate to what is known about mutagens from epidemiologic studies and experimental studies?
2. Is it possible to derive quantitative estimates of the risk of heritable mutations in humans from experimental evidence in animals or from somatic-cell mutation tests in human beings? If it is not, what kinds of information are necessary before such extrapolation is possible?
3. How likely is it that a substance will require a more stringent standard as a mutagen than it will as a carcinogen or for other toxic effects?
4. How will information from the new technologies for detecting heritable mutations that are described in this assessment change our perception of the kinds of "adverse effects" against which regulation should be directed?

The discussion in the remainder of this chapter addresses these questions.

### A Regulatory Definition of a Germ-Cell Mutagen

Strategies for regulating mutagens to protect public health cannot today rely on data from current studies of heritable mutations in human beings. Just as is the case in regulating carcinogens, a regulatory definition must serve as a substitute, particularly for making judgments about the potential risks of new substances.

Certain lessons can be learned from experience in regulating carcinogens. (For a discussion of the issues surrounding carcinogen regulation, see 145. ) The most convincing evidence for carcinogenicity, from well-conducted epidemiologic studies, is that human beings have developed cancer after exposure to a given agent. If an increase in genetic *disease* could be convincingly shown to be related to a specific agent, there would certainly be no problem in acting against that agent. The spirit of the regulatory laws, however, embody the concept of taking protective action before people are harmed. In the absence of direct evidence from human beings, regulators must rely on indirect evidence from a variety of experimental test systems which will *never be absolutely predictive* of effects in human beings. A regulatory definition of a mutagen will be pragmatic and rely on

information that it is possible to collect, and on a number of untested assumptions.

EPA is the first U.S. regulatory agency to have proposed "Guidelines for Mutagenicity Risk Assessment" (154). The guidelines require evidence of: 1) mutagenic activity and 2) chemical interactions in the mammalian gonad. The decision to regulate is to be based on a "weight-of-evidence" determination. EPA has proposed no formal method for quantitative extrapolation by which acceptable exposure levels could be set.

According to the EPA guidelines, evidence for mutagenic activity may come from tests that detect point mutations and structural or numerical chromosome aberrations. Structural aberrations include deficiencies, duplications, inversions, and translocations. In the absence of evidence of heritable mutations in human beings, evidence from a variety of experimental test systems may be invoked. For mutagens that cause point mutations, whole animals tests (e.g., the mouse specific-locus test) provide the highest degree of evidence, but these tests are relatively more expensive than short-term tests, and there is a limited capacity for laboratories to perform them. Other tests for point mutations include those in bacteria, eukaryotic micro-organisms, higher plants, insects, and mammalian somatic cells.

Structural chromosome aberrations can be detected either in somatic or germ cells in different assays. The organisms used include higher plants, insects, fish, birds, and several species of mammals. Mutagens that cause numerical changes in chromosomes may be missed by the tests that directly measure DNA damage. Tests specifically directed at detecting changes in chromosome number are not as well developed as are those for point mutations or structural changes in chromosomes. Tests are in various stages of development in fungi, *Drosophila*, mammalian cells in culture, and intact mammals, including mammalian germ-cells tests.

Results from tests that measure endpoints other than mutagenicity directly may also be used in judging the potential mutagenicity of a substance. DNA damage, unscheduled DNA synthesis in mammalian somatic and germ cells, mitotic recombination and gene conversion in yeast, and

sister chromatid exchange in mammalian somatic and germ cells are cited by EPA as tests that provide evidence known to be correlated with mutagenicity, though they measure other genetic events.

Evidence from various kinds of mutagenicity tests is weighted with regard to the relationship of the test to human germ-cell mutation. Greater weight will be given to results from tests in: 1) germ cells over somatic cells, 2) mammalian cells over submammalian cells, and 3) eukaryotic cells over prokaryotic cells.

EPA lists two classes of evidence for chemical interactions in the mammalian gonad: sufficient and suggestive. Sufficient evidence is from studies in whole mammals that demonstrate, for example, unscheduled DNA synthesis, sister chromatid exchange, or chromosomal aberrations in germ cells. Adverse effects on the gonads or on reproductive outcomes after exposure, which are consistent with the substance reaching the gonads but which do not indicate direct interaction with DNA, are considered as providing suggestive evidence.

The final step in EPA's mutagenicity risk assessment is the weight-of-evidence determination, which classifies the evidence for potential germ-cell mutagenicity as "sufficient," "suggestive," or "limited." In this step, results of tests plus any information about effects in human beings is evaluated. Sufficient evidence consists of a positive mammalian germ-cell test. In addition, positive responses in at least two different test systems, at least one of which is in mammalian cells, and evidence of germ-cell interaction, together constitute sufficient evidence. Evidence of lesser quantity and/or quality of both mutagenic response and germ-cell activity constitute suggestive evidence. Limited evidence consists of positive results in either mutagenicity assays or tests for chemical interactions in the gonad, but not both.

EPA's guidelines became final in September 1985. Currently and for the foreseeable future, the greatest value of EPA's guidelines is the recognition of germ-cell mutagenicity as a legitimate endpoint to consider in assessing the potential adverse effects of substances in the environment.

## Quantitative Extrapolation

If the levels of risk from suspected human germ-cell mutagens is to be estimated in the absence of direct evidence of harm in human beings, data from experimental systems must be used in a “quantitative extrapolation.” The experimental systems are basically those mentioned in the EPA guidelines discussed in the previous section, and those discussed elsewhere in this report. The three categories of tests are: 1) whole animal heritable mutation studies; 2) animal somatic-cell mutation studies, either *in vivo* or *in vitro*; and 3) human somatic-cell mutation studies, either *in vivo* or *in vitro*. Unfortunately, the kind of information (i.e., measures of human mutations) that would link results from these three categories of tests to human heritable mutations is scanty. It is encouraging, however, that using tests available now, such information can be generated at least for some substances. For EtO and EDB, for instance, mutagenicity data are available in both somatic and germ-cell systems in animals, and some somatic cell (cytogenetic) data are available from human beings exposed at known occupational levels.

Obtaining more information to fill in the arrows of the extrapolation “parallelograms” presented in chapter 7 of this report should be a high priority for regulators. In fact, EPA’s Reproductive Effects Assessment Group has collaborated with other groups in the Federal Government to fund such studies (168). Without the kind of information that would come from coordinated studies in several test systems, there is little chance of writing a successful regulation that limits exposure to a specific level (short of a complete ban for an agent acknowledged to be unacceptably risky at any level). Given the experience with carcinogens, a regulation that states an exposure level without adequate experimental evidence and theory behind it will not survive a court challenge, which, in the United States today, appears to be the final test of a regulation.

Even with good experimental data, some of the same problems that plague extrapolating from animals to humans to determine acceptable exposure levels for carcinogens are certain to hinder quantitative extrapolation for estimating levels of mutagenic risk at specific levels of exposure. In

carcinogen extrapolation, there still are unresolved controversies about the appropriate conversion factors between species and about the shape of dose-response curves. The latter is important because most animal bioassays test extremely high doses in relation to the animals’ body weights, while humans are generally exposed at lower levels over longer periods of time. Though the details of extrapolation for mutagenicity differ from those for carcinogenicity, the problems will undoubtedly be similar. Right now, there is not enough information about the relationships between results in various test systems to address intelligently the practical problems of actually performing extrapolations.

Given the appropriate information, it may become possible to carry out quantitative extrapolations for mutagenicity. When the time comes, the regulatory agencies will need to require that the appropriate tests are done, either by manufacturers or by the Federal Government. The testing requirement may take various forms, which may vary by statute.

## Mutagenicity and Carcinogenicity

As mentioned previously, while the regulatory apparatus exists for acting against human germ-cell mutagens, in fact no regulations based on germ-cell mutagenesis exist except for radiation exposure limits. There are several reasons for this. First, there are no proven human germ-cell mutagens, and only a limited number of presumptive human mutagens known from animal tests. Second, it has often been thought that regulations based on demonstrated carcinogenicity would automatically protect against mutagenicity as well. In fact, however, this may not always be true. Voytek (167) reported a preliminary assessment indicating that the risk of heritable genetic disease in the first generation after exposure to EDB was greater than the lifetime risk of cancer in the exposed individuals, based on an extrapolation from animal data.

It is widely held that a somatic mutation is a necessary step in the development of cancer. Many substances that are mutagenic in bacteria, in cells in culture, and in *Drosophila* also are carcinogenic in laboratory rats, mice, or both. Short-

term tests based on mutagenicity in these lower organisms are therefore used as screens for carcinogenicity. The most widely used screening test for carcinogenicity is the Ames test, which measures mutagenicity in strains of the bacterium *Salmonella*. Under some statutes (e.g., TSCA), negative results in short-term tests, meaning that the substance is not mutagenic in those systems, can obviate the need for a long-term bioassay, a savings of up to \$1 million to a manufacturer (145). Positive results in short-term tests, meaning that the substance is mutagenic in these systems, have not been accepted as a basis for regulation under any statute, but they have probably halted the development of new chemicals. Manufacturers know that positive mutagenicity tests may trigger the requirement for a long-term bioassay, which in a high proportion of cases will turn out positive. The product, whatever it is, might never get to market. Rather than risk a financial loss, many manufacturers simply will not proceed with that product.

### **New Methods for Measuring Mutation Rates and Their Potential Effects on Regulation**

The new techniques for detecting heritable mutations and the somatic-cell techniques that eventually may be used to predict germ-cell mutagenesis will lead to the consideration of effects that are increasingly removed from measurable or even hypothetical adverse health effects in humans. Mutagenicity endpoints may be detected that could be more sensitive than those currently used to predict carcinogenicity. In the regulatory context, judgments will have to be made, most likely in the absence of certain knowledge of effects,

about the appropriate actions to be triggered by demonstrations of various kinds of changes in DNA, detectable by various analytic methods, that can be convincingly linked to specific exposures. From a public health standpoint, it is most appropriate to act under the assumption that mutations of any kind are deleterious, and that environmental agents at levels that cause any reliably detected changes in the DNA should be subject to available regulatory controls. This does not get around the problem of defining "safe" or "acceptable" exposure levels, however. Animal experiments will be needed to explore the quantitative relation between subtle changes detectable anywhere in DNA and the levels of adverse effects that might be observed in the animal.

At present, testing for safety is a significant part of the research and development investment in new products, whether they are chemicals, drugs, or food additives; determining the risks of substances already in the environment is a significant task for the Federal Government. If they become available, new mutagenicity testing technologies using experimental animals could either impose significant new testing requirements in addition to those already in place, or could replace some expensive and not entirely reliable tests for carcinogens. Regardless of the methods used to detect mutations, the relation between specific types of changes in DNA and health effects will have to be studied experimentally to shed light on the meaning of a "positive result." Until this knowledge is available, the ability to detect an effect without knowing the likelihood of any health consequences for human beings will remain a thorny public policy question which scientists, regulators, and politicians must address.