39. J. P. Kennett and P. Huddlestun, *Quat. Res.* **2**, 38 (1972).
40. R. A. Stacey, *Nature (Lond.)* **250**, 133 (1974).
41. S. Moorbath, H. Sigurdsson, R. Goodwin, *Earth Planet. Sci. Lett.* **4**, 197 (1968).
42. H. Sigurdsson, *Iceland and Mid-Ocean Ridges*, S. Bjornsson, Ed. (Societas Scientarium Islandica, Reykjavik, 1967), pp. 32–49.
43. ———, personal communication.
44. R. J. Adie, *Antarct. Geol. Geophys. Int. Union Geol. Sci. Ser. B No. 1* (1972), pp. 137–1141.
45. W. Dort, *ibid.*, pp. 645–652.
46. V. E. Fuchs and T. T. Peterson, *Geol. Mag.* **84**, 322 (1947).
47. P. A. Mohr, *Bull. Volcanol.* **32**, 5 (1968).
48. J. M. Ade-Hall, P. H. Reynolds, P. Dagley, A. E. Mussett, T. P. Hubbard, E. Klitzsch, *Can. J. Earth Sci.* **11**, 998 (1974).
49. G. Siedner and A. Horowitz, *Nature (Lond.)* **250**, 23 (1974).
50. W. A. Berggren, in *Initial Reports of the Deep Sea Drilling Project* (Government Printing Office, Washington, D.C., 1972), vol. 12, pp. 953–964.
51. N. J. Shackleton and J. P. Kennett, in *ibid.*, vol. 29, in press.
52. J. P. Kennett and P. Vella, in *ibid.*, vol. 29, in press.
53. W. J. Humphreys, *Physics of the Air* (McGraw-Hill, New York, 1940); H. Wexler, *Sci. Am.* **186**, 74 (1952); H. H. Lamb, *The Changing Climate* (Methuen, London, 1966); *Climate, Present, Past, and Future* (Methuen, London, 1972), vol. 1; J. P. Kennett and N. D. Watkins, *Nature (Lond.)* **227**, 930 (1970); J. C. Schofield, *N.Z. J. Geol. Geophys.* **13**, 737 (1970); A. J. Gow and T. Williamson. *Earth Planet. Sci. Lett.* **13**, 210 (1971).
54. J. Chappell, *Quat. Res.* **3**, 221 (1973).
55. H. Stille, *Grundfragen der Vergleichenden Tektonik* (Borntraeger, Berlin, 1924).
56. J. Gilluly, *Geol. Soc. Am. Bull.* **60**, 561 (1949).
57. M. Brookfield, *Earth Planet. Sci. Lett.* **12**, 419 (1971).
58. H. Kuno, *Geol. Surv. Can. Pap. 66* (1965), pp. 317–336; W. R. Dickinson, *J. Geophys. Res.* **73**, 2261 (1968); D. H. Green and A. E. Ringwood, *Contrib. Mineral. Petrol.* **18**, 105 (1968); T. Hatherton and W. R. Dickinson, *J. Geophys. Res.* **74**, 5301 (1969); D. L. Turcotte and E. R. Oxburgh, *Phys. Earth Planet. Interiors* **1**, 381 (1968); E. R. Oxburgh and D. L. Turcotte, *Geol. Soc. Am. Bull.* **81**, 1665 (1970); *J. Geophys. Res.* **76**, 1315 (1971).
59. K. Mogi, *Tectonophysics* **22**, 265 (1974).
60. P. R. Vogt, G. L. Johnson, T. L. Holcombe, J. G. Gilg, O. E. Avery, *ibid.* **12**, 211 (1971).
61. Tj. H. Van Andel and T. C. Moore, *Nature (Lond.)* **226**, 328 (1970).
62. R. J. Blakely, *J. Geophys. Res.* **79**, 2979 (1974).
63. J. R. Heirtzler, G. O. Dickson, E. M. Herron, W. C. Putman, X. Le Pichon, *ibid.* **73**, 2119 (1968); J. D. Phillips, *Science* **157**, 920 (1967); ———, G. Thompson, R. P. Von Herzen, V. T. Bowen, *J. Geophys. Res.* **74**, 3069 (1969); Tj. H. Van Andel and C. O. Bowin, *ibid.* **73**, 1279 (1968).
64. F. J. Vine, *Science* **154**, 1405 (1966).
65. R. K. Mathews, *Earth Planet. Sci. Lett.* **5**, 459 (1969).
66. N. Mörner, *Tellus* **24**, 586 (1972).
67. F. J. Mauk and M. J. S. Johnson, *J. Geophys. Res.* **78**, 3356 (1973).
68. W. L. Hamilton, *ibid.*, p. 3363.
69. W. A. Berggren, in *Symposium on Messinian Events in the Mediterranean*, C. W. Drooger, Ed. (North-Holland, Amsterdam, 1974), pp. 10–20; *Lethaia*, **5**, 195 (1972); Tj. H. Van Andel, G. R. Heath, T. C. Moore, *Geol. Soc. Am. Mem.*, in press.
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Environmental Mutagenic Hazards

Mutagenicity screening is now both feasible and necessary for chemicals entering the environment.

It has become increasingly clear in recent years that man is environmentally exposed to a wide variety of chemicals, some of which are mutagens (agents that induce mutations). The possibility therefore exists that human beings may be exposed, now or in the near future, to chemicals with individually or collectively powerful mutagenic effects. We describe here the ways in which environmental mutagens may be detected, possible methods of estimating the resulting adverse effects on human health, and regulatory principles that bear on the problem of preventing environmental mutagenesis.

Mutation consists of abrupt heritable changes in the composition or arrangement of genes, which are composed of deoxyribonucleic acid (DNA). Most mutations producing effects large enough to be observed are deleterious, although other mutations may produce effects of little or no consequence, and certain rare mutations may even be advantageous. The magnitudes of spontaneous mutation rates, the way selection acts on various gene combinations, and the size and structure of human populations are sufficient to maintain a rich source of genetic variability. An artificially increased mutation rate, however, is potentially capable of producing a general decline in genetic health unless balanced by increased selection against deleterious mutant genes; while such selection occurs extensively in most natural populations, the efficacy of modern medicine may increasingly tend to reduce selection against deleterious traits in many human populations.

Many geneticists believe that man's genes constitute his most precious heritage, and that a deterioration in gene quality can result in a corresponding decrease in the quality of life. Steady progress in the control of infectious diseases, lengthening human life spans, and improved procedures for identifying genetic disorders have revealed an important residue of genetic disease in human populations. An impressive proportion of hospital admittances, for instance, are now recognized as reflecting genetic disabilities (*1*). The prospects for directly curing the resulting genetic diseases, in contrast to merely alleviating their symptoms, are poor, and are not likely to improve in the near future. Furthermore, the wide variety of mechanisms by which radiations and chemicals induce mutations (*2*) make it very unlikely that generalized schemes can be devised to protect against mutagens, except by avoiding them in the first place.

Considerations such as these have recently led geneticists throughout the world to seek test systems capable of detecting environmental mutagens. A number of reports about the environmental mutagenesis problem have already appeared (*3*, *4*), and a special sec-

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tion of the journal *Mutation Research* (*5*) has been reserved for papers bearing on the subject. Chemical mutagens and their detection have been the subject of several books (*6*), and an important information retrieval service is now available (*7*). A number of mutagenicity tests are available with the result that some scientific basis already exists for determining what chemical compounds are mutagenic, and for deciding how to limit man's exposure to mutagenically active compounds. This article describes some of these mutagenicity test systems, the significance of data obtained from them, and some ways in which these data can be applied to the protection of human populations.

Screening Systems for Environmental Mutagens

By the time that the effects of an increased human mutation rate become manifest, genetic damage will already have occurred. Furthermore, it is quite possible for substantial environmentally induced genetic damage to go forever undetected, since many deleterious mutations are already present in the human gene pool, and since the same kinds of abnormalities sometimes occur for other, nonmutational reasons. Since there may often be no way to identify environmental mutagenesis as the specific cause of observed abnormalities, it is crucial to identify potential mutagens *before* they can induce genetic damage in the population at large: sensitive and reliable test systems are needed which can be used to screen large numbers of potential mutagens. Since trace chemicals that have passed imperfect screening systems might still produce weak but cumulatively important mutagenic effects, it is also important to develop efficient methods for monitoring human populations for abnormalities likely to have arisen by mutation.

Mutations can arise in both somatic and germ line cells. Somatic mutation, and its probably close correlate, carcinogenesis, are already recognized as immediate toxicological problems. In this article, however, our primary concern is with the genetic health of future generations, and we therefore focus upon germ line mutations. For our purposes, the greatest significance of somatic mutation will be its use as a monitoring device. We will consider first the desirable characteristics of an ideal set of screening systems, and next the capabilities of the currently available systems, together with the kinds of additional information that will be needed to apply the results obtained from screening programs.

Characteristics of the Ideal Screening System

Sensitivity and reproducibility. The sensitivity of a test system must be sufficient to detect with ease and statistical accuracy a small mutational effect. A small effect for our purposes consists of one that would produce even a small increase over the spontaneous human mutation rate, whether in particular individuals or in the population at large. Very large concentrations of chemicals, much larger than those to which human beings are likely to be exposed, can be tested in most experimental systems. Furthermore, with the exception of mammalian specific locus and dominant lethal tests, most screening systems employ large numbers of organisms. Sensitivity therefore need not often be a limiting factor in mutagen screening (except in the intact mammal, where the number of animals involved is always relatively small).

Reproducibility is a more demanding criterion. High levels of reproducibility must be demonstrated both within any given laboratory and among different laboratories. This can be achieved only by adopting standardized protocols and by training those conducting the tests to high levels of technical competence. Reproducibility is also desirable in the form of similarity of response among different test systems, which permits a more confident interpretation of positive or negative screening data. Because of metabolic factors, however, and also perhaps because of differences in the composition of prokaryotic and eukaryotic chromosomes, different test systems frequently do not yield uniformly positive or negative responses.

Detecting the whole mutagenic spectrum. With the exception of certain viruses, the genetic material of all organisms consists of DNA, often complexed with ribonucleic acid (RNA) and proteins. It is becoming increasingly clear that the basic types of mutations occur ubiquitously (*2*). In principle, therefore, mutagenicity can be scored in any organism. The significance of a mutational response is greatly enhanced, however, in those organisms whose genetics have been most extensively studied; it is important, for instance, to possess sufficient background information to determine whether a mutational response represents all or only certain possible types of genetic alterations.

Being an error process, mutation consists of all possible changes in the genetic material (excluding recombination and segregation). Furthermore, the severity of any given mutation depends both upon the importance of the affected gene or genes and upon the nature of the mutational lesion itself. Eventually, therefore, suitable test systems must readily detect all classes of mutations, including those with relatively minor effects upon gene function. The kinds of mutations that require detection, although not necessarily simultaneously in the same system, consist of the following:

1) Changes in chromosome number. Changes in chromosome number commonly result in drastic gene imbalance, and are likely to be lethal early in development. The most common viable changes are monosomy and trisomy.

2) Changes resulting from chromosome breaks. These consist of deletions, duplications, inversions, and translocations. Small deletions in homozygous condition and large deletions in heterozygous condition are likely to be highly deleterious and to produce early lethality, whereas many of the other types may allow development but produce genetic disease in the affected individual. Rearrangements (inversions and translocations) can be detected cytologically so long as they involve fairly large proportions of chromosomes; they can also be detected by changed linkage relationships in suitable organisms. Chromosome breaks per se can also be detected. Many chromosome breaks are repaired, however, or lead to the death of the afflicted germ line cell without further deleterious consequences: there is as yet no evidence that clearly demonstrates that breaks themselves constitute heritable mutations. While we do not believe that the induction of breaks alone is a valid index of heritable genetic damage, the appearance of breaks should be interpreted as a warning signal, since it is likely that concomitant chromosomal rearrangements would frequently be detected if screening were sufficiently extended.

3) Single gene mutations. These consist of base pair substitutions and of

additions or deletions of base pairs. Base pair addition and deletion mutations usually inactivate the affected gene completely, so that their severity depends upon the nature of the target gene itself. Most base pair substitutions, on the other hand, only mildly affect the target gene. In either case, however, point mutations are likely to allow the afflicted individual to survive and reproduce, and may thus be transmitted and affect subsequent generations. In terms of human suffering, therefore, the summed effects of single gene mutations probably exceed the deleterious effects of changes in chromosome number or arrangement.

Basic units and the spontaneous baseline. Estimates of mutation rates in man are based on grossly deleterious traits. Studies on man to date indicate an average mutation rate of 10^{-5} to 10^{-6} per locus per sexual generation (*8*), and may even exceed one per diploid genome per sexual generation. The evidence for the second value is indirect, and depends upon estimates of the number of loci in the human genome and upon extrapolation from *Drosophila*. Whether these rates are characteristic is unknown; the possibility that subtle biochemical traits mutate at higher rates has recently been raised (*9*). Nearly 2000 genetically determined human defects are already known (*10*), but these undoubtedly represent only a fraction of the total loci. The number of loci in *Drosophila*, whose total amount of DNA per cell is about 20-fold less than that of man, is on the order of 5000 (*11*), and it is therefore reasonable to assume up to 10^5 loci for the human genome. As a very approximate estimate, therefore, 10^{-5} mutations per locus per human generation times 10^5 loci gives about one mutation per genome per human sexual generation. On the other hand, a recent report (*12*) suggests that the number of loci in man may not be much greater than the number in *Drosophila*, but at the same time suggests that human loci may be intrinsically much more sensitive to (radiation-induced) mutagenesis than are *Drosophila* loci; thus the mutability of the entire human genome would still be similar to that of the entire *Drosophila* genome, which may be as high as one per diploid genome per sexual generation (*13*).

It will be important when extrapolating mutation rates from test systems to man to employ certain basic units. Two units are suggested for adoption at this time, one based upon the spontaneous mutation rate and one upon radiation equivalents. The suggested units are:

1) The rate-doubling concentration. The concentration of chemical that produces as much genetic damage as occurs spontaneously in the same period of time in a particular test system (for instance, 10 spontaneous mutations plus 10 induced mutations equals 20 total mutations). Given suitable additional information, this unit could be converted into the *human rate-doubling concentration*, which could conveniently be expressed in milligrams per kilogram of whole body weight.

2) The rem-equivalent-chemical (REC). A vast store of information is already available concerning radiation-induced mutagenesis, the standard unit of measurement being the rem (radiation-equivalent-man). The REC is that dose or product of concentration multiplied by time which produces an amount of genetic damage equal to that produced by 1 rem of chronic irradiation. As a very simple example, REC's might be calculated as follows. Suppose that in a test system, 1 rem of radiation produces 1000 mutations and that for every kilogram of body weight 1 mg of a chemical acting over 5 days produces 50 mutations. Then 1 mg/kg of the chemical applied over 1 year would produce $(50 \times 365/5)/1000$, or 3.65 REC; and the same dose applied over the average human reproductive life-span (30 years) would produce 30×3.65, or 110 REC. (Note, however, that this calculation assumes implicitly that the response of the organism, as modulated by numerous pharmacological factors, remains constant in time.)

It is important to note that in extrapolating from a test system to man, it is frequently unknown whether the ratio of chemical mutagenicity to radiation mutagenicity remains constant and is linear with time, and whether the test system genes are similar to average human genes. Different genes vary considerably in their mutational responses to different mutagens (some of which, for instance, may affect only special sequences within the gene), and even larger variations are observed among different reversion tests. Furthermore, physiological states (such as the active versus the repressed condition) frequently influence mutation rates. Only the extensive accumulation of comparative information can indicate the reliability of extrapolation procedures. It has recently been shown, however, that the effects of radiation are remarkably constant in diverse biological systems when the results are adjusted for the amount of DNA per nucleus (*12*).

We therefore recommend that experiments be initiated with several selected chemicals representing different classes of mutagens to see whether the same generalization can be made with chemical mutagens; specific locus tests, for instance, can now be conducted with a number of systems, including microorganisms, *Drosophila*, the mouse, certain plant systems, and cultured human cells. The reliability of such a comparison would be strengthened by determining the amount of the chemical mutagen which actually penetrates to the genetic material.

Metabolic factors. Comparative studies of mutagenicity in prokaryotic and higher eukaryotic systems, and biochemical analyses of the fates of administered compounds, have provided many instances where chemicals are mutagenically inert until they are metabolically activated. Conversely, many mutagenically active compounds are metabolically converted to inactive derivatives, or are so active chemically that they spontaneously hydrolyze or react with (for instance) serum components to produce inactive products. Ideal test systems should therefore be capable of carrying out metabolic functions similar to those characteristic of man. For example, prokaryotic test systems can incorporate suitable human or other mammalian tissue fractions (such as liver microsomes), or mammalian organisms can be treated with a chemical and their appropriate tissue extracts (including urine) can then be applied to microbial test systems (*14*). Any detailed examination of a particular compound should include an analysis of its metabolic fates (at least in experimental animals), and such analyses should extend to sufficiently large numbers of individuals so that genetically determined variability in metabolic activities is likely to be discerned.

Dosimetry. It is common laboratory practice to introduce test substances into a mammal both by natural routes (such as inhalation, ingestion, or absorption through the skin) and by artificial routes (such as intraperitoneal or subcutaneous injection). It has long been evident in general toxicological testing that different routes of administration can strongly influence the out-

come. Exposure routes become especially significant when one concentrates specifically on the gonadal effects of an agent, and it is particularly important to note that the metabolic disposition of a compound depends not only upon the biochemical capabilities of mammalian tissues, but also upon the activities of organisms present in the gut. The conversion of cyclamate to cyclohexylamine by gut organisms, for instance, is the first step in the activation of this artificial sweetener to a mutagenic derivative. Intact-mammal test systems should therefore be presented with test compounds in ways which reflect as closely as possible the realities of natural exposure routes. While quantitative dosimetry may thereby be made more difficult, net gonadal dosimetry may be determined empirically regardless of the route of administration, as discussed below.

Quantitative extrapolation from test systems to man should be based not simply upon single-dose tests, but instead upon dose-response curves. When dose-response curves take the form of straight lines on an arithmetical scale, the lowest-dose points remain close to the line, and the line extrapolates to the spontaneous rate at zero dose, interpolation is fully justified. Curvilinear relationships, however, present special problems, since they suggest either that the mutagenic response may become insignificant at very low doses, or even that true thresholds may exist.

As far as we are aware, no information exists proving or disproving the existence of a threshold response to a mutagen in any system; when a mutagen active at a high concentration produces no detectable effect at a low concentration, it is generally impossible to determine with confidence that sufficient statistical accuracy has been achieved to conclude that an effect is truly absent. It therefore seems to us that the best practice, when extrapolating from curvilinear dose-response curves, consists of interpolating linearly between the spontaneous rate at zero dose and the response from the lowest dose tested for which reliable data exist. It is unlikely that this procedure will underestimate the risk. If there is a threshold, or a deviation from linearity in that direction, this procedure will overestimate the risk, which we assume to be the prudent policy to follow in a matter of this importance. As more data on low doses accumulate, the extrapolation line can be regularly revised.

Mutagen interactions. Although the sources of ionizing radiations are diverse, qualitatively different radiation doses can be summed in fairly reliable ways to produce estimates of total mutagenic effects (*4*). Summation is likely to be much more difficult in the case of chemical mutagens, however, for there are both experimental and theoretical grounds for anticipating that significant interactions will occur among such compounds. We are particularly concerned with the possibility of synergism, either between two mutagens, or between a mutagen and a nonmutagenic agent which enhances its mutagenicity. At present there are few guidelines to assist in deciding which of the myriad of possible combinations of compounds should be tested for synergistic or enhancing interactions. Two groups of compounds that are suspect, however, are those that inhibit DNA repair processes (such as caffeine), and those that either induce enzymatic activities in mammalian tissues which are likely subsequently to activate compounds to mutagenic derivatives, or inhibit enzymatic activities which are capable of deactivating the mutagenic potential of chemicals. However, there is little that can be done now except to assume, until information to the contrary accumulates, that all mutagens produce simple additive effects. At the low exposure levels which we hope will be found to occur in human populations this is a reasonable initial procedure.

Characteristics of Currently Employed Screening Systems

A number of mutagen screening systems have now been developed to the point of practical application. Detailed descriptions of these systems are available (*15*), and need not be given here. Instead, we have tabulated them in two ways: according to their ability to detect various types of genetic damage, and according to their operational characteristics.

Systems that are currently being put to at least some use are listed in Table

Table. 1. Types of genetic damage detected by currently employed mutagen screening systems.

Screening system		Type of damage detected						
		Chromosome aberrations				Gene mutations		
Category	Organism	Dominant lethality	Translocations	Deletions and duplications	Nondisjunction	Forward or reverse or both	Multiple specific locus	Induced recombination
Bacterial	*Salmonella typhimurium*					+		
	Escherichia coli					+		
Fungal	*Neurospora crassa*			+	+	+	+	
	Aspergillus nidulans				+	+	+	+
	Yeasts	+			+	+	+	+
Plant	*Vicia faba*		+	+	+			
	Tradescantia paludosa		+	+	+	+		
Insect	*Drosophila melanogaster*	+	+	+	+	+	+	+
	Habrobracon juglandis	+	+			+	+	+
	Bombyx mori	+				+	+	
Mammalian cell culture	Chinese hamster		+	+	+	+		
	Mouse lymphoma		+	+	+	+		
Intact mammal	Mouse	+	+	+	+		+	
	Rat	+	+	+	+			
	Man		+	+	+			

1. We have omitted from the table the larger number of test systems now under development but not yet employed in screening. The dominant lethal response is listed in the chromosome aberration group since a large proportion of dominant lethal mutations do in fact consist of chromosomal aberrations. Among the gene mutations, the multiple specific locus tests simultaneously screen forward mutations at from several loci (as in the mouse) to several hundred loci (as in the sex-linked recessive lethal screening system in which *Drosophila* is used). Since an impressive correlation exists between mutagens and agents which enhance rates of recombination (*16*), we have also included a final column indicating which systems have been employed to screen directly for induced increases in recombination rates.

Most of the nonmammalian test systems (except for the plant, *Habrobracon*, and *Bombyx* systems), and also the mammalian cell culture systems, have been adapted for use with metabolic activation systems. Three methods are currently employed for coupling the two types of systems. In the first, metabolically active tissue extracts (such as rodent liver microsomes) are added to a submammalian test system along with the suspect compound. In the second, an intact mammal (either rodent or man) is exposed to the suspect compound, and tissue extracts (including body fluids such as blood and urine) are collected and added directly to a submammalian test system. In the third (the host-mediated assay), a microbial or cultured mammalian cell population is introduced into an appropriate rodent body cavity, the intact animal is exposed to the suspect compound, and the test cell population is later recovered and assayed for mutational damage. It should also be noted that *Drosophila* possesses a microsomal enzyme system similar to that found in mammalian liver, and has been used to identify mutagens requiring microsomal activation.

Among the screening systems listed in Table 1 which can detect gene mutations, only a few (the bacteria, *Neurospora*, and the yeasts) permit the identification of the specific types of molecular lesions which are induced.

Table 2 summarizes some of the operational characteristics of current test systems. It is too early to assess the eventual operating efficiencies of these systems, and we have therefore tended to use simple qualitative comparisons. The factors which seem to be of most immediate importance are: the times required to conduct the tests, given a fully staffed and equipped laboratory; the operating costs (materials and personnel); the initial investment costs; and the relative ease of mutation detection, including the magnitudes of the responses obtained with known mutagens, the degree to which the tests lend themselves to quantitation (for example, the feasibility of obtaining dose-response curves), and test reproducibility. Where operating characteristics cannot yet be determined with any reliability, or where the test system is not actually in significant use (for instance, *Aspergillus nidulans, Habrobracon juglandis*, and *Bombyx mori*), it has simply been omitted from the table.

Correlative Information Required for Decision-Making

The identification of a compound as a mutagen is only the first step in estimating the hazard it may pose to man. In order to estimate the level of human exposure and to set priorities for testing, additional information is required concerning the amounts and distribution patterns of the compounds in the environment, its persistence both without and within the human body, its metabolic disposition, and the possibly varying susceptibilities among exposed individuals of differing genotypes and living habits.

Production levels. Primary produc-

Table 2. Operational characteristics of mutagen screening systems.

Test system	Time to run test	Operating costs*	Initial investment costs	Relative ease of detection†	
				Gene mutations	Chromosome aberrations
Microorganisms with metabolic activation:					
Salmonella typhimurium	2 to 3 days	Very low	Low	Excellent	
Escherichia coli	2 to 3 days	Very low	Low	Excellent	
Yeasts	3 to 5 days	Very low	Low	Good	Unknown
Neurospora crassa	1 to 3 weeks	Moderate	Moderate	Very good	Good
Cultured mammalian cells with metabolic activation	2 to 5 weeks	Moderate to high	Moderate	Excellent to fair	Unknown
Host-mediated assay with:					
Microorganisms	2 to 7 days	Low to moderate	Low to moderate	Good	
Mammalian cells	2 to 5 weeks	Moderate to high	Moderate	Unknown	Good
Body fluid analysis	Variable	Variable	Low to moderate	Variable	
Plants:					
Vicia faba	3 to 8 days	Low	Low		Relevance unclear
Tradescantia paludosa	2 to 5 weeks	Low to moderate	Moderate	Potentially excellent	
Insects:					
Drosophila melanogaster:					
Gene mutations	2 to 7 weeks	Moderate	Moderate	Good to excellent	
Chromosome aberrations	2 to 7 weeks	Moderate	Moderate		Good to excellent
Mammals:					
Dominant lethal mutations	2 to 4 months	Moderate to high	Moderate		Unknown
Translocations	5 to 7 months	Moderate to high	Moderate		Potentially very good
Blood or bone marrow cytogenetics	1 to 5 weeks	Moderate	Moderate		Potentially good
Specific locus mutations	2 to 3 months	High to very high	High to very high	Unknown	

* Operating costs vary widely depending upon the protocol specified and upon the number of substances tested simultaneously. Very approximately, very low is $1,000; low is $1,000 to $5,000; moderate is $3,000 to $10,000; high is $10,000 to $20,000; and very high is $25,000 upward. † Since most of these test systems do not detect all classes of gene mutations or chromosome aberrations (see Table 1), these columns refer only to the detectable mutations.

tion levels can in principle be ascertained from information supplied by manufacturers and importers. In practice, however, such information is frequently inaccessible, being considered proprietary information. It therefore becomes necessary for regulatory agencies to possess legal authority to obtain such information. (The Toxic Substances Act now pending in Congress may provide for such mandatory disclosure.)

Distribution patterns. It is usually much more difficult to determine the distribution of mutagenically active compounds in the environment than to determine gross production levels. The most significant aspects of distribution patterns are those steps at which human beings can become exposed to the chemicals in question. For medicinal drugs, for instance, it is necessary to obtain information about typical dosage schedules, about the sizes and age distributions of the treated populations, and in some cases about the extent to which the drugs are disseminated illegally. For agricultural chemicals it is necessary to discover the areas of the country in which they are used and the proportion of applied compounds which enter food chains. For food additives it is necessary to survey both the concentrations of such substances in numerous food products and the total amounts of these foods that are consumed.

It is particularly important when ascertaining distribution patterns to determine both average and maximum exposure levels and conditions and durations of exposure: Are there important groups (such as children, industrial and agricultural workers, or ill persons) being exposed to unusually high levels of a compound? It seems likely that this will almost always be the case, but to measure the size and level of exposure of such populations may often be difficult. Where it is reasonable to expect such information to be obtainable, however, it should become the responsibility of manufacturers and distributors to gather and transmit it to the relevant regulatory agencies.

Persistence. The environmental mutagenic efficiency of a chemical will in practice depend strongly upon its persistence. Persistence must be considered at two levels: in the environment, and in the human body. The half-lives of compounds in the external environment are likely to depend strongly upon the local water and soil, temperature, *p*H, solar radiation flux, microbial ecology, and so on, and extensive information may have to be sought to make realistic estimates of half-lives. As a routine adjunct to mutagenicity testing, therefore, suitable information should regularly be sought about the persistence of any mutagen in the external environment, and any regulatory scheme that requires mutagenicity testing should also require measurements of environmental persistence on a suitably extensive scale. (Information of this type is already required, for instance, under Environmental Protection Agency guidelines for the registration of new pesticides.)

It is also important to obtain data concerning the persistence of compounds within the human body. As in the case of persistence in the environment, responsibility for mutagenicity testing should also be coupled with responsibility for providing data on relevant endogenous half-lives of mutagens.

Metabolic disposition. Since it is already clear that mutagen (and especially carcinogen) activation and inactivation frequently occur as a result of metabolism, it will be important to obtain data on the human metabolic disposition both of compounds of established mutagenicity, and of compounds whose structures suggest the possibility of metabolic activation to a mutagenically active state. Whole-animal, specific-tissue, and tissue-extract studies will each be needed. As far as possible, the entire spectrum of conversion products should be monitored. Since extensive pharmacogenetic experience has already revealed much genetically determined individual variability in the metabolic disposition of chemicals, the possibility of individual variation in metabolic disposition of mutagens should also be considered.

The genetically significant concentration. The gonads are the organs at risk in the case of heritable mutations. For a compound to act as a germ line mutagen, it must reach the chromosomes of the reproductive cells of the gonads. Anatomical barriers at the tissue and cellular level, however, determine the ability of a chemical to affect germ cells. The blood-testis barrier (*17*), for instance, could significantly protect male germ cells. Spermatogonial cells appear to be located between the two major elements of this barrier and may be slightly exposed to blood components, whereas spermatocytes and mature sperm are exposed only to those substances that are selectively transported across the tubular epithelium and Sertoli cells. At present, very few substances have been studied for their abilities to penetrate these barriers. Furthermore, no such barrier has yet been reported for ovaries. The immature ova, however, are surrounded by a layer of follicular cells, across which substances must move in order to reach the ovum. A broad assessment of the significance of gonadal tissue barriers for the mutation process must await suitable physiological studies; comparative measurements of somatic and germinal mutation rates, however, may also provide insights into the importance of such barriers.

For assessing genetic risk, then, it becomes necessary to measure the genetically significant concentration (GSC) of mutagens: the GSC is the concentration of the active form of a mutagen in the immediate neighborhood of the genetic material. An appropriate measure of the GSC could, for instance, consist of the amount of the compound reacted (or complexed) with the germ line DNA of experimental animals, and such information could readily be obtained by the use of radioactively labeled compounds. Measurements of this type are now possible: alkylation of sperm DNA has been measured both in *Drosophila* and in the mouse (*18*). A regular part of the testing of compounds already distributed in nature, or seriously considered for future distribution, should therefore include measurements of the extent to which they actually reach the germ cells.

Where sufficiently detailed information is available, it will be advisable to calculate the effect of a mutagen upon the total population in a manner which takes into account the reproductive age distribution. The GSC for the population is then obtained by weighting the gonadal concentration in each individual by his or her expectation of future children. Specifically, the weight W for an individual of age y is

$$W = \sum_{x=y}^{m} l_{yx} b_x$$

where l_{yx} is the probability of surviving from age y to age x, b_x is the probability of reproducing at age x, and m is the maximum reproductive age. The genetically significant concentration is then given by $\mathrm{GSC} = \Sigma WC / \Sigma W$ where C is the gonadal concentration and the

summation is over all individuals in the population.

The population average for the two sexes is the simple average of the average GSC in males and in females, even if the sexes are not equally frequent in the population (for the good biological reason that each child has exactly one mother and one father). If in addition there are differences in sensitivity among different age or sex groups, then the different groups should be weighted by their specific sensitivities and then summed.

Individual variability. Fundamental studies of mutagenesis have clearly established the existence of powerful genetic factors controlling both spontaneous and induced mutation rates, and natural populations commonly contain individuals of both high and low susceptibility. When considering the mutagenic hazards of environmental chemicals, therefore, one must distinguish at least in principle between the average susceptibility of the population and the increased susceptibility of some individuals.

In the case of man, there is already some evidence that some individuals may be unusually susceptible to chemical mutagens. The inducibility of aryl hydrocarbon hydroxylase, for instance, is probably a single gene polymorphism, and this enzyme may be of great importance in determining susceptibility to carcinogenesis (and presumably therefore also to mutagenesis) by means of its ability to convert certain compounds to active states (*19*). Less general examples of conditions that may alter human susceptibility to mutagens and carcinogens are xeroderma pigmentosum, Bloom's syndrome, and Fanconi's anemia and progeria, all of which result in sharply enhanced frequencies of carcinogenesis and chromosome damage.

It does not seem possible at present to determine quantitatively what fraction of the human population is at increased mutational risk because of genetically determined high mutagenic susceptibility, and this factor cannot therefore now be taken into account when average susceptibilities are being computed. The main significance of variable susceptibility lies in its implications for the choice of suitable organisms for screening programs. Most screening systems employ specially chosen (that is, highly inbred) strains of organisms, which may not be representative of natural populations. It is therefore desirable to obtain comparative individual data on mutagenic susceptibility, particularly for man. One possible approach would be to determine frequencies of variant cells in different individuals, as has been done to a limited extent, for instance, for azaguanine resistance in human fibroblasts (*20*). As a general principle, mutagenicity screening should, where possible, be conducted with genetically diverse populations.

Risk Analysis

The fundamental purpose of mutagen screening is to obtain data relevant to mutagenesis in human beings. Two major problems arise, however, in the application of mutagen screening data to the protection of human populations: the choice of methods for quantitatively extrapolating from test systems to man, and the choice of criteria for defining acceptable versus unacceptable risks.

Extrapolation from test systems to man. As a general principle, it seems most desirable to extrapolate to man from those systems which are closest to man, such as human and mouse specific locus tests (both in vivo and in vitro) and mammalian (including human) cytogenetic tests. Quantitative extrapolation from microbial systems, or from reversion tests in which tissue cultured mutant mammalian cells are used, does not yet appear to be possible, and these tests therefore serve mainly as qualitative indicators of the presence or absence of mutagenicity.

Quantitative extrapolation from test systems to man can be attempted, as indicated previously, by using either the factor of increase over the spontaneous background, or the ratio of chemical to radiation mutagenesis. Regardless of which method is used, however, only rarely do we now have any assurance that the relevant ratios are constant over the various test systems (but see the appendix to this article). Our confidence in these extrapolation methods will increase as more and more data are accumulated on comparative system responses to a wide variety of chemicals.

Some specific attempts at extrapolation from test systems to man, in which we have employed data on potentially important environmental mutagens, are presented in the appendix. We are perfectly aware that these calculations are controversial, but they are good examples of the kind of approach which should be attempted.

The nature of acceptable risk. Given a reasonable calculation of the genetic hazard posed by an environmental mutagen, it then becomes necessary to consider how acceptable such a risk will be to the population at large. The guiding principle in all cases should be that no risk whatsoever is acceptable when the mutagenic compound presents no clear benefits, or when an alternative nonmutagenic compound is available. Thus a proposal to introduce a new compound which is mutagenic but may provide substantial benefits should also demonstrate convincingly that no alternative compound is available; in addition, when a useful compound already in distribution is discovered to be mutagenic, vigorous efforts should be initiated to replace it with a less hazardous compound, or to develop new substitutes.

Society is only slowly developing methods to measure broadly and quantitatively the benefits and costs associated with the use of specific materials and processes. For instance, the 1972 BEIR report (*4*) includes an appendix entitled "An attempt to measure the economic cost of radiation" in which an estimated dollar cost was placed on radiation exposure: "The total future cost of one man-rem [that is, a dose of 1 rem to one man] in terms of health costs paid in present dollars, is between $12 and $120." The procedures employed to derive this estimate can in principle be extended to chemical mutagens when the activities of such mutagens are measurable in GSC and REC units. Equivalent calculations could also be performed in terms of the spontaneous mutation rate if the rate-doubling concentration of a chemical was known. Comparisons of the resulting values with monetary estimates of benefits could then provide risk/benefit ratios which could in turn be used to assist decision-making concerning the restrictions to be placed upon the distribution of environmental mutagens. The continued development of such measures should obviously be promoted.

In the absence of information suitable for quantitative comparisons of costs and benefits, a decision as to what constitutes an acceptable risk is necessarily somewhat arbitrary. It is useful, however, to begin with the estimate that clinically recognizable genetic diseases currently appear to affect about 6 percent of the population during their

lifetime (*4*). (This may, however, be a substantial underestimate because of difficulties in recognizing some genetic diseases, particularly those of low penetrance or of multigenic nature.) When assessing the genetic hazards posed by ionizing radiations, the BEIR committee (*4*) computed the effects likely to be produced by the current maximum permissible dose of man-made radiation (excluding medical radiation), 0.17 rem per year. Over the average 30-year reproductive age span this dose rate sums to 5 rem per person. Using a fairly conservative rate-doubling dose of 40 rem (*4*), one would expect this amount of radiation to produce an additional 0.75 percent (5/40 of 6 percent) of genetically defective individuals in the population at equilibrium. Only a very small proportion of this upper limit, however, is actually experienced at present (about 0.15 rem out of the total of 5 rem). (For purposes of comparison, natural radiation produces a dose of about 3 rem per generation, and medical radiation a dose of about 2 rem per generation.) Taking at face value the recommendations of the BEIR committee concerning the acceptable genetic hazards of man-made radiation, we therefore propose that the total mutagenic exposure from man-made chemicals as well as radiations (but still excluding medical radiations), expressed, for instance, as the sum of rem's and REC's, be limited to the same extent, namely to the equivalent of 5 rem per generation. On the 40-rem doubling dose assumption, this corresponds to a 12.5 percent [(5/40) $\times$ 100] increase over the present mutational load (*21*).

This limit is currently intended to exclude radiations and chemicals administered medically. It is conceivable, however, that medical sources too should someday be included in the maximum permissible mutagenic dose delivered to the entire population. It is also possible that the currently estimated mutation rate-doubling dose of 20 to 200 rem (*4*) will be revised upward on the basis of new information (*22*). Even should this occur, however, we recommend that *no* upward adjustments be made in the limit of a 12.5 percent increase over the present spontaneous mutational load.

We further recommend that no *single* mutagenic agent should be allowed to exceed 10 percent of the 5-REC budget allotted to all mutagenic agents. This limit should be absolute, and no compound producing even substantial benefits should be disseminated beyond a level producing this effect. Although the maximum permissible dose would only permit ten such mutagens to be simultaneously distributed to their maximum individual levels, it seems unlikely that so many highly dangerous agents would, in fact, be so widely distributed. If they actually were, it would be a discovery of the most serious implications for human health.

While our main concern is necessarily with the average population, we recognize that high-risk subpopulations also exist, encompassing, for instance, certain industrial and agricultural workers. We therefore recommend that the maximum permissible mutagenic exposure *to individuals* who are still within their reproductive life-span be limited to a tenfold excess over the average maximum permissible exposure level. (Note, however, that this level of exposure might more than double their genetic risk expectancy. Every attempt should therefore be made to inform these individuals of their increased risk status.) It should be understood, however, that these individuals, when averaged with the entire population, should not cause the exposure level of the average population to rise above the recommended limit.

Regulatory Principles

Mutagenicity evaluation is an area of toxicology that should be handled by regulatory agencies in the same ways as are other toxicological problems.

Regulatory agencies. Regulation should be the responsibility of federal agencies, since neither private manufacturers nor state governments appear to possess the necessary capabilities. A number of federal agencies, in fact, already possess at least some of the relevant regulatory responsibilities (*23*). The burden of testing should rest with manufacturers, however, since the federal government lacks the extensive facilities, personnel, and budgets required for such testing, and since general toxicological testing is already the responsibility of manufacturers.

A regulatory agency should depend for its risk-benefit evaluations upon committees composed of expert geneticists and toxicologists capable of developing risk estimates, of economists, of industrial safety evaluation personnel, and of representatives of the public at large. The regulatory agency should also be able to promote rapid testing in critical areas, making use not only of the facilities of governmental laboratories but also of independent investigators throughout the country and abroad. The agency should also be prepared to coordinate closely with relevant testing, research and regulatory agencies in other countries.

The testing systems now developed, and the range of laboratories now qualified to perform tests, appear sufficient to provide a rapid response to a preliminary indication of mutagenicity in a moderate number of compounds whose distribution suggests the possibility of significant risk. Specifically, a battery of rapid but reliable tests could now be assigned, performed, and evaluated within at most a few months if deemed sufficiently important in particular instances.

Informed toxicologists and geneticists, however, should certainly not wait for the development of a comprehensive system of regulation in order to make known their concerns about the hazards posed by mutagenic environmental contaminants. Furthermore, as they discover mutagenic activities in compounds which they know or suspect to be distributed in the environment, they should so inform appropriate governmental agencies (*23, 24*).

Regulation of compounds already in distribution. There appear to be substantial differences in appropriate regulatory principles for compounds already in distribution compared to compounds yet to be distributed. Furthermore, the number of compounds already in widespread distribution is much greater than the number of new compounds being introduced annually. The two groups will therefore be considered separately, beginning with compounds already in distribution.

First, attempts should be made to identify suspect substances. Suspect substances may be any that are added to or taken up in the production of foods, crops, animals used for food, drinks, drugs and drug preparations, cosmetics and toiletries, or that are, under normal conditions of preparation, handling, distribution, and use, capable of being ingested, breathed, or absorbed by human beings. Particularly suspect substances are those that have not previously been tested for mutagenicity but are related chemically to known mutagens or are distributed in unusually large amounts.

All suspect substances should be screened promptly in the already available, rapid, and inexpensive microbial systems coupled with metabolic activation systems. Positive results in any such system would indicate that the compound should, with a high priority, be tested in appropriate higher animal systems. Furthermore, in establishing priorities for testing in higher animal systems, the number of persons at risk and their exposure levels should be taken into account; additional priority should be assigned to substances to which people in childhood or in their reproductive years might be especially exposed. Examples of high priority compounds are drugs such as aspirin, food additives such as nitrites, industrial and household chemicals, colors and dyes, agricultural chemicals, and packaging materials (especially polymerizing agents).

Regulation of compounds newly proposed for distribution. Substantial numbers of compounds are newly put onto the market each year, and human beings are likely to be exposed to many of them. It is much easier to monitor the possible toxic effects of these compounds than of the far larger number of compounds already in distribution. All new compounds under the jurisdiction of a regulatory agency should therefore be subjected to the best available mutagenicity screening tests. A compound found to be mutagenic in mammalian systems should not be distributed without regulation; production levels, distribution patterns in space and time, exposure levels, human pharmacological disposition, and mutagenic potency should all be taken in account to ensure that benefits to the population at risk are clearly greater than the corresponding risks.

While mutagenicity tests with mammalian systems in vivo or in vitro are highly desirable, the rapid and inexpensive submammalian tests are particularly useful for initial screening programs involving the larger number of compounds which are investigated during the selection of marketable compounds. Mutagenicity observed in these submammalian tests correlates well with mutagenicity or carcinogenicity observed in mammalian tests, particularly when metabolic activation systems are included in the submammalian screens. Compounds that are proposed for actual distribution, however, should also be subjected to mammalian screening tests.

Future Directions

New and improved test systems. We fully recognize that the test systems now available do not provide a very broad base for screening, either in their abilities to detect all forms of heritable mutations or in their relevance to mutation in man. We therefore urge that a number of new systems be developed or improved, to wit:

1) Human somatic mutation in vivo. A system is urgently needed for monitoring the cells of individuals, particularly those at greater than average risk. The system should screen readily obtainable cells capable of continued division in vitro sufficient to establish the heritability of putative mutations.

2) Mammalian meiosis in vitro. While some somatic mutations can now be scored in cultured cells, mutations arising in germ line cells can only be scored by conventional breeding experiments. A system in vitro that could support the entire meiotic process, and whose products could be screened for mutations, would therefore be of the greatest importance for mutagenicity testing as well, of course, as for fundamental studies of the meiotic process itself.

3) Genetically significant concentrations. Methods should be developed to greatly expand the number of compounds for which the GSC can be determined and compared with specific mutagenicity in mammalian test systems.

4) Population monitoring. Methods should be developed to survey biochemical, cytogenetic, and phenotypic characteristics of large human populations (1,000,000 or more individuals), in order to obtain better information about the true spontaneous human mutation rate, natural variations in this rate in certain subgroups of the population, and possible increases in the mutation rate which might result from environmental mutagens.

5) Human somatic mutation in vitro. Both new and improved systems for screening conditional lethal mutations are needed to enlarge the number and reliability of human specific locus systems. In addition, the nature of human specific locus mutations (genetic or epigenetic; entire locus or only restricted sites mutable) must be worked out. The current amount of effort required to test chemicals in such systems needs to be sharply reduced by developing improved test systems. It will also be important to couple the systems for conditioned lethal screening with specific-organ or organ-extract systems capable of metabolic activation; the metabolic activation systems now in use are highly toxic to cultured mammalian cells.

6) Gene transfer in tissue cultured cells. Many conventional modes of genetic analysis are not yet applicable to cultured mammalian cells because of the unavailability of a parasexual gene transfer system. The development of such a system would provide a fundamental tool for genetic analysis, with consequent improvement of our understanding of the mutation process in higher eukaryotic cells.

7) Chromosomal aberrations in microbial eukaryotes. Cytogenetic tests are now feasible only with the higher eukaryotes. It would be of great significance for determining comparative rates of point versus chromosomal mutation to possess a system capable of detecting chromosomal aberrations in a microbial eukaryote.

Initiation of mass screening. Even those systems now available are suitable for screening large numbers of suspect compounds. Screening should therefore be initiated as rapidly and as extensively as possible, with the financial support both of industry and of federal agencies. We recognize that screening programs are not particularly suitable for support from funds appropriated for basic research, except for those aspects listed above, and that National Science Foundation and National Institutes of Health research grant support is not the best way to promote screening. Contract support, however, seems fully suitable for screening programs, and the level of such funding should be considerably expanded, together with informational programs designed to attract suitable investigators.

Mass screening will also require many more trained personnel. Consideration should therefore be given to initiating training programs to provide highly qualified technicians and supervisors.

Both mammalian and submammalian test systems possess individual advantages and disadvantages, and both types of systems are likely to be used in the immediate future. It is therefore important to accumulate comparative information on their differential responsiveness to numerous compounds, that is, to determine what types of compounds produce uniformly positive or negative responses in both types of sys-

tem, and what types of compounds produce positive responses in one type of system but negative responses in the other.

Conclusions

Human populations are now exposed to a wide variety of compounds never before encountered in the history of man. Many of these compounds are clearly mutagenic to lower organisms, and there are sound biological reasons to conclude that at least some are also mutagenic to man. Since the vast majority of detectable mutations are deleterious, an artificially increased human mutation rate would be expected to be harmful in proportion to the increase.

A number of test systems are now being developed and perfected to the point where they can detect chemical mutagenesis reliably and sensitively. No system by itself meets all the requirements of an ideal system, but the available systems collectively provide important information about the mutagenic potential of many, perhaps most, chemical compounds.

When data from the best available mutagenicity screening systems are combined with information about the distribution of compounds in the environment and their metabolic fates in the body, it becomes possible to make at least preliminary estimates of the health hazards posed by environmental mutagens. It is therefore already reasonable to begin to regulate the distribution of chemical compounds. In general, no mutagenic compound should be distributed unless it serves a truly useful purpose and unless no efficacious substitute is available. We recommend specific limits for the environmental distribution of mutagenic agents, including both ionizing radiations and chemical compounds, such that the resulting genetic damage does not exceed a 12.5 percent increase over the spontaneous mutational background.

Table 3. Quantitation of mutagenic effects of hycanthone methanesulfonate, ethyl methanesulfonate (EMS) and nitrite.

Test system	Calculations
	Hycanthone methanesulfonate (HTD, 3 mg/kg)*
Specific locus mutations in cultured mouse cells	1. Same mutant frequency produced by 50 mg/kg hycanthone (17 HTD) and 120 rad x-rays: 7.2 REC/HTD
	2. Locus tested was twice as sensitive as the average locus: 3.6 REC/HTD
	3. Threefold correction factor for acute rate: 1.2 REC/HTD
Chromosome aberrations in cultured human cells	1. Seven HTD induced 1.0 percent translocations: 1.43×10^{-3} translocations per HTD
	2. Only 2.5 percent of translocations transmitted to viable offspring: $1.43 \times 10^{-3} \times 2.5 \times 10^{-2}$ corresponds to 36×10^{-6} live-born abnormal offspring per HTD
	3. One rad produces 12×10^{-6} live-born unbalanced translocations: $36 \times 10^{-6}/12 \times 10^{-6}$ corresponds to 3 REC/HTD
	4. Threefold correction factor for acute rate: 1 REC/HTD
Recessive lethal mutations in postmeiotic *Drosophila* male germ cells	1. Same mutant frequency produced by 100 HTD hycanthone and 355 rad x-rays: 3.55 REC/HTD
	2. Mammalian spermatogonia are about 1.5-fold more sensitive than postmeiotic *Drosophila* germ cell mixture: 5.34 REC/HTD
	3. Threefold correction factor for acute rate: 1.8 REC/HTD
	Ethyl methanesulfonate
Specific locus mutations in cultured rodent cells	Same mutant frequency produced by 1240 mg/kg EMS and 1500 to 2000 rad x-rays: REC, 0.62 to 0.83 mg/kg
Mouse dominant lethal mutations	Same mutant frequency produced by 248 mg/kg EMS and 400 rad x-rays: REC, 0.62 mg/kg
Mouse translocation mutations	Same mutant frequency produced by 248 mg/kg EMS and 325 rad x-rays: REC, 0.76 mg/kg
Specific locus mutations in mouse spermatozoa	Same mutant frequency produced by 248 mg/kg EMS and 100 rad x-rays: REC, 2.5 mg/kg (but upper confidence limit of EMS data includes REC of 0.62 mg/kg)
	Nitrite
Recessive lethal mutations in *Drosophila* spermatozoa	1. Same mutant frequency produced by 767 mg/kg nitrite and 66 rad x-rays: REC, 15.6 mg/kg
	2. Mammalian spermatogonia are about twofold more sensitive than *Drosophila* spermatozoa: REC, 7.8 mg/kg
	3. Average human nitrite consumption: 0.17 mg/kg per day; 1862 mg/kg per 30 years, therefore 239 REC per generation
	4. Threefold correction factor for acute rate: 80 REC per generation
	5. Sensitive period for mutagenesis is less than 10 percent of spermatogonial cell cycle: upper limit, 8 REC per generation

* HTD, human therapeutic dose.

Appendix

We have attempted to estimate the mutagenicity of three compounds in REC units (Table 3). The first, hycanthone methanesulfonate, has been widely applied in the treatment of schistosomiasis. The second, ethyl methanesulfonate (EMS), is a standard laboratory mutagen, but certainly not an environmental hazard. The third, nitrite, is a common food additive (used as a preservative and to enhance flavor and meat color) and also appears in the environment as a result of the microbial processing of both agricultural and natural nitrates. *Our calculations are intended to be exemplary, but hardly definitive.* The GCS's (genetically significant concentrations), for instance, are generally unknown, as are many other relevant factors.

In making comparisons between chemical and radiation mutagenesis, we have frequently corrected for a radiation dose rate effect: since acute doses are commonly administered in testing procedures, whereas chronic or low-dose irradiation produces only about one-third as many mutations as does acute irradiation (see *4*, p. 61), we have assumed that the same holds for environmental chemical mutagenesis. Furthermore, in mice the mutation rate induced by chronic irradiation is much lower in the female than in the male, about 20 times lower in females with mature oocytes and even lower than that if the oocytes are in earlier stages of development (*4*, pp. 65–66). Since it is unclear whether the same will be true for chemical mutagenesis in man, some of our calculated REC values may be overestimated by a factor of 2.

Hycanthone methanesulfonate. Three different screening systems have been used to assess hycanthone mutagenicity, and all three yield remarkably similar results.

1) Specific locus mutations in cul-

tured mouse somatic cells (*25*). A dose of 10^{-4} molar hycanthone induced the same mutant frequency in the thymidine kinase locus as did 120 rad of x-rays in the same system, and the dose-response curve was approximately linear. This dose of hycanthone corresponds to about 50 mg/kg, or about 17 human therapeutic doses (HTD, 3 mg/kg). Therefore 120 rad per 17 HTD corresponds to 7.2 REC per HTD. The x-ray–induced specific locus mutation rate in this particular system (about 5×10^{-7} mutations per rad) is about twice as large as the average specific locus rate (*12*), so that the HTD probably corresponds to between 3.6 and 7.2 REC. When corrected for the difference between acute and chronic dosage schedules, these figures are reduced to between 1.2 and 2.4 REC per HTD.

2) Chromosome aberrations in cultured human somatic cells (*26*). A dose of $4 \times 10^{-5}M$ hycanthone (7 HTD) induced a translocation frequency of 0.010. If we assume that the response is linear and that the mutation rate is the same in human spermatogonial cells, this corresponds to 0.010/7 or 1.43×10^{-3} translocations per HTD. Only about 2.5 percent of these, however, would be transmitted through meiosis to live-born abnormal offspring. Therefore, $0.025 \times 1.43 \times 10^{-3}$ corresponds to 36×10^{-6} live-born abnormal offspring. The BEIR report (*4*, p. 55) estimated that 1 rad (or 1 rem, which is very similar) would produce 12×10^{-6} unbalanced translocations among the first-generation offspring, and as a result, the HTD of hycanthone would correspond to 36/12 or 3 REC. When corrected for the difference between acute and chronic x-ray dosages, this figure becomes about 1 REC per HTD.

3) Sex-linked recessive lethal mutations in *Drosophila* (*27*). A hycanthone dose of 300 mg/kg (100 HTD) induced 1.72 percent mutations in postmeiotic germ cells sampled 2 to 6 days after treatment, a frequency which would also be produced by 355 rad of x-rays (*28*). (No significant increase in mutation rate was observed in cells treated in the spermatogonial stage, but the small size of the sample—less than 1000 cells—does not warrant the conclusion that no mutations were induced in these cells.) Hence 1 HTD would correspond to 355/100 or 3.55 REC. When corrected for the fact that mammalian spermatogonia are about 1.5 times more sensitive to irradiation than *Drosophila* postmeiotic cell mixtures, and for the threefold decreased mutagenic efficiency of chronically administered doses, this figure becomes about 1.8 REC per HTD.

Ethyl methanesulfonate. This has been used as a standard mutagen during the development of most mutagen screening systems. Here we will show that diverse mammalian systems respond in a quantitatively similar manner, an EMS dose of 0.01*M* (1240 mg/kg) corresponding to a radiation dose of 1000 to 2000 rad.

1) Specific locus mutations in cultured rodent cells (*29*). At the mouse thymidine kinase locus, 0.01*M* EMS produced the same mutant frequency as did about 4000 rad; but this published estimate is probably about twofold too high (*30*), and the correct value is therefore closer to 2000 rad. At the Chinese hamster 8-azaguanine resistance locus, 0.01*M* EMS produced the same mutant frequency as did about 1500 rad.

2) Mouse dominant lethal and translocation mutations. A concentration of 0.002*M* EMS induced 47 percent dominant lethal mutations in sperm samples 1 to 21 days after treatment (*31*), and a similar rate (48 percent) was induced by 400 rad administered to mature sperm. If we assume a linear response, therefore, 0.01*M* EMS would have produced the same effect as 2000 rad. Furthermore, 0.002*M* EMS induced about 10 percent of translocations in sperm when F_1 male offspring were tested (*32*), and about 325 rad of acute x-irradiation would produce about 10.5 percent translocations in F_1 male offspring of treated males (*33*). If we assume linearity, therefore, 0.01*M* EMS would have produced the same effect as about 1600 rad.

3) Mouse specific locus mutations (*32*). Two specific locus mutations were produced among 4818 progeny after treatment of spermatozoa with 0.002*M* EMS, and a dose of 300 to 400 rad would have produced 6 to 8 mutations in a sample of this size. (We employed the mutation rate corresponding to acute radiation for the spermatozoa data, since there is no evidence for a dose rate effect in postmeiotic cells.) The upper 95 percent confidence limits of the observed EMS mutation rate include both values predicted from the x-ray data. An EMS dose of 0.01*M* could therefore correspond to 1500 to 2000 rad.

In EMS experiments involving specific locus mutations induced in spermatogonia, no mutations were observed among 14,400 offspring from treated parents. If the mutation rate induced by chronic x-irradiation is taken to be 8×10^{-8} per locus per rad, the seven loci sampled would have responded to 300 to 400 rad of irradiation with a mutant yield of $8 \times 10^{-8} \times 7 \times (300$ to $400) \times 14{,}400$, or 2.4 to 3.2. Since both of these values are again within the 95 percent confidence limits for the x-ray data, an EMS dose of 0.01*M* could again correspond to 1500 to 2000 rad.

Nitrite. The highest eukaryote for which nitrite mutagenesis data are available is *Drosophila* (*34*). Males of average weight (0.9×10^{-6} kg) consumed 5×10^{-4} ml of 0.02*M* $NaNO_2$ (molecular weight, 69) in a 1-day experiment. The average dose was therefore 767 mg/kg. The resulting induced sex-linked recessive lethal mutation rate was 0.13 percent. If the x-ray doubling dose for mature spermatozoa is taken to be 66 rad (*28*), this mutation frequency would have been produced by 49 rad. Therefore, 1 REC corresponds to 767/49 or 15.6 mg/kg. Since mammalian spermatogonia are about twice as sensitive as *Drosophila* spermatozoa, this figure is reduced to 7.8 mg/kg. The average amount of nitrite consumed by human beings is about 0.17 mg/kg per day (*35*). Over a 30-year period, therefore, the average person consumes $0.17 \times 365 \times 30$ or 1862 mg/kg, which corresponds to 1862/7.8 or 239 REC per generation. Since acute doses were used in the radiation experiments the threefold reduction factor to convert to chronic doses reduces this value to 80 REC. Other data (*36*), however, can be interpreted to suggest that the sensitive period for mutagenesis during the spermatogonial cell cycle lasts less than 10 percent of the cycle (*36*). As a result, our *upper estimate* of the level of environmental nitrite mutagenesis is 8 REC per generation. If the doubling dose is taken as 40 REC, then this level of nitrite mutagenesis would correspond to 20 percent of the spontaneous mutation rate in human beings. This calculation, based on a single-dose experiment, is highly uncertain. It calls attention, however, to a clear need to explore the mutagenicity of nitrites much more fully, in order to understand whether nitrites do in fact constitute an important mutagenic hazard.

References and Notes

1. B. Childs, S. M. Miller, A. G. Bearn, in *Mutagenic Effects of Environmental Contaminants*, H. E. Sutton and M. I. Harris, Eds. (Academic Press, New York, 1972), pp. 3–14.
2. J. W. Drake, *The Molecular Basis of Mutation* (Holden-Day, San Francisco, 1970).
3. For instance: J. F. Crow, *Scientist and Citizen* **1968**, 113 (June–July 1968); E. M. Mrak (chairman), *Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health* (Department of Health, Education, and Welfare, Washington, D.C., December 1969); Food and Drug Advisory Committee on Protocols for Safety Evalution, *Toxicol. Appl. Pharmacol.* **16**, 264 (1970); WHO Scientific Group, *The Evaluation and Testing of Drugs for Mutagenicity; Principles and Problems, WHO Tech. Rep. Ser. No. 482* (1971); H. C. Grice, Ed., *The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity* (Health and Welfare Canada, Ottawa, 1973); C. Ramel, Ed., *Evaluation of Genetic Risks of Environmental Chemicals* (Ambio Special Report, Royal Swedish Academy of Sciences, Stockholm, 1973); F. J. de Serres and W. Sheridan, Eds., *The Evaluation of Chemical Mutagenicity Data in Relation to Population Risk. Environmental Health Perspectives*, experimental issue No. 6, HEW Publ. No. NIH 74-218 (National Institute of Environmental Health Sciences, Bethesda, Md., 1973). Furthermore, radiation hazards are specifically reviewed in *Ionizing Radiation: Levels and Effects* (United Nations Scientific Committee on the Effects of Atomic Radiation, 1972), vols. 1 and 2, and in the BEIR report (see *4*).
4. Advisory Committee on the Biological Effects of Ionizing Radiations [BEIR], *The Effects on Populations of Exposure to Low Levels of Ionizing Radiations* (National Academy of Sciences–National Research Council, Washington, D.C., 1972).
5. F. H. Sobels, Ed., *Mutation Research* (Elsevier, Amsterdam).
6. L. Fishbein, W. G. Flamm, H. L. Falk, *Chemical Mutagens. Environmental Effects on Biological Systems* (Academic Press, New York, 1970); F. Vogel and G. Rohrborn, Eds., *Chemical Mutagenesis in Mammals and Man* (Springer-Verlag, New York, 1970); A. Hollaender, Ed., *Chemical Mutagens. Principles and Methods for Their Detection* (Plenum, New York, 1971–73), vols. 1–3; H. E. Sutton and M. I. Harris, Eds., *Mutagenic Effects of Environmental Contaminants* (Academic Press, New York, 1972).
7. This consists of a computerized bibliographical service that can supply information about many specific compounds, as well as more general information. Interested persons should contact the Environmental Mutagen Information Center, Biology Division, Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, Tennessee 37830.
8. A. C. Stevenson and C. B. Kerr, *Mutat. Res.* **4**, 339 (1967); L. L. Cavalli-Sforza and W. F. Bodmer, *The Genetics of Human Populations* (Freeman, San Francisco, 1971).
9. J. V. Neel, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3311 (1973); see also H. Harris, D. A. Hopkinson, E. B. Robson, *Ann. Hum. Genet.* **37**, 237 (1974).
10. V. A. McKusick, *Mendelian Inheritance in Man* (Johns Hopkins Press, Baltimore, ed. 3, 1971).
11. B. H. Judd, M. W. Shen, T. C. Kaufman, *Genetics* **71**, 139 (1972).
12. S. Abrahamson, M. A. Bender, A. D. Conger, S. Wolff, *Nature (Lond.)* **245**, 460 (1973).
13. T. Mukai, S. I. Chigusa, L. E. Mettler, J. F. Crow, *Genetics* **72**, 335 (1972).
14. B. N. Ames, W. E. Durston, E. Yamasaki, F. D. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2281 (1973); W. E. Durston and B. N. Ames, *ibid.* **71**, 737 (1974).
15. A. Hollaender, Ed., *Chemical Mutagens. Principles and Methods for Their Detection*, (Plenum, New York, 1971–73), vols. 1–3.
16. F. Zimmermann, *Mutat. Res.* **11**, 327 (1971); D. J. Brusick and V. W. Mayer, in *The Evaluation of Chemical Mutagenicity Data in Relation to Population Risk. Environmental Health Perspectives*, F. J. de Serres and W. Sheridan, Eds., experimental issue No. 6, HEW Publ. No. NIH 74-218 (National Institute of Environmental Health Sciences, Bethesda, Md., 1973), p. 83.
17. B. P. Setchell, J. K. Voglmayr, G. M. H. Waites, *J. Physiol. (Lond.)* **200**, 73 (1969); M. Dym and D. W. Fawcett, *Biol. Reprod.* **3**, 308 (1970); B. P. Setchell, *Anat. Rec.* **196**, 424 (1971).
18. G. A. Sega, P. A. Gee, W. R. Lee, *Mutat. Res.* **16**, 203 (1972); P. A. Gee, G. A. Sega, W. R. Lee, *ibid.*, p. 215; G. A. Sega, R. B. Cumming, M. F. Walton, *ibid.* **24**, 317 (1974).
19. G. Kellermann, C. R. Shaw, M. Luyten-Kellermann, *N. Engl. J. Med.* **289**, 934 (1973).
20. R. DeMars and K. R. Held, *Humangenetik* **16**, 87 (1972).
21. An alternative method of defining acceptable risk is to work from some other clearly defined risk which is already implicitly considered to be acceptable. Automobile accident deaths, for instance, might provide such an index: their frequency represents a balance between economic forces and individual choice which yields rather slowly to attempts at improvement, and reflects the degree of suffering which people are willing to accept (however much they may disparage it) in order to operate automobiles at an acceptable monetary cost. Motor vehicle accident deaths numbered 56,600 in 1972 [National Safety Council, *Accident Facts* (National Safety Council, Chicago, Ill., 1973)]. Compared to the 3,260,000 births in 1972, therefore, the motor vehicle accident death rate was 1.7 percent. Since this death rate is being slowly reduced, it could be argued that about half this rate would constitute an acceptable risk, a value similar to the 0.75 percent upper limit we have proposed for artificially induced genetic damage.
22. J. V. Neel, H. Kato, W. J. Schull, *Genetics* **76**, 311 (1974).
23. The Food and Drug Administration regulates all compounds which might occur in foods, except for pesticides (including herbicides); all drug products; cosmetics and certain related products; and radiations, including medical sources but excluding those associated with energy production. The Environmental Protection Agency regulates pesticides (including herbicides); compounds polluting water and the atmosphere; and a broad category of toxic agents proposed for distribution, except for those in the food and drug categories. The Atomic Energy Agency regulates radiations released during energy production. The Consumer Products Safety Commission regulates toxic compounds (particularly those likely to occur in household products), except for those in the food and drug categories which are already in distribution. The Environmental Mutagenesis Branch of the National Institute of Environmental Health Sciences is also a concerned institution, but does not possess regulatory powers.
24. If unsure of whom to inform, we suggest Dr. Frederick J. de Serres, Chairman, Interagency Panel on Environmental Mutagenesis, Environmental Mutagenesis Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, N.C. 27709.
25. D. Clive, W. G. Flamm, M. R. Machesko, *Mutat. Res.* **14**, 262 (1972). (More recent but also unpublished information, however, suggests a higher mutation rate, corresponding to about 6 REC per HTD.)
26. R. J. Preston and F. G. Pearson, *Annual Report 1972* (Oak Ridge National Laboratory, Oak Ridge, Tenn.).
27. A. G. A. C. Knaap and P. G. N. Kramers, *Mutat. Res.* **21**, 38 (1973).
28. S. Abrahamson, in *Proceedings of the First Interdisciplinary Conference on Selected Effects of a General War* (Defense Atomic Support Agency, Information and Analysis Center, General Electric, TEMPO, Santa Barbara, Calif., 1968), vol. 1, p. 280.
29. D. Clive, W. G. Flamm, J. B. Patterson, in *Chemical Mutagens. Principles and Methods for Their Detection*, A. Hollaender, Ed. (Plenum, New York, 1973), vol. 3, pp. 79–104.
30. D. Clive, perosnal communication.
31. B. M. Cattanach, *Mutat. Res.* **13**, 371 (1971).
32. ———, in *Chemical Mutagens. Principles and Methods for Their Detection*, A. Hollaender, Ed. (Plenum, New York, 1971), vol. 2, pp. 535–539.
33. A. G. Searle, C. E. Ford, E. P. Evans, C. V. Beechey, M. D. Burtenshaw, H. M. Clegg, *Mutat. Res.* **22**, 157 (1974); A. Leonard and G. Deknudt, *Can. J. Genet. Cytol.* **10**, 495 (1968).
34. R. Valencia, S. Abrahamson, P. Wagoner, L. Mansfield, *Mutat. Res.* **21**, 240 (1973).
35. R. G. Cassens, personal communication.
36. P. Oftedal, *Hereditas* **60**, 177 (1968).