The Mutagenicity of Pesticides
Concepts and Evaluation

Samuel S. Epstein and Marvin S. Legator

with a foreword by Joshua Lederberg

The MIT Press
Cambridge, Massachusetts, and London, England

This book was designed by
the MIT Press Design Department.
It was set in Univers 685/689
Printed on 60 lb. P&S Offset
by Halliday Lithograph Corp.
and bound by The Colonial Press Inc.
in the United States of America

ISBN (hard cover) 0 262 05008 0

Library of Congress catalog card number: 70-130274

Foreword

Socially responsible scientists should be concerned about the potential hazards of chemically induced mutation for at least three reasons. The most important is also the most remote in the scale of time: the human nature that defines our posterity is energized by our cultural tradition; but it is also bounded by the integrity of the genetic information of which each generation is the vessel.

Second, genetic impairments already account for a very large part of our existing burden of disease and premature death. If we give proper weight to the genetic component of many common diseases which have a more complex etiology than the textbook examples of Mendelian defects, we can calculate that at least 25 percent of our health burden is of genetic origin. This figure is a very conservative estimate in view of the genetic component of such griefs as schizophrenia, diabetes, atherosclerosis, mental retardation, early senility, and many congenital malformations. In fact, the genetic factor in disease is bound to increase to an even larger proportion, for as we deal with infectious disease and other environmental insults, the genetic legacy of the species will compete only with traumatic accidents as the major factor in health.

Finally, experimental evidence of mutagenic capability should be a danger signal that a compound may also be capable of other, somatic hazards through its action on DNA and other cellular constituents. For example, the demonstration of chromosome breakage in cultured cells exposed to cyclohexylamine was the (administratively neglected) forerunner of the eventual inculpation of the parent compound, cyclamate, as a cancer hazard.[3]

Our existing genetic load is a summation of three kinds of process: the historical accumulation of recessive gene mutations reappearing from time to time as their heterozygous carriers chance to mate; the immediate manifestation of dominant mutations and chromosome anomalies, which are only rarely propagated; and the paradoxical segregational load, where deleterious recessives had been stabilized within the population through some present, or more often historical, advantage of the heterozygotes.

Any assessment of the social and personal costs of mutation must take account both of absolute and of relative measures. (And of course we must use the same perspective in weighing the social and personal benefits claimed for a given environmental additive.) A 10 percent increase in the existing, "spontaneous" mutation rate is, in effect, the standard that has been adopted as the "maximum acceptable" level of public exposure to radiation by responsible regulatory bodies.[4] This level can be defended on the argument that we neglect to take a number of measures that could probably improve the mutation index to a comparable degree. It can be attacked by reciting the absolute level of eventual biological injury that might come from public exposure at such a level, were this in fact to occur from the proliferation of nuclear power plants and unregulated weapons tests.

A rational approach to the assessment of chemical hazards likewise calls for a detailed *quantitative* examination of risk, if only to expose the policy assumptions to the same degree of public understanding and debate as pertains to atomic energy. In both sets of situations, we are plagued by serious uncertainties about the numerical estimates for induced mutation in man, perhaps much worse for any chemical than for radiation. Given the problem of the number of new, suspicious compounds now pervading the environment, we face a formidable task in putting our genetic house in order.

For many purposes, we could dispose with a quantitative analysis, simply because most of our

assays are so *in*sensitive that a compound that scores positively as a mutagen must have a portentous effect in any but the most peculiar circumstances. Nevertheless, the labeling of compounds as "mutagenic" or "nonmutagenic" may, particularly if we pursue the development of assays for sensitivity rather than selectivity, be regarded as simplistic, both in quantitative and in qualitative terms. However, when there are murky doubts about an issue as important as mutational damage, they must be resolved in favor of the species.

The sobriety with which we face the task of setting up rational criteria for decision must be robust enough to withstand some inevitable ridicule. The very first compound to be reported[1] in the published literature as mutagenic was *allyl isothiocyanate*, or mustard oil, well known as a natural constituent of horseradish and other widely used condiments. (This should not be confused with mustard gas, research on which was classified during World War II, and which was later revealed to be a mutagen of uncommon potency.) Whether allyl isothiocyanate is mutagenic in mammals is not known, and it is too soon to condemn a foodstuff having such a venerable tradition. This is a concession to that tradition, rather than to any factual assurance that mustard-eaters have a mutation rate demonstrably unaltered by their diet. Further research may allay or confirm these suspicions; if they are confirmed, it would not be the first time that a common dietary article was found to be harmful to some consumers despite centuries of common use (cf. the role of wheat gluten, only recently understood in the etiology of celiac disease.)

This kind of diffidence in the face of custom and uncertainty does not extend to efforts to promulgate a compound like mustard oil for uses lacking an ancient precedent. It has, for example, been advocated as a way of denaturing hobby glues to deter sniffing, an admirable social purpose if it reached the roots of the problem. The FDA's approval of mustard oil as an obviously safe additive is the entire argument against unwonted side effects. This must be balanced against a long-buried observation of skin tumors in mice painted with mustard oil;[7] which is also another example of the predictive value of mutagenicity for carcinogenicity. Theoretically, isothiocyanates can be expected to function as alkylating agents.

Mustard oil is not a pesticide (except, perhaps, as a natural one evolved by cruciferous plants); why mention it here? Mainly because, little as we know

about it, more is known about mustard oil than about most pesticides. Furthermore, most of our concerns about pesticides are prototypes of our general concerns about environmental additives. We should admit, at the outset, that pesticides must be differentiated from food additives in the dilemmas that they raise about the cost-benefit equation. (Some critics believe, however, that pesticides are overpromoted to the point where economic benefits would be left intact or even improved if they were used more selectively; and the environmental load could then be cut by a factor of 10 or 100.)

It will encourage a fuller exposure of the magnitude and incidence of economic benefits of potentially hazardous chemicals if we do suggest some quantitative standards of acceptable mutagenicity. I believe that the present standards for population exposure to radiation should and will (at least de facto) be made more stringent, to about 1 percent of the spontaneous rate, and that this is a reasonable standard for the maximum tolerable mutagenic effect of any environmental chemical (better, for them in aggregate).

Accepting, for present argument, the formal arguments of the UN advisory group,[8] I translate this standard into a rate of about one recessive mutation per1,000 gametes (10^{-7} per nominal locus) per generation of typical exposure. Dominant mutations and chromosome aberrations may deserve even more stringent scrutiny, in view of the immediacy of their personal and social cost. The corresponding standard of 50 per million induced, viable chromosome anomalies and 2 per million dominant mutations entails a raw social cost of over $100 million. It is probably at the margin of ultimate detectability even by extrapolation from experimental assays; so that at least a billion-dollar argument needs to be put up by the defendant of any additive that gives a positive experimental score in such tests.

The costs of recessive mutations are much more difficult to estimate, being quite sensitive to the proportion of the *mutational* to the *segregational* load. At equilibrium, a 1 percent increase in the mutation rate will generate an estimated *economic* loss of about $1 billion per year (measured in the 1970 economy of the United States) but taking at least ten generations to approach full impact.

An extremely conservative estimate would then put the near-term annual tax connected to this standard at about $100 million for the recessives. These

calculations give no weight to such costs (or savings) as may attend the gradual deterioration of intelligence and other complex functions as a consequence of cumulative genetic damage. Nor do they put a value on heartache.

These estimates are surely subject to an uncertainty of a factor of ten or so. They predicate the value of a human life as between $50,000 and $1 million per capita, depending on the age at which disability or death occurs and the level of custodial care entailed by it, as well as loss of economic productivity. They assign no value to early prenatal losses, though some would regard these as beneficial for the aim of zero population growth. This kind of cost-accounting is morally insufferable, but we must find some de facto standard of value in making hard decisions. If lives are valued at much more than a "million per bod," there is little evidence of this from the pragmatic behavior of the community or of most individuals in the choices they make in their daily lives.[9] However, these choices are made in a hindered market where the cost of safety is a side issue, more often obscured than intelligently ventilated.

On the other hand, a pesticide manufacturer would fire his director of public relations if he were to advertise that he calculated a life at less than his own annual income.

A health cost (from the "acceptable" standard) of $200 million per year is a grievous burden in absolute terms, but is immediately lost in an overall budget of over $100 billion. (Of this, $60 billion is direct health care; the indirect economic costs of disease, injury, and premature senescence are open-ended.) This is to say that a level of risk that approaches the intolerable, once we are well aware of it, may be impossible to verify by direct measurements of disease diffused throughout the population! In exceptional circumstances, an effect like the peculiar malformations induced by thalidomide comes to the surface, and then achieves a visibility and notoriety all out of proportion to other agents. *If the malformation induced by thalidomide were a mental retardation of 10 percent of the I.Q., instead of a highly characteristic and unusual deformation of the limbs, in an equal number of subjects, we would be unaware of it to this day.*

All this is to say that we must look to extrapolations from laboratory measures for the *only* reliable indication of mutagenicity in the human population!

Someday, it will be argued that the standard risk

should be elevated in a particular case, for example, were there to be a demonstrable net social benefit of, say, $1 billion per year from the use of an agent that elevated the mutation rate by 5 percent. The argument should not be rejected out of hand. For example, I believe that an acceleration of health research by $1 billion per year would improve the genetic and the overall health climate so as to more than outweigh the penalties of more mutations. If there were a harmonious redistribution of the resource benefit, we could foresee an advantageous tradeoff. The problem is to produce that harmony, to ensure that the people who bear the risk and eventually pay the price will also reap the benefits. Perhaps we will invent a tax on pesticides, earmarked for compensatory research. This makes sense only if we have exhausted alternative sources of income for such restorative purposes.

Should pesticides be particularly suspect as mutagens? Or would their pesticidal activity bear only an accidental relationship to genetic damage? One should answer this in relation to how much we know about the mechanism of action of the compound, and particularly the basis of its *specificity*. The fundamental cellular processes of all organisms are remarkably similar, the more so the closer we go to the genetic foundations. The DNA of the bacterium, the insect, the weed plant, the rodent pest, and of man has precisely the same architecture. For this reason, the most suspicious agents should be the disinfectants, the compounds that act directly on cells, and indeed most of these probably do act on the DNA of the microbe as the target. Other pesticides may also act on DNA, but owe their specificity to details of penetration, or of metabolism in the pest which will deliver the final toxic molecule to the cellular target. Finally, most pesticides probably act as enzyme inhibitors, but may have mutagenic effects as (1) incidental side effects of their own structure; (2) further metabolism in the mammalian system to genetically active products, or (3) as side effects of the inhibition of cellular enzymes. At the very least, pesticidal action does not disqualify a compound as a mutagen, and in some cases this may be very closely related to its intended mode of action. Only empirical studies, of the kind outlined in this monograph, can give a conclusive answer.

These approaches are ingenious and already well enough calibrated to be a sensible basis of regulatory policy—at minimum the routine screening of every proposed new compound through several tests.

However, this is only a start.

I do not believe that any routine arbitrary procedure will cover all of the potential hazards involved in extrapolating from laboratory data to man. Many new discoveries, some of fundamental importance to biological theory, will be made from unanticipated findings of harmful biological effects—just as the discovery of genetic damage by x-rays was a flash of creative genius when first surmised by H. J. Muller. In most cases it will be metabolic products of a pesticide that will cause trouble, not the initial compound, and it is imperative that we have a clear picture of their biochemical pathways in man. With such data, an investigator might, for example, realize that some biochemical deviants in the human population will be uniquely sensitive; or that the pesticide will interact with some drug or other environmental additive; or that some stage in fetal life may be uniquely sensitive. In any case, the system of evaluation must display all that is known about a new compound so as to assure the most creative thinking by pluralistic critics. This recommendation runs counter to existing policy which regards safety-evaluation data on a pesticide as the private property of the sponsor.

This purpose is probably unachievable, despite the most socially minded intentions, in the context of sponsor-directed research. I would advocate, instead, that the responsibility for safety-testing be in the hands of disinterested third parties, funded by fees taxed to the sponsors. This would also allow a fair allocation of costs to the coattail riders who will jump into competition now only after a pioneer has paid the initial costs of an evaluation out of his own pocket. It would also afford some leeway in selecting which tests are most likely to elicit damning news, a discretion that obviously cannot be vested in interested sponsors.

When chemical mutagenesis was a matter of speculation 30 years ago, many geneticists believed that its realization was indispensable to understanding the chemistry of the genetic material. History has revealed the opposite—other biophysical studies have given us the main clues about the structure of DNA, and we are just beginning to understand the complexity of the cellular processes that result in mutation in the light of that knowledge. We still do not know the local chemical change involved in radiation damage to DNA, while mutagenesis by base-substitution has been analyzed in so much detail that it is the textbook example of molecular

pharmacology.[5] But for many other chemical mutagens there is much more to be learned, perhaps mostly in the metabolism and repair or misrepair of the DNA once it has suffered its primary damage.

This kind of insight already has great promise for more powerful assays of mutagenic potentiality in human cells, for example by direct measurement of the extent of DNA repair in cells subject to experimental or environmental insult, along the lines of Cleaver's studies on repair-deficient genotypes of man.[2]

Geneticists must not now overlook the other side of the coin—the enormous value of reliable measures to *decrease* the spontaneous mutation rate. There has been very little followup of the pioneering work on anti-mutagenic chemicals by Novick and Szilard two decades ago.[6]

Joshua Lederberg

References

1. Auerbach, Charlotte and Robson, J. M.
1944. Production of mutations by allyl isothiocyanate. *Nature 154*: 81.

2. Cleaver, J. E.
1969. Xeroderma Pigmentosum: A Human Disease in Which an Initial Stage of DNA Repair is Defective. *Proc. N.A.S., 63*: 428–435.

3. Some of the history of this incident is recounted by R. Egeberg, et al. 1970, Report of the Medical Advisory Group on Cyclamates, *J.A.M.A., 211*: 1358–1361. See also Mintz, Morton, Rise and Fall of Cyclamates, *Washington Post*, Oct. 26, 1969 (reprinted in the Congressional Record, Oct. 27, 1969, S–13256–8).

4. Federal Radiation Council.
May 13, 1960. Radiation Protection Standards Report No. 1. See also Federal Register, 4402, May 18, 1960.

5. Goldstein, Avram, Aronow, Lewis, and Kalman, S. M.
1968. Principles of Drug Action. Harper and Row, N.Y.

6. Novick, A., and Szilard, L.
1952. Antimutagens. *Nature 170*: 962–927.

7. Rusch, H. P., Bosch, Dorothy, and Boutwell, R. K.
1955. The influence of irritants on mitotic activity and tumor formation in mouse epidermis. *Acta Un. Int. Canc., 11*: 699–703.

8. Scientific Committee on the Effects of Atomic Radiation.
1966. Report to U.N. General Assembly, Official Records, 21st session. Suppl. 14 (A/6314).

9. Starr, C.
1969. Social benefit versus technological risk. *Science 165*: 1232–1238.