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# Perspectives on Testing for Toxic Agents

## by Norton Nelson*

**A series of observations and comments are made with respect to several areas of toxicology: these are briefly discussed. Some innovative areas receive discussion as representing substantial progress made in the field of toxicology in recent years. Topics included raise a number of questions: what agents should we test, and how should we go about selecting them; what is the importance of allowing for genetic diversity in carrying out tests that are meaningful for humans; and what is the relevance of studies in pharmacokinetics in the laboratory to humans. Human studies, because of ethical considerations, must be indirect, through access to available autopsy and surgical human tissues. Also, drug trials and clinical studies must be exploited.**

**Cancer testing and evaluation is briefly commented on. Systemic toxicity is considered in respect to possible improved ways of determining the "NOEL," that is, the no-observed-effect level. Suggestions for improving study of mixtures of chemicals are considered. The rapid advances in molecular biology have significantly strengthened our ability to trace the action of chemicals in the body from exposure to disease. It is very important that training in toxicology be based on a sound disciplinary training in one of the classic fields of the biomedical sciences, such as biochemistry, pharmacology, molecular biology.**

**It is concluded that advances in the past decade have made the practice of toxicology a much more scientific endeavor, especially in its use of the latest developments in basic biomedical sciences.**

## Introduction

I would like to use the broad license given me by the title to make a series of comments on a field that I have participated in and observed for a number of years. These observations lead me to the conclusion that although much solid and impressive progress has been made over the years, a few other areas have, in a relative sense, lagged.

My view of the boundaries of toxicology may not be accepted by all. I include information gathered from the study of human experience (epidemiology) and the use of surgically available human tissues within my definition, always, of course, with full regard for ethical considerations, which exclude human experiments.

## What Should We Test?

What chemicals should we test? As illustrated in Figure 1 (*1*), the study carried out by the National Academy of Sciences under the sponsorship of the National Institute of Environmental Health Sciences (NIEHS) illustrates the very large number of untested or inadequately tested chemicals. The same study proposed a method for establishing priorities for the testing of chemicals. This may or may not be the best technique; there are others. One technique developed under the National Science Foundation a few years back had to do with a priority assessment technique that depended on the total amount of material produced, modified by what was called its "release rate" (*2*). The release rate is the fraction of the material that goes back immediately into a new synthetic process. Obviously, it is the release rate of a chemical that determines more directly than total production whether or not the chemical is likely to become widespread in the community; of course, the extent of handling within a plant can, in some degree, alter occupational exposure.

The current premarket notification system which our present Toxic Substances Act requires is very general; thus, the very minimum amount of information is required before a chemical is permitted to enter into manufacturing and use. Currently the system is very largely based on little more than structure-activity relationships. It is true that EPA can, on the basis of their study, request additional testing under the present law. It may well be that Congress should reexamine the Toxic Substances Act and see whether more stringent requirements for premarket testing should not be required as, in fact, is the case in some other countries. Obviously, the testing responsibilities would be different depending on whether the responsibility should be that of manufacturer or whether the chemical is already in the common domain for which no one manufacturer has specific responsibilities. There should clearly be more information available before manufacturing proceeds.

## Genetic Diversity

There is an element of partial self-delusion involved in the choice of inbred strains in toxicity tests. Inbred

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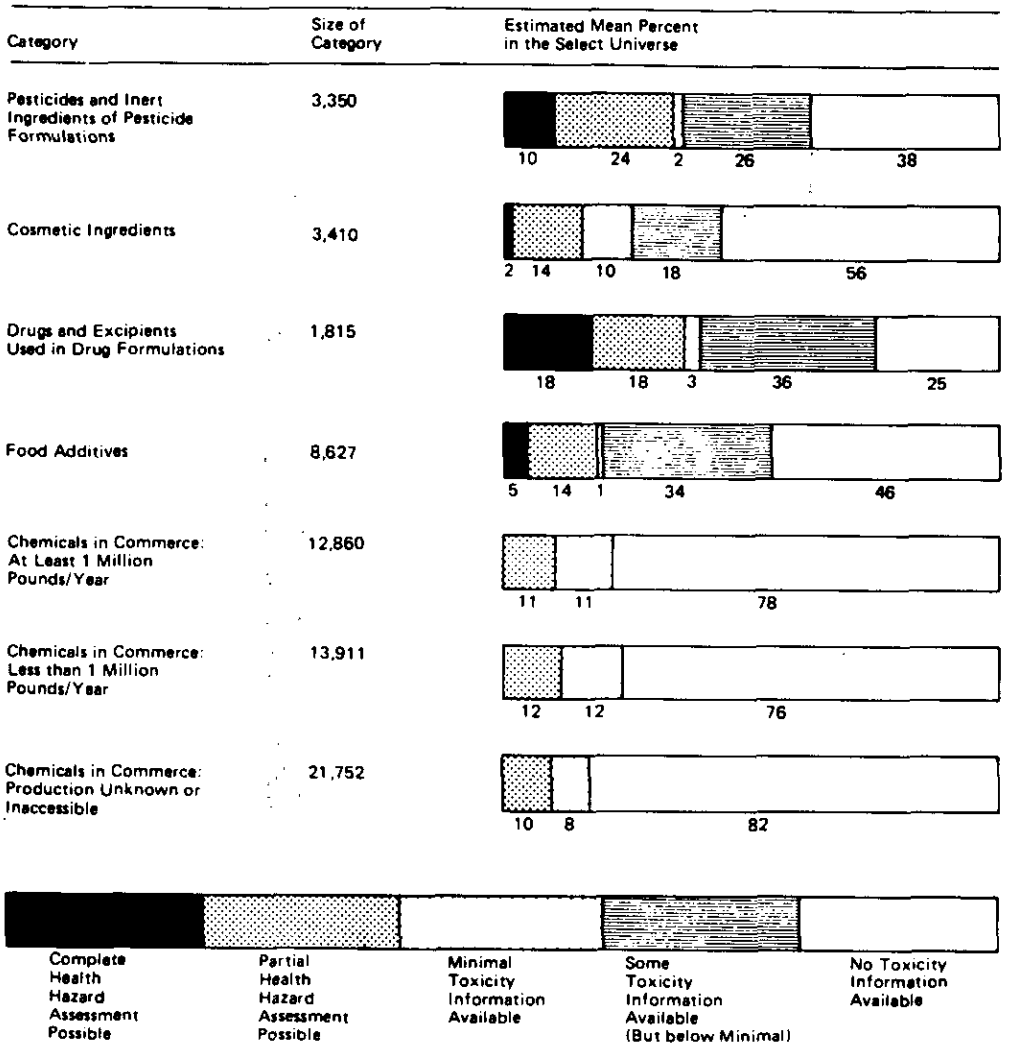


FIGURE 1. Ability to conduct health-hazard assessment of substances in seven categories of select universe.

strains, of course, lead to more orderly and repeatable tests, which increase the likelihood of getting more comparability in tests carried out in different laboratories and at different times. This is, of course, a desirable objective, but we should also keep in mind that another objective in toxicological appraisals is to use the strain or strains that best mimic the human responses or, failing that, the most sensitive strain. The approach based on using the strain or species that is either most sensitive or most faithfully mimics human response is rarely supported by any actual evidence to support such choices.

One possible approach to this objective might be the addition of at least one genetically diverse species or strain alongside the highly studied inbred strains. Obviously, pharmacokinetic data supporting parallels between humans and test animals can be very helpful in choosing the test strain.

We are well aware of the wide range in susceptibilities in humans and at least give lip service to the need for protection of the susceptible components of the population. Nevertheless, we live to a substantial degree in ignorance of the range of susceptibilities. One can, for example, look at the work of Harris (*3*), which dealt with the capabilities of human tissues to bind aromatic hydrocarbons as at least one measure of the very wide diversity in biochemical interaction in humans (Fig. 2). With the current major advances in genetic characterization, this field is capable of advancing very rapidly.

Another example is from the work by the Palmes group (Fig. 3) (*4*) on the wide diversity in the dimensional aspects of human pulmonary structure. This diversity influences susceptibility because particle deposition will be altered by the differences in small airway dimensions in the pulmonary system; variations in the extent and pattern of deposition will alter response to inhaled toxic particles.

As laboratory tests for toxicity expand and proliferate and become more routinized, one sees fewer and fewer studies on larger animals such as dogs, cats, and pri-

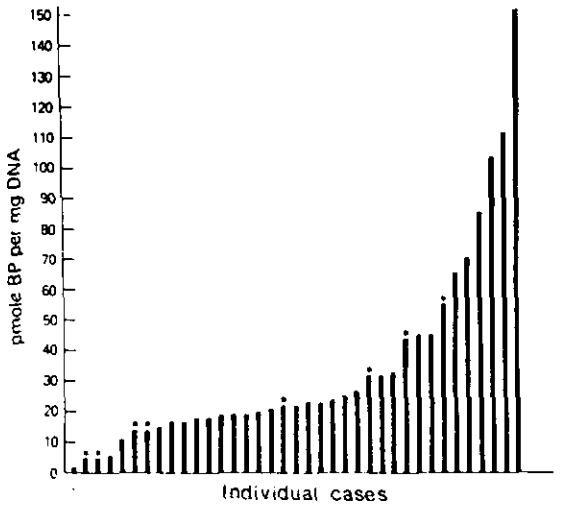


FIGURE 2. The interindividual variation in the binding levels of BP to DNA in cultured human bronchi. The values marked with an asterisk are from patients without lung cancer and the remainder are from lung cancer patients.

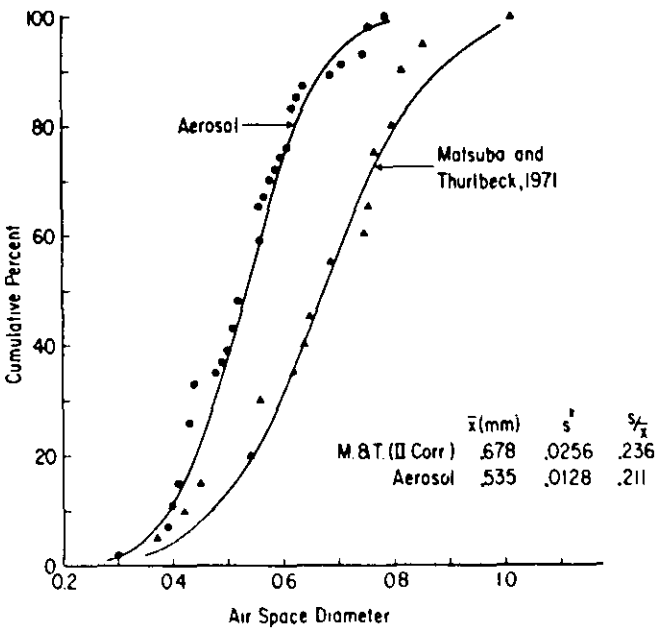


FIGURE 3. Distribution of airspace diameters by aerosol persistence compared with diameters of small (< 2mm) nonalveolated airways found by Matsuba and Thurlbeck (*20*). The ordinate refers to the percent of airspaces less than the stated diameter.

mates; these have never been studied in large numbers but, nevertheless, have been sometimes extremely useful in disclosing patterns of response that rodents do not show. Thus, the specificity of the dog in responding to bladder cancer with aromatic amines (mimicking human responses) would have been missed had we been dependent on the current procedures using rodents only.

## Pharmacokinetics

For many years toxicologists have urged the importance of the understanding of the comparative behavior of chemicals in the test species and man for valid prediction of effects in man. This requires a pharmacokinetic model of exposure and selective entry into various parts of the body where it is metabolized or excreted, or both. This is shown in Figure 4 where we illustrate a somewhat simplified mammal capable of taking up external chemicals through the lungs, through the skin, and through the mouth; subsequently, the agent or its products move through the organism subject to a variety of processes of diffusion, circulation, metabolic alteration, and eventually excretion. This scenario represents those classical processes which we collectively call the pharmacokinetic handling of chemical agents, which we have grappled with for many years; but we are now becoming more and more aware of the vital importance of such considerations in the study of the effects of foreign chemicals on the mammalian organism.

Both species diversity and genetic diversity within species force us to use a variety of approaches to attempt to secure valid predictions for humans through the study of both human and nonhuman organisms.

## Human Studies

As noted earlier, the realm of toxicology should not stop with laboratory studies. There are many sources of information from human populations that can be used to strengthen our attempts to protect humans from

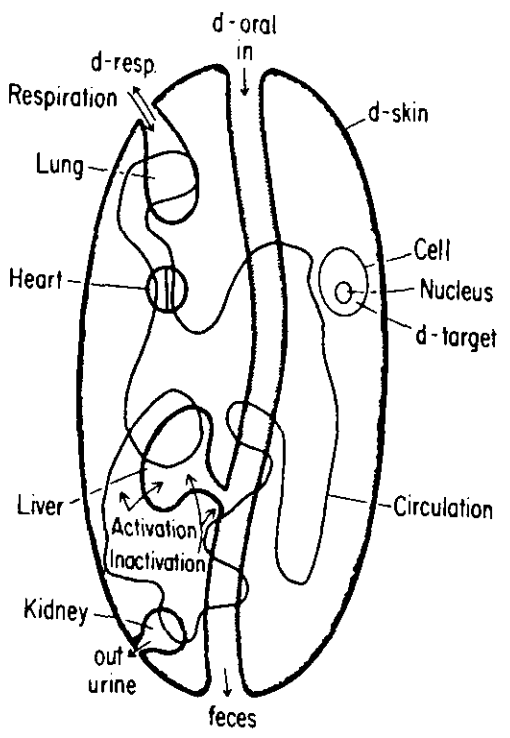


FIGURE 4. A mammal (slightly simplified).

chemical threats (*6*). These sources include fuller use of drug trials, fuller use of available human tissues as practiced by Harris (*3*), and fuller use of those examples in clinical medicine that can inform on human responses. A major advance over the years has been the emergence of ethical considerations that have prevented reckless and needless experimentation on humans. There are, nevertheless, while avoiding this, data that can be very instructive if we exploit the information being collected in our hospitals and clinics. It is in this sense that NIEHS plans to develop a series of clinical and research training appointments in which toxicological interests are linked to clinical units for the fuller exploitation of human experiences to help provide information on human responses.

## Comments on Cancer Testing and Evaluation

Our understanding of the mechanisms of cancer induction have improved dramatically over the last 15 years; many profoundly important advances have been made. With the emergence of the oncogene, we have some more exciting years ahead. On the other hand, the ancient PHS Publication 149 (*7*) has been since 1948, and still is, a highly useful and fruitful source of information.

Although the use of quantitative risk assessment (QRS) is now more or less routine in the evaluation of carcinogens, it may be too comfortably accepted by many people. Those who practice it, as well as those who have observed its use, need a healthy skepticism as to the validity and reliability of these procedures. Nevertheless, QRS is an indispensable step in the evaluation of carcinogenic potency. Moreover, the use of QRS has led to a more critical evaluation of the basic data in relating to carcinogenesis and has the potential of improving protocol design and test procedures.

The Ames (*8*) procedure has been an extraordinarily powerful tool for assessing mutagenicity and carcinogenicity; it promises still broader reliability with newer supplementary procedures (*9*) in expediting the very heavy burden of testing chemicals for carcinogenicity.

There is sometimes a tendency in our study of the biological effects of xenobiotics to assume that by naming the process or the response, we have solved the biological complexities involved. A prime example is the use of the term "promotion" in the multistage carcinogenesis model. The assumption appears to be sometimes made that all promoting mechanisms are biologically similar. Is the response to phorbol ester on the skin of mice after a single application of benzopyrene a truly comparable mechanism to that present in the use of barbituates following initiation in the study of rodent liver cancer? Premature classification based on operational similarities can sometimes discourage seeking underlying biological differences.

A somewhat similar position is perhaps evident in the field of metal carcinogenesis. An understanding of the processes by which metal compounds induce malignancy has been hindered sometimes by the assumption that metals *per se* have similar biological mechanisms in their carcinogenic action. In fact, there may be as much difference among the various species of metal compounds as there is from one group of metals to another. Accordingly, one should not seek for easy classification that may obscure the real differences lying behind the biological processes.

## Systemic Toxicity

The evaluation of noncarcinogenic responses have not been conceptually changed in any major way over the years. We still depend largely on tests in laboratory animals for the detection of a range of dose responses, including the estimation of a no-effect level. As noted below, short-term tests still play only a modest role in evaluating systemic toxicity.

The "O" in the NOEL (*10*) recognizes that the no-observed-effect level states the reality as to what was or was not seen. However, at its crudest, this is an area in no man's land, in which the NOEL is highly influenced (even wholly dependent) on the size of the group and the interval in spacing of the doses. It makes use of no more than two points in what might otherwise be a well-conducted multidose study. More recently, and elsewhere (*5*), I have suggested a different approach toward the estimation of what might be called a "computed NOEL." A computed NOEL would be based on the fitting of the dose-response data to any one of the classical models (logit, probit) over the range of test dosage. That is to say, the NOEL is calculated, from the earliest detected effect all the way up to the highest dose, which still shows the earliest effect detected. For this purpose, it matters relatively little which dose-response curve is used, over the normal group size; it will not make much difference which curve is chosen. One now computes the lowest frequency of occurrence that is likely to be observed (or missed) with the particular group size used. If one then takes the dose from the fitted curve at that point, one has chosen a point on the curve that is at the borderline of observing or not observing an effect. This can thus be regarded as a computed, or a synthetic, NOEL. Such a NOEL has the immense advantage that one can use all the points that are relevant and available and can do so within the context of their statistical validity so that confidence limits can be calculated.

What one then does with this computed or synthetic NOEL is a separate issue, not really very much different than what one would do with a so-called observed NOEL. A logical approach is to determine the computed NOEL and draw a line down to zero or the normal background occurrence. One may then, in a risk management sense, choose the appropriate risk point; indeed, the slope of that extrapolated line may serve as an index of potency. Conversely, one may choose to divide it by any one of the commonly used safety factors, 10, 100, or 1000, depending on the risk management

circumstances imposed by nontoxicological considerations.

Attempts to develop short-term tests for other systems and organs along the lines of the Ames test (*8*) have been much less promising and are certain to be more difficult, since, at least in part, the underlying biological processes are not as well understood (a statement perhaps many would challenge). A recent study that systematically sought improved short-term test procedures in isolated systems for end points other than genotoxicity has produced many useful approaches to the problem but did not, in fact, succeed in identifying major simplified tests of the effect of chemicals on entire organs or organ systems (*11*). This review was only a beginning and needs substantially more work; such studies should be given strong encouragement and support.

## Mixtures

Toxicologists live uncomfortably with the assumption that humans are exposed to pure chemicals when they know that, almost invariably, mixtures are involved. Often the mixtures are very complex. The evaluation of toxicity based on information from pure chemicals is often predictive of the toxicity of mixtures. Repeated endeavors have been made to develop what may be called a "calculus" for deducing the effect of mixtures from data on single chemicals. This has largely failed except where the chemicals have similar effects; this was very well examined many years ago (*12*). A recent systematic and extended study (SGOMSEC 3) (*13*) has a number of useful proposals for study of mixtures.

This may be an area in which an orderly application of some of the newer computer techniques for dissection of complex molecules with attribution of different modes of action to separate components could usefully be explored to characterize mixtures of chemicals. This would suggest the consideration of a deliberate set of studies in which mathematical and computer approaches (*14–16*) could be applied to the analysis of biological tests of carefully selected mixtures as an approach to the development of practical techniques for studying the biological responses to mixtures of unrelated chemicals.

## Short Cuts

We are confronted with an extraordinary amount of needed information on chemicals not previously tested or inadequately tested in the past. This will require much more work, but also a great deal of ingenuity in finding more efficient and informative ways to deal with what would otherwise be an overwhelming task. We thus seek short cuts.

There are currently in toxicology some simplifications which, although perhaps useful, do not instill immediate confidence that they produce accurate results. One is the relationship between toxicity and carcinogenicity (*17*). That there should be a correlation is not really very surprising; however, it would be very surprising if, in fact, there were not many exceptions to the partial parallelism that has been observed. It could well be that study of the exceptions may be more useful than study of the parallelism; the basis for the differences may be very instructive.

In the face of the enormous mass of new information required, there is search for simplifying assumptions both in the conduct of tests and in their evaluation. This has led, in some instances, to the search for much simplified, even single, indices of toxicity. This is an endeavor which has some utility in setting priorities for limited purposes but has intrinsically many dangers in the likelihood of obscuring the very important features which can only be expressed in terms of a narrative statement which includes the appropriate qualifications. We should be very wary of inappropriate use of oversimplification, despite the fact that they may have justifiable applications in limited areas.

A very promising example of what should not be regarded as a short cut, but as a broad spectrum of accelerated toxicity tests that deserves careful study as producing a well-rounded body of information, is contained in the NTP study on methyl isocyanate (*18*). This exercise welded together proven toxicological techniques of the past with very recent advances in developing information on a wide range of important end points. This entire study was completed in some 8 months and yielded a remarkably broad and rich body of information which will certainly provide, as a minimum, a basis for more detailed studies and in some cases, will eliminate the need for more extended studies. It can set an example of efficient choice and application of our advancing skills in toxicology.

Thus, the full exploitation of the advances in toxicology of the last few years, building on the still relevant remnants of the past, can provide much improved ways of dealing with the awesome body of needed but missing information.

## Markers

The word "marker" has come into fashion to replace the word "indicators." These indicators or markers are, indeed, a very interesting mixture of older topics for which we now have somewhat different names and very much newer knowledge which emerges directly out of the very forefront of the science that has been developing in the last few years, molecular biology.

Figure 5 (*19*) represents such a blending of the old and the new. This blending is, in fact, a filling in of gaps of ignorance that confronted us just a few years ago. Whereas, at one time, we spoke of exposure and the effect without dwelling in any great detail on the biological events lying between exposure and effect, we now can speak often with very great circumstantiality and detail about the events occurring between exposure and the effect or disease itself.

Figure 5 originated under a contract between NIEHS and EPA with the Council on Environmental Quality and the National Science Foundation (*19*). It is now

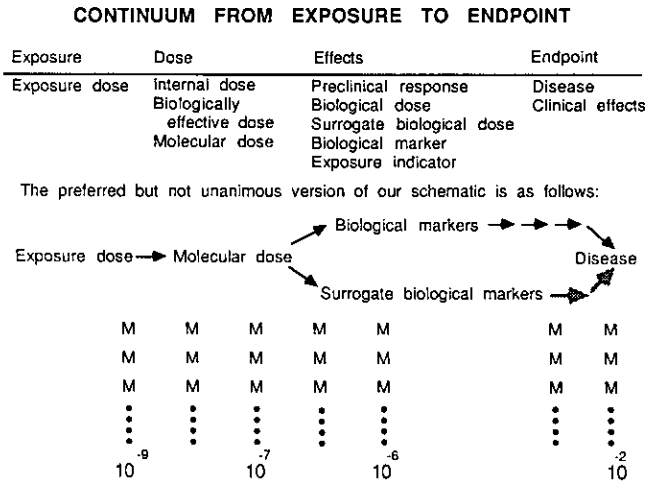


FIGURE 5. Markers of exposure and effects.

possible to identify a series of steps lying between exposure and the penultimate or ultimate effect. The term markers (or indicators) has been widely adopted now to cover the whole series of stages or transitions between external exposure and ultimate effect, which may be disease or death; one can now identify some of the specific biochemical events that are associated with these progressive markers.

The term marker as used here represents a sequential or parallel step or transition in the movement of events from early exposure to overt biochemical disorder, onto an easily visible effect, and, in some cases, outright disease. The chart is deliberately rather fuzzy with respect to the number of marker transitions or marker events because there are still many blanks to be filled and because they can serve to cover many different phenomena. Figure 5 starts with external exposure, progresses through absorption and movement to an internal dose, hence, to a biologically effective dose or molecular dose (where they may be called biological markers or exposure indicators), onto a preclinical response from which stage they pass on to clinical effects and disease. In some cases, one might consider surrogate biological markers in addition or instead of the actual biological markers. The uptake of ethylene oxide or other alkylating agents through interaction with hemoglobin could be such a surrogate marker; that is, an agent that might be deliberately administered in order to trace the extent to which it does interact with biochemical molecules. Or, one may look at a cell which is accessible, e.g., a leukocyte as a surrogate cell for one less accessible.

Looking at the bottom of the Figure 5, one can see that there are numerical exponents which are intended to crudely indicate the level of detectability or the level of involvement of the phenomenon at several stages. The stages may start, for example, with the part per billion detectability for the exposure dose, progress through a detection of the molecular dose at the level of one in 10 million, to a biological marker at a level of one in a million, finally to disease where the marker may be detectable only at the order of 1%. There are obviously a series of steps in between many of these markers which, at this stage, may defy detection or analysis.

The filling in of what were until recently blank spaces in a chart such as this is in itself a major accomplishment in science. In many instances, a marker can identify specific entities, e.g., an adduct on a protein or an adduct on DNA, which not only marks evidence of interaction, but may in itself be a marker of an altered biochemical unit which can have grave consequences, e.g., through a mutated DNA which can lead eventually to cancer induction in the tissues involved. Such increased sensitivity and specificity gives promise of being able to detect at a much earlier stage the progression of events that may have grave consequences for the bearer of particular markers. As such, the promise of earlier detection of the action of toxicants can have important public health benefits.

Obviously, all such markers do not carry with them such grave consequences; some may be simply transitional interactions with a protein or biochemical unit which when that particular protein or biochemical unit is replaced will disappear forever. But conceptually, we can now begin to look more closely at the intervening events between exposure to a foreign chemical, the nature of its action, and the likelihood of the outcome of the biological interaction.

## Training

The field of toxicology is perhaps not as new as is sometimes supposed. In the 1940s, training programs were rare and essentially nonexistent in the sense of being specifically designed to train toxicologists. The recruitment into the field came from a few disciplines, heavily pharmacology and biochemistry, with smaller numbers from other allied fields, including chemistry in all its branches. I direct my attention to a career in which the objective is to improve our understanding of the mechanisms of action of toxicants either in general biological terms or specifically aimed at a contribution to the eventual incorporation of such information into the test armamentarium, and to improve efficiency and sensitivity of the detection of injury.

Nothing can substitute for a solid basic disciplinary training in one of the classic fields of science, be it pharmacology, biochemistry, cell biology, or, even better, a selection of these fields with a clear focus on the application to toxicology. This may be longer and more drawn out than normal training restricted to a classic field of science, but I believe it to be the best training for those who are searching to understand how chemicals produce injury.

A well-rounded predoctoral and postdoctoral training within the classic disciplines teaches one not only the basic techniques (transient though they be) in the biological sciences but, most importantly, teaches a very important lesson, that is, how to recognize the uncertainties encountered in biological research and the need

to be constantly ready to alter one's approach, to alter one's thinking, and to instill the requisite skepticism in looking at a body of data to test it for its soundness, coherence, and relevance.

It should further be said that this implies a substantial period of time in the direct conduct of research. Indeed, I would feel that the specifics of that research are relatively unimportant. It is, however, very important to work closely with a mentor of critical mind and imagination who is willing to give the neophyte a loose rein for trial and error in the learning experience. One should not, in this arena, be too much concerned by the name that is applied to the course of study. Some programs called toxicology are, unfortunately, routinely oriented to the simplest kind of repetitive testing. Others, in fact, have at their base the ingredients of an excellent basic discipline in one or several of the biological sciences. Obviously, whatever be the major route, total restriction to the biological sciences is, of course, shortsighted in these days when competence in the physical techniques and computer resources with grounding in the physical and mathematical sciences is essential.

One can accept that training for the more basic practice of toxicology can be done within any qualified basic department of biological science. However, there is an important bonus when such basic training occurs within a program committed to the basic field of toxicology; this brings directly and at an optimal period an orientation in the breadth and linkages of the field.

In summary, toxicology as a field has matured and grown into a science that can now, and does, command the commitment of the best scientists in the biomedical arena. Indeed, it is returning new and fruitful ideas to the basic sciences from which its own inspirations and advances were drawn.

## REFERENCES


1. National Academy of Sciences/National Research Council Report on Toxicity Testing Strategies to Determine Needs and Priorities. National Academy Press, Washington, DC, 1984.
2. Stephenson, M. E. An approach to the identification of organic compounds hazardous to the environmental and human health. Ecotoxicol. Environ. Saf. 1: 39–49 (1977).
3. Harris, C. C., Autrup, H., Connor, R., Barrett, L. A., McDowell, E. M., and Trump, B. F. Interindividual variation a binding of benzo[a]pyrene to DNA in cultured human bronchi. Science 194: 1067–1069 (1976).
4. Lapp, N. L., Hankinson, J. L., Amandus, H., and Palmes, E. D. Variability in the size of airspaces in normal human lungs as estimated by aerosols. Thorax 30: 293–299 (1975).
5. Nelson, N. Use of toxicity test data in the estimation of risks to human health. In: Assessment and Management of Chemical Risks, ACS Symposium Series No. 239 (J. V. Rodricks and R. G. Tardiff, Eds.), American Chemical Society, 1984, pp. 13–22.
6. Nelson, N. Epidemiology and Toxicology. Presented at the Annual meeting of the Collegium Ramazzini in Bologna, Italy, October, 1985. New York Academy of Sciences Press, New York, in press.
7. Hartwell, J. L. U.S. Public Health Service Publication No. 149, Washington, DC, 1951. Continuing Volumes to 1980 and Cumulative Indexes, November 1984.
8. Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31: 347–364 (1975).
9. International Programme on Chemical Safety, Environmental Health Criteria 47, Summary Report on the Evaluation of Short-term Tests for Carcinogens (Collaborative Study on In Vitro Tests). World Health Organization, Geneva, 1985.
10. National Academy of Sciences. Principles for Evaluating Chemicals in the Environment. National Academy of Sciences, Washington, DC, 1973.
11. Nelson, N. Chairman Scientific Group on Methodologies for the Safety Evaluation of Chemicals Workshop held in Ottawa, Canada, August 1984, The Predictive Value of Short-Term Tests for Non-Genotoxic Effects. John Wiley and Sons, Sussex, England, in press.
12. Bliss, C. I. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26: 585–615 (1939).
13. Nelson, N. Scientific Group on Methodologies for the Safety Evaluation of Chemicals Workshop held in Guildford, England, August 1983, Methods for Assessing the Effects of Mixtures of Chemicals. John Wiley and Sons, Sussex, England, in press.
14. Rouvray, D. H. Predicting chemistry from topology. Am. Sci. 254: 40–47 (1986).
15. Herndon, W. C., and Szentpaly, L. V. Theoretical model of activation of carcinogenic polycyclic benzenoid aromatic hydrocarbons. Possible new classes of carcinogenic aromatic hydrocarbons. J. Mol. Struct. 148: 141–152 (1986).
16. Klopman, G., Frierson, M. R., and Rosenkranz, H. S. Computer analysis of toxicological data bases: Mutagenicity of aromatic amines in salmonella tester strains. Environ. Mutat. 7: 625–644 (1985).
17. Zeise, L., Crouch, E. A. C., and Wilson, R. A possible relationship between toxicity and carcinogenicity. J. Am. Coll. Toxicol. 5: 137–151 (1986).
18. McConnell, E. E., Bucher, J. R., Schwetz, B. A., Gupta, B. N., Shelby, M. D., Luster, M., Brody, A. R., Richter, C., Stevens, M. A., and Adkins, B., Jr. The toxicity of methyl isocyanate. Summary, in press.
19. Executive Office of the President, Council on Environmental Quality. Report on Long-Term Environmental Research and Development. Washington, DC, 1985.
20. Matsuba, K., and Thurlbeck, W. M. The number and dimensions of small airways in nonemphysematous lungs. Am. Rev. Respir. Dis. 104: 516–524 (1971).