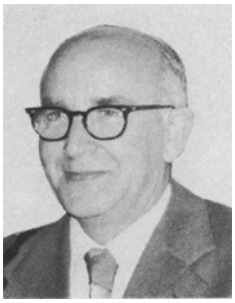

Human Health Effects: Importance of Chronic and Genetic Evaluation on New Large Volume Chemicals Disposed into the Environment with Potential Entry into the Food Chain

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ABSTRACT

In the process of benefit-risk assessment, it is the responsibility of the scientist to provide qualitative and quantitative estimates of risk associated with human and environmental exposure to a compound. In order to do this, a systematic approach to safety evaluation is proposed. The need is stressed to take account of the current revolution in toxicological thought and practice. Particular emphasis is laid on the early introduction of studies on metabolism and pharmacokinetics, the results of which act as a guide to the determination of doses and duration of subchronic and long term studies. Chronic and genetic evaluations are regarded as the superstructure resting on well laid foundations. Consideration of tests for genetic effects emphasizes the useful but limited role of microbial assays of mutagenic potential, in contrast to the very real value of using mammalian systems in vitro or in vivo. All have a long way to go in terms of standardization, validation, and exploration of a wider range of chemical classes. Long term toxicity tests are intended to delineate the dose-response relationships of adverse effects, including carcinogenicity. Without exception, all such relationships are subject to mathematical analysis to provide risk estimates that can be used in conjunction with data concerning anticipated human and environmental exposures. Risk estimates should also be applied to the potential for entry into the food chain, taking into account all factors relating to this phenomenon.

BENEFIT-RISK ASSESSMENT AND ITS ELEMENTS

Benefit-risk assessment is fundamental to the decision to introduce any new chemical on the market today. Quite apart from regulatory considerations, every manufacturer has sound economic incentives, as well as moral and ethical responsibilities, to make sure that a new chemical meets the necessary criteria for safety to man and to the environment. A broader judgment needs to be made of benefits and risks associated with the new chemical and of the balance between benefits and risks. Analysis and evaluation of benefits is a societal decision (Table I). On the other hand, risk assessment and interpretation is the province of the scientist. The view is held in many quarters that, apart from the purely technical role of delineating the toxic properties and potential for harm, the scientist should have no further input into the risk-benefit discussion. In fact, the imprecision and uncertainties of biological data render it essential that the scientist, and particularly the toxicologist, participate in the decision-making process to provide the benefit of specialized knowledge and understanding (1).

ROLE OF THE SCIENTIST IN RISK ASSESSMENT

As will become evident in the course of this presentation, risk assessment depends on sufficiency of first-rate data. The issue of quality vs. quantity is discussed. What should be stressed here is the importance of designing studies that are appropriate to the particular chemical under consideration and to the types of exposure of man and the environment to the chemical under conditions of use. As the studies progress, questions and scientific issues emerge that need specific investigation. It is a serious error to ignore these in the naive belief that once a series of prescribed discrete toxicologic "packages" have been developed, the sum of the resulting information suffices for safety evaluation. Not infrequently the truth falls between the cracks! In other words, interpretation of the results may be impossible without additional data from more specific and sharply focused investigations.

A word of caution, however. In clarifying the significance, weaknesses, and uncertainties of the available data, the scientist is always tempted to fall back on the need for further research. Suggestions for ways of strengthening and clarifying the data are appropriate but do not necessarily imply that what has already been achieved is inadequate for the purpose of reaching a practical decision. To achieve reliable overall assessment of the significance of the findings in terms of hazard or conditions of safe use calls for expert judgment of a high order. This is a source of many problems since not infrequently it is the lawyer, consumer advocate, or other lay member of the community who seeks to reach his or her own assessment of risk. Wisdom dictates that they seek the help of professional experts.

A key source of information should be mentioned,

TABLE I

Benefits: Elements of the Societal Decision

1.	Advantages to the individual
	Practical: convenience, health and hygiene
	Esthetic
2.	Advantages to Society
	Economic
	Conservation of resources
3.	Advantages to environment

TABLE II

Environmental Considerations

1.	Pollution: water, land, air
2.	Effects on biota: wildlife, vegetation
3.	Esthetic values
4.	Economic damage: property
	natural resources
	energy resources

TABLE III

Food Chain Implications

1.	Stability and persistence	
2.	Availability to relevant organisms	
3.	Potential for biomagnification	
4.	Toxic consequences:	along food chain at peak of food chain to man

namely evidence of the effects of the compound in man. These data may be forthcoming from special studies on human volunteers exposed under controlled conditions, from periodic review of reported effects after marketing, or from epidemiologic studies. The problems that confront the investigator in this area merit much fuller discussion than can be afforded them here.

NONTOXICOLOGICAL FACETS OF RISK ASSESSMENT

At all stages in safety evaluation it is very helpful to know the nature and levels of anticipated exposure to a compound, as well as to its accompanying impurities, degradation products, metabolites, and other compounds formed by biodegradation in man and the environment. The earlier that this information can be made available, the better. To secure the data, analytical procedures have to be developed. These are essential in revealing some aspects of the environmental concerns that need to be considered (Table II).

There are special implications arising from the possible entry of the compound into the food chain (Table III). Experiences with such environmental contaminants as the chlorinated pesticides, phthalate esters, polychlorinated biphenyls, and hexachlorobenzene have provided clear precedents for the study of compounds entering the food chain. Perhaps the most important step along the way is the analysis of human milk to ascertain the qualitative and quantitative aspects of materials that are already in the food chain and to detect new compounds.

TOXICOLOGY AS THE KEY ACTIVITY IN SAFETY EVALUATION

The objective of toxicological investigations is to define the dose-response relationships that exist with regard to the variety of effects that the compound is capable of eliciting in living systems. The hierarchy of tests is so structured that we proceed from general information that is valuable to those who have to manufacture and handle the compound in bulk, through a series of stages in the decision tree that involve progressive increments in time and effort invested in the compound. Simultaneously, as a rule, technical development proceeds in parallel with safety evaluation.

The harsh realities of present-day regulations are such that decisions committing oneself to substantial investments in toxicity and related testing efforts have to be made much earlier than heretofore. Accordingly, more sophisticated approaches are called for in the initial phases of the investigation, involving the combined skills and efforts of a multidisciplinary team that can apply critical tests to reveal the toxic potential of the compound and attempt to clarify the meaning of the effects observed, in advance of the more routine studies, or at least concurrently with them. The tests that may be applied are many and varied, but basically they hinge upon gaining an understanding of the behavior of the compound in the body and of the action of the body on the compound – a variety of items of information collectively referred to as "metabolism and pharmacokinetics." In the case of a compound that enters the environment and possibly the food chain, the common thread that runs through the whole gamut of organisms and ecosystems, from man to soil bacteria, is the metabolism and pharmacokinetics of the compound under various circumstances. The influence of dose and route of exposure, as well as the metabolic bases for species and organ specificities of toxic actions, are illuminated by the information gained through combination of biochemical toxicology with morphological approaches.

The examples cited above illustrate the key role of the toxicologist in directing the investigation of the compound along the most meaningful lines dictated by the developing information on the metabolic and pharmacokinetic characteristics of the compound and its biological properties. The entire strategy calls for insight, based on training, expertise, and experience. Neither official "guidelines" nor collections of detailed "recipes" can serve or substitute for knowledge and mature judgment.

CURRENT REVOLUTION IN TOXICOLOGICAL THOUGHT AND PRACTICE

These thoughts lead to a consideration of the climate of opinion regarding environmental hazards. There is increasing emphasis on the so-called "irreversible" long term effects: carcinogenic, mutagenic, and teratogenic actions. Although there is ample justification for legitimate concern about such effects, a totally disproportionate anxiety over environmental carcinogens has been stimulated by what Dr. A.C. Upton (the newly appointed director of the National Cancer Institute) considers to be a foolish "dogmatic statement that 90% of human cancer is caused by environmental factors" (2). For years this frequently repeated assertion was interpreted as referring entirely to man-made chemicals; but now chemicals are regarded as contributory causes of only a very small proportion of human cancer, the major villain now being called "lifestyle" (3).

At regular intervals some new soothsayer announces the coming of a cancer "epidemic." Ames (4) is the latest prophet of doom, having just become aware of the presence in natural food of a plethora of carcinogens and mutagens, especially when the food has been cooked. Thus, in fact, the cancer "epidemic" has been with us from time immemorial. It is in the area of naturally occurring carcinogens and mutagens that analytical advances provide a cutting edge for discovery of fresh hazards. The thermal energy analyzer (TEA) has revealed the endogenous formation of appreciable levels of dimethylnitrosamine and diethylnitrosamine, both of them potent carcinogens and mutagens, during and after the consumption of a rather prosaic lunch (5). TEA has also made possible the discovery of nitrosamines present in a variety of amines and related products, as indicated by the following list (6): alkylamine salts, alkanolamine salts, dinitroaniline derivatives, substituted ureas, alkylcarbamates, dithiocarbamates, amides, secondary and tertiary amines, quaternary ammonium compounds, guanidines, triazines, hydrazides, and the compound phenothiazine.

Another trend with important implications is the belief that in many instances carcinogenesis is initiated by a somatic mutation. Some even believe that if a compound is a mutagen, it has not only carcinogenic potential but, with sufficient determination and dedicated effort, it can also be shown to be a teratogen. Tests for mutagenic potential thus assume great importance, and the superficial simplicity of bacterial tests such as that developed by Ames fosters the illusion that "short-cuts" are at hand that will render obsolete the old, cumbersome, expensive, and time-consuming procedures used to assess the potential for "irreversible" effects. Alas, the day of liberation has not yet dawned! The significance to man, or to other organisms, of tests carried out with bacteria remains unclear.

Metabolic activation is an essential factor in mutagenesis

TABLE IV

Plan of Work Preceding Chronic Studies

1. Physical and chemical properties; specification
2. Analytical procedures: compound and impurities
3. Acute toxicity and irritancy; sensitization potential
4. Metabolism and pharmacokinetics
5. Subchronic studies
6. Reproduction and teratogenicity

TABLE V

Acute Toxicity

	a. Single dose	b. Repeated doses
1.	Clinical observations:	behavioral pharmacological
2.	Gross pathology	
3.	Microscopic pathology	

TABLE VI

Metabolism and Pharmacokinetics

1.	Metabolic studies in vitro	
2.	Radioautography	
3.	Metabolism:	qualitative quantitative
4.	Pharmacokinetics:	dose-time effects mathematical modeling
5.	Environmental:	degradation biotransformation

tests or in vitro carcinogenesis tests, converting "promutagens" and "procarcinogens" into chemically reactive metabolites capable of undergoing covalent binding with cellular and other macromolecules. Evidence of covalently bound derivatives of a compound, present in proteins and/or nucleic acids, is a valuable index of exposure and suggestive evidence of the potential for adverse effects. DNA damage brought about by a compound or its chemically reactive metabolites is subject to excision repair, as well as postreplication repair, and the rate of repair in specific organs such as the nervous system may prove to be a limiting factor in the capacity to avoid development of neoplasia. All these considerations emphasize the central role which is increasingly played by metabolism and pharmacokinetics in safety evaluation, not only for purposes of design of studies and interpretation of results, but also for insight into mechanisms of, and factors influencing, toxic action.

TESTS PRECEDING CHRONIC AND GENETIC TOXICOLOGICAL STUDIES

Foundations necessary for the superstructure we are about to discuss are indicated in outline form in Tables IV-VII. Only some general comments are appropriate here. Selection of the product to be tested is a crucial and often difficult decision. For instance, does one use the technical product or a highly purified form of the compound? By and large, it is advisable to start with the actual product intended for use. If analysis reveals the presence of obviously unacceptable or even questionably significant impurities, one should consider how they can be eliminated or minimized before engaging in substantial toxicological effort with the compound.

Pursuing the analogy of a sound foundation, the dose-response relationship is the key item of information that should emerge from the preliminary studies. Metabolic and

TABLE VII

Subchronic Studies

1. Dose-effect relationships
2. Target organ and other effects
3. No-adverse-effect level
4. Foundation for long term tests

TABLE VIII

Genetic Toxicology

1.	Gene mutations:	forward reversions
2.	Chromosomal aberrations:	germ cells somatic cells
3.	Effects on DNA repair	

TABLE IX

Long Term Tests

1.	Objectives:	toxicity, carcinogenicity
2.	Conflicts:	"Maximum Tolerated Dose" threshold extrapolation to low doses
3.	Problems:	species and strains, diets dose selection background "noise," senility

pharmacokinetic investigations should have contrasted the behavior of low and high doses in several mammalian species, especially those contemplated for use in the long term tests, and perhaps even in some of the species to be used in the genetic tests.

GENETIC TOXICOLOGY AND ITS CURRENTLY UNRESOLVED PROBLEMS

The principal concerns of genetic tests are indicated in Table VIII. Unfortunately this field has been dogged by the promises and perception that it offers short-cuts, well-established methods, and easy answers. Far from doing that, it is a treacherous quagmire which may well prove to be an unjustified burial ground for many valuable compounds. To avoid this danger, a course of conduct is necessary based on critical and realistic appraisal of the state of the art: the merits, weaknesses, and consequent priorities of the available test procedures.

Foremost in everyone's mind is the Ames test, and related microbial tests, in which a positive result is popularly believed to correlate closely with carcinogenic potential. It is more in keeping with the facts to state that, in most instances, given a compound that is known, believed, or desired to be carcinogenic, some organism can be discovered or procedure devised to give a positive mutagenic result. At that point, to attempt to establish that the positive result is fallacious, or of no significance to man or other mammalian organism, may prove to be a herculean task. Thus the superficially attractive simplicity of bacterial tests is an illusion. Most effectively, such tests should be used for preliminary screening purposes, as a guide to the elimination of undesirable impurities, or for screening the excreta or tissues of test animals for the possible presence of potentially mutagenic metabolites.

Other tests using mammalian cells in culture, as well as intact animals that have been exposed to the compound of interest, are well on the way to acceptance as useful and reliable procedures. The entire field is too new, too full of ferment, for adequate standardization and validation to

have occurred. A variety of collaborative studies now in progress may achieve these goals with respect to a selected number of tests and on the basis of a narrow spectrum of classes of known carcinogens and structurally related non-carcinogens. We have yet to venture forth into the wider world of industrial chemicals, intermediary metabolites present in the body, and natural components of food, among many other categories. Despite all these considerations, there is mounting pressure to adopt immediately a system of genetic tests for regulatory purposes, and one can only view with grim foreboding the harvest of confusion that will result from the seeds about to be sown so prematurely.

LONG TERM TOXICOLOGY AND ITS FUNDAMENTAL WEAKNESSES

The long term tests have two objectives, namely assessment of chronic toxic effects and determination of whether or not the compound has carcinogenic potential in animals (Table IX). At one time it was common practice to perform a separate and independent test for each purpose. For obvious reasons it is advantageous to try to meet both needs by means of a single test.

This mariage de convenance introduces many discordant elements. It means, for example, that the use of a single control group leaves too much to chance, and that historical control data assume great importance. Efforts are now in progress, at long last, in the U.S. to compile a national collection of historical data concerning spontaneous tumor incidence and other pathological changes in the commonly used Fischer 344 rat. Most important of the conflicts between the two objectives of the test is the compulsion to go to unreasonable lengths in raising the highest dosage level to the so-called "Maximum Tolerated Dose" (MTD), lest a negative outcome of the study be castigated as non-significant on the grounds that a subcarcinogenic dose of the compound was used. There seems to be no escape from the pernicious fallacies underlying the illogical and unscientific concept on which the MTD is based. Having established that such doses overwhelm the body's defensive and adaptive mechanisms and distort metabolic pathways, we are told that maybe these abnormal pathways in animals are the normal ones in man. Having demonstrated the possibility of neoplastic artifacts that arise through the predisposing action of nonspecific injury caused by the astronomical doses, we are told that the concept of "chronic irritation" was abandoned as a cause of cancer over 50 years ago. Having before us the evidence of the multiplicity of metabolic pathways for any carcinogen, very few of which lead to activation, we constantly hear the reaffirmation of the hypothesis that one molecule of a carcinogen suffices to induce cancer. The further we progress in the study of the intimacies of molecular biology, with mounting evidence pointing to the existence of thresholds for carcinogens, the more adamantly the belief in that Mastodon of Toxicology, the standard carcinogenesis bioassay, holds sway. This procedure makes no attempt to develop dose-response data, in the belief that a carcinogen is a carcinogen at any dose, under any circumstances.

Statisticians have spent years in developing increasingly complex mathematical models in order to extrapolate from what are sometimes the absurdities of MTD down to the low-level exposure of real life. At last the realization is growing that there is nothing unique about neoplasia, that all toxic responses can be treated on the same basis by constructing dose-response curves, to which mathematical modeling can be applied in order to provide quantitative *risk estimates.* From these calculations safe conditions of use of the material can be developed and decisions reached on "virtually safe" levels for human use and environmental exposure. Repugnant as this idea may appear to the cancer establishment, is it not time to abolish the artificial line of demarcation between carcinogens introduced by Nature and those for which man is responsible? In both situations strict control is essential, based on ranking by relative potency and reliable estimates of risk at various levels of exposure.

Turning now to the long term toxicity test, selection of species and strains of experimental animals, use of standard diets, the type of caging, and other features of the protocol represent a compromise. There are no hard and fast answers to these problems. Nonetheless, by the time a total of over 2,500 animals of two species have been used, ending up with about 100,000 tissues and many millions of items of data, the successful completion of such a study provides substantial insight into the toxicological properties of the compound. At present no short term test can substitute for this massive effort. Even the best work needs skilled interpretation, which depends not only on the findings in control groups but also on the investigator's familiarity with the historical incidence of spontaneous disease and pathological changes in the particular species and strain of animals used, as well as the effects of senility for which allowance must be made.

IDEAL VS. PRACTICAL APPROACH TO SAFETY EVALUATION

In the area of human and environmental safety, as the spectrum of investigations grows, so also does the importance of *meaningful* research, rather than voluminous stereotyped studies. Reference was made to this subject earlier but it bears repetition here, in the face of proliferating official "checklists," "guidelines," and catalogs of "required procedures." Rather than seeking merely to obey regulations, how can one best achieve a corpus of knowledge that is directed at the heart of the issues raised by a particular compound? So many factors and variables in so many fields of expertise are involved that the advice of an appropriate group of experts can be most helpful in defining what is to be regarded as relevant and essential information, in the light of findings at various stages in the progress of the studies. In particular, selection of tests having high predictive value is to be preferred to attempts to cover the entire waterfront indiscriminately.

Selectivity in the approach to safety evaluation becomes even more critical when dealing with a compound that shows a tendency toward bioaccumulation – of the compound itself, or of decomposition products, or of both. Analysis of body burdens of these agents assumes importance in relation to studies of the pharmacokinetic behavior of the test compound and its degradation products in various species. Save in very exceptional cases, the power and sensitivity of analytical methods have eliminated the need for "relay" tests as a way of assessing the toxicity of tissue residues. In addition to the familiar laboratory models of ecosystems, and the species of birds, fish, etc., on which tests need to be carried out to satisfy regulations, enthusiasts and protagonists have suggested a wide variety of flora and fauna for purposes of environmental testing and monitoring. The extent to which it is practical and useful to become involved in such environmental studies depends on the general order of toxicity of the compound, the speed and extent of its environmental degradation, and the toxicity of its degradation products. Once again, decisions that must be made depend on informed judgment.

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