Def Sci J, Vol 37, No 2, April 1987, pp 85-97.

Generation of Toxicological Data on Chemicals in the U.S.A.

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ABSTRACT

The paper reviews the current trends of toxicological testing of chemicals in the U.S.A. and the methods of data generation for risk assessment and regulatory purposes. The recent survey conducted by NAS/NRC revealed that only a small fraction of widely used chemicals have been tested toxicologically. In spite of developments in short-term toxicological tests, using non-mammalian test species continue to be indispensable.

In evaluating carcinogenicity tests, the current approach is generally based on the assumption that there is not threshold.

Under the regulation of the Toxic Substances Act 1977 (U.S.A.) the responsibility of providing toxicological data lies with the manufacturer. The National Toxicology Programme emerged as a new governmental agency for toxicological evaluation since 1978 integrating a number of pre-existing testing activities in U.S.A. A number of private testing laboratories which can provide test data on contract basis also have come into existence in U.S.A. The guidelines published for risk assessment by the Environmental Protection Agency is expected to provide standards for the conduct of toxicological tests used for regulatory purposes. The newly formed Board on Environmental Studies and Toxicology reviews and evaluates the toxicological issues.

1. INTRODUCTION

The toxicological testing of chemicals in the U.S. has expanded greatly in the last two decades, largely as a result of legislation requiring such testing for the protection of human health and the environment. The resources available for the development, analysis, and compilation of toxicological data also have expanded correspondingly.

Received 29 September 1986

2. TOXICOLOGY AND EPIDEMIOLOGY

A major objective of toxicological evaluation is to predict the probable health (or ecological) effects of a chemical in advance, so that appropriate controls can be instituted (or substitutes found) in time to prevent undue human exposure and resulting injury. With the successful application of this approach through toxicological testing, epidemiological studies should, in principle, no longer be necessary as a means of identifying toxic substances, although they will remain important in the follow-up of control measures to confirm that the intended purpose of preventing injury has been fulfilled. The relative strengths and weaknesses of toxicology, as compared with epidemiology, are largely self evident (Table 1)[1] and need no further discussion herein.

Table 1. Some attributes of toxicology and epidemiology : comparitive strengths and limitations (from reference 1).

Attribute or Application	Toxicology	Epidemiology
Relevancy	Uncertain	Excellent
Control of variables :		
Exposure, Environment, Confounding factors	Excellent	Poor
Identifying casual factors	Excellent	Poor
Size of population	Limited	Can be very large
Sensitivity	Poor	Poor
Genetic diversity	Normally deliberately narrow	Broad
Intercurrent disease	Controllable	Not controllable
Study of mechanisms	Easily accessible	Ethical hindrances
	Uncertain relevancy	Directly relevant
Diagnostic tests	Unrestricted (except interview)	Severely restricted

3. THE EXTENT OF THE NEED FOR TOXICOLOGICAL DATA

The U.S. National Academy of Sciences/National Research Council (NAS/NRC) recently conducted a major survey of the extent and quality of testing of chemicals in current use.[2] For logistical reasons, the survey was limited to machine-retrievable lists of chemicals, which comprised a 'universe' of some 65,000 entries (53,500 discrete molecular entities). The chemicals were classified into 7 categories according to use or production volume, including pesticides, food additives, drugs, cosmetics, and chemicals of commerce, and were then examined by an appropriate stratified sampling technique. Representative substances in each category were evaluated as to the percentages tested and the thoroughness and quality of the tests.

Among the substances in different categories, drugs and medicinal products were found to be the most extensively tested, followed by pesticides (Fig. 1). These

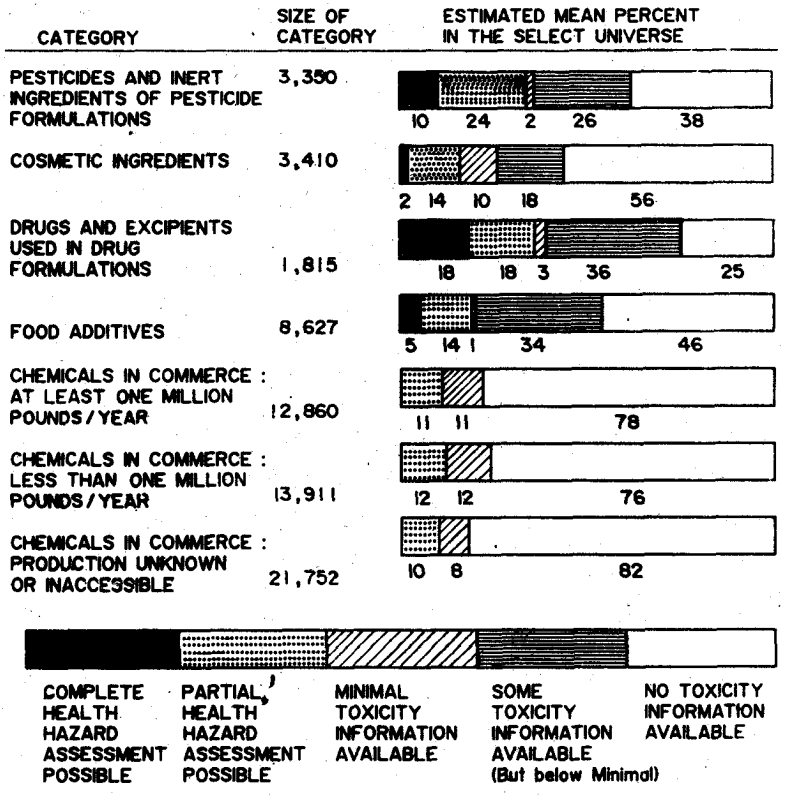

Figure 1. Availability and adequacy of toxicological data for chemicals within different use and production categories (reproduced from ref.2).

differences, reflect the relatively strict and long-standing legal requirements for the testing of pesticides and drugs.

Although more than 60 per cent of pesticides were found to have had some degree of toxicological evaluation, only about 10 per cent were deemed to have been evaluated with sufficient thoroughness to enable a complete assessment of their risks to health (Fig. 1). Of the total 'universe' of 65,000 entries, more than 40,000 of the chemicals were in large-scale commercial use, and these were found to have had very limited testing; in fact, there were none for which the data were deemed adequate to enable a complete health hazard assessment (Fig. 1). Hence, despite much public concern and the development of elaborate testing resources in the United States, only a small fraction of chemicals to which there is widespread human exposure have been tested toxicologically in a way that would be considered adequate.

4. CURRENT TRENDS IN TOXICITY TESTING

4.1 Types of Tests

Toxicity testing in the U.S., as elsewhere, depends largely on the use of laboratory animals. There has been considerable pressure to develop test systems for replacing

mammals with non-mammalian test species and with short-term isolated test systems, but success in the development of such systems has been modest thus far. Nevertheless, these approaches are being actively explored.

A short-term, non-mammalian test that has enjoyed considerable success is, of course, the bacterial reverse mutation assay system, as developed by Ames and other workers.[3] This system has provided toxicologists with an efficient and informative technique for studying the genetic toxicity of chemicals and has achieved an important role in the screening of chemicals for potential carcinogenicity. Nevertheless, it is not regarded by most workers as capable of replacing long-term animal tests. A recent review under the auspices of the International Programme on Chemical Safety (IPCS)[4], which systematically examined a battery of short-term tests for carcinogenicity and mutagenicity, concluded that some supplementation of the Ames Test is necessary to satisfy even the initial steps of testing for carcinogenicity. This IPCS document, summarizing a worldwide study of short-term tests for carcinogenicity, concluded that among the various assays investigated the induction of chromosomal aberrations, cell transformation, gene mutation in mammalian cells, and aneuploidy in yeast gave encouraging results when used to supplement the bacterial reversion test system. Thus, although considerable progress has been made with the powerful new short-term tests which have become available for examining the toxicity of chemicals, the definitive test for carcinogenicity still remains the lifetime bioassay in rodents.

A recent study of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals[5] examined the possible availability of short-term tests for evaluating other types of chemical toxicity to various organ systems (skin, liver, lung, immune system, hemopoietic system, etc.). The general conclusion of this study was that no short-term tests for overall organ toxicity are available as yet, although there exist many tests for specific toxic endpoints.

Accordingly, the use of classical animal tests still remains the definitive basis for the most toxicological evaluations. These tests involve exposure of animals by ingestion, inhalation, skin application, or other means, with subsequent observation for adverse effects. The tests are being constantly expanded and improved, and now include many more refined biochemical and functional tests than have been available heretofore.

A limitation in current procedures, as in past procedures, is the uncertain reliability of extrapolation among different species and among different levels of dose. It is generally recognized that appropriate allowance for pharmacokinetic differences can markedly improve the extrapolation from a test animal species to humans. Hence use of pharmacokinetic data has become more or less routine in the studies of the National Toxicology Programme (NTP), discussed elsewhere in this report.

For practical reasons, the procedures currently in use in toxicity testing involve the exposure primarily of rats and mice. It appears that in recent decades, there has been a decrease in the use of primates and other large animals, such as dogs and rabbits, in toxicity testing within the U.S.

There has been a growing refinement in environmental controls along with other advances in testing procedures. The use of inbred animals and their maintenance in pathogen-free circumstances has become routine. As concerns the number of animals per test group, group sizes have tended to become larger in recent years; i.e., in the standard NTP bioassay, there are 50-60 animals of each species and sex per dose group. A carcinogenicity study carried out at the National Centre for Toxicological Research several years ago (the so-called ED01 study)[6], which aimed at identifying increases in cancer incidence at the 1 per cent level, required such large test groups (of the order of 2,000-3,000 mice per group) that a study of its type is not likely to be repeated in the near future.

Although a two-year, or lifetime, rodent study is regarded as imperative for the detection of carcinogenicity, there is a growing body of opinion that a 90-day study may be adequate for the detection of most other forms of systemic toxicity, including reproductive injury. For detection of the overall effects of chemicals on reproduction, however, multi-generation studies are customary; a newer and somewhat more efficient alternative for this purpose is the fertility assessment by continuous breeding assay system[7], which is being explored by NTP.

4.2 Interpretation and Application of the Data

Until 30 years ago, it was generally assumed that there was a 'threshold' below which chemicals could be considered 'safe'. With further understanding of the mode of action of carcinogens, this concept has since given way to the assumption, for public health purposes, that carcinogens operate without threshold and hence that any level of exposure to a carcinogen may conceivably produce some risk of a malignant lesion.

The threshold concept is still in extensive use for many other toxic endpoints, however, with the result that the relevant evaluation procedures are still based on the so-called *no observed effect level* (NOEL) in test animals. This level, determined experimentally, is decreased by a safety factor – often 100 (10 for species differences and 10 to allow for uncertainty or human variability) – to arrive at an estimate of the threshold for the human population and to determine 'acceptable' levels from which allowable daily intakes (ADI), maximum allowable concentrations (MAC), and threshold limit values (TLV) can be derived.

With the abandonment of the threshold hypothesis for carcinogenicity, the NOEL approach for assessing the risks of carcinogens has been supplanted in the U.S.A. by the use of quantitative risk assessment.[8] Although quantitative risk assessment had been applied previously to prediction of the carcinogenic effects of low-level ionizing radiation, it was a new approach for evaluating the toxicity of chemicals. The rationale for the approach was based on the premise that a dose-response curve for a given chemical could be derived from epidemiological data or animal test results that would enable prediction of the risk of carcinogenicity following exposure to the chemical at low doses (Fig. 2).[9] Parenthetically, it should be noted that some workers have continued to challenge the applicability of the 'no threshold' concept to chemicals that are not genotoxic as determined, for example, by the Ames Test.

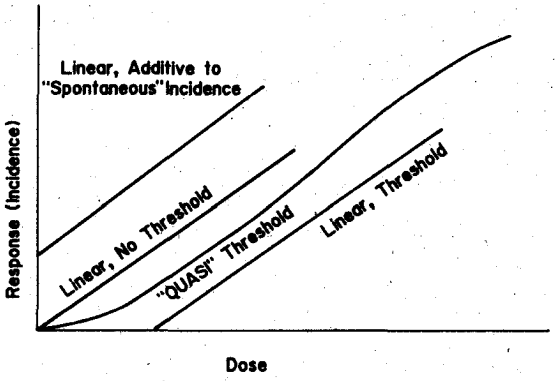

Figure 2. Simplified diagram of representative hypothetical dose–response relationships for carcinogens (reproduced from ref.9).

As mentioned above, the relatively small number of animals that can feasibly be used in carcinogenicity tests have only limited capability for detecting the low rates of response that would be socially acceptable in human populations. Because high test doses must be used in order to produce detectable rates of response, extrapolation of the results to far lower doses is required in order to assess the risks at dose levels relevant to human exposure. The extrapolation model selected for this purpose has a major influence on the predicted dose-response relationship and hence on the estimate of risk at low levels of exposure (Table 2).[10] Although the models used in the past have varied widely, there has been a tendency to concentrate increasingly on the Doll and Armitage model[11], which is thought to have greater biological validity than others. This model, when used as the basis for extrapolation, has been adapted to force linearity near the zero dose intercept.

Table 2. Extrapolated values of 'safe' doses, as estimated with three different dose-response models, each of which conforms equally well to the responses observed in the 2 to 50% response range (from reference 10).

	Probit curve	Logistic curve	One-particle curve
TD_1	0.040	0.022	0.0144
$TD_{0.1}$	0.0155	0.00315	0.00144
$TD_{0.0001}$	0.00136	0.0000098	0.00000144
$TD_{0.000001}$	0.000412	0.00000016	0.000000014
$TD_1 / TD_{0.000001}$	100	100,000	1,000,000

In addition to extrapolating from high dose levels to lower dose levels in interpreting toxicological test data for purposes of risk assessment, it is also necessary to extrapolate from the test animal to man. There is always uncertainty, however, as to how faithfully the response of any other animal species parallels the human response. Concern about this problem has led to the conviction that pharmacokinetic studies should be used more fully in toxicological evaluations[12], with the result that such studies are now a standard part of toxicity testing in the NTP.

The application, by regulators, of mathematical models relating cancer incidence to dose has varied from time to time and from agent to agent. Thus, the level of exposure that has been estimated to correspond to an acceptable level of risk has varied, depending on the particular regulatory application. Furthermore, the estimate has usually been based on a 'risk-vs-benefit' decision, rather than on a purely scientific judgement. Hence, the lifetime risks of cancer judged to be acceptable have ranged from 1 in 1000 (occasionally higher) down to 1 in a million exposed persons.

In recent years, it has become customary to distinguish between: (i) the estimation of risk – qualitative as well as quantitative – which is regarded as a largely scientific and value-free process (although risk estimates are in some degree value-laden, depending on the assumptions going into the calculations) and (ii) the application of the resulting risk estimate to a specific regulatory problem. This distinction has been discussed in a recent NAS/NRC report[13], which suggests that the first step, 'risk assessment', is essentially a scientific endeavor, whereas the second step, 'risk management' (that is, the application of risk assessment to a specific control or regulatory problem) entails consideration of various socio-political and philosophical issues (e.g., determining the level of risk that the public is willing to accept for the benefits secured). 'Risk management' is thus regarded as a trans-scientific matter, with the result that some agencies meticulously separate it from "risk assessment", as a second and distinct step.

5. RESPONSIBILITY FOR GENERATING THE DATA

In the regulation of many chemicals in the U.S.A., it has long been held that the manufacturer of a substance ordinarily has the responsibilty to prove that no harm will arise from its proposed use. Thus, the manufacturer of a chemical intended as a food additive must supply toxicological data demonstrating the safety of the substance under the proposed pattern of use. It is noteworthy in this context that the food regulations in the U.S.A. contain the Delaney Clause, which stipulates that a chemical known to cause cancer in man or animals may not be used as deliberate food additive. Pesticides, however, which may be inadvertent contaminants in food commodities, are not specifically covered by the Delaney Clause unless they concentrate in processed food. In the regulation of raw food commodities, Environmental Protection Agency (EPA) has used the quantitative risk assessment approach for evaluating pesticides, rather than an all-or-none approach, so as not to exclude pesticides which are important in the maintenance of the food supply or in agricultural practice. The pesticide manufacturer is expected to supply the appropriate data for such calculations.

Under the Toxic Substances Control Act (TSCA) of 1977[14], the manufacturers of chemicals newly going into commerce are required to notify EPA and to supply data bearing on the toxicity of their proposed products. The burden of evidence requiring additional data rests on EPA, so that a decision by the Agency to permit a manufacturer to proceed may depend on little more than evaluation of structure-activity relationships. Nevertheless, EPA can find that because of possible widespread exposure or potential unreasonable risk, the submission of additional data

may be required; on the basis of this it can deny permission to manufacture a new chemical. The manufacturer of a chemical may also be required to submit additional toxicity data as production of the chemical proceeds.

6. PERFORMERS OF TOXICOLOGIC STUDIES

For 'old' chemicals, which are not on the TSCA 'inventory', there is no established requirement for toxicity testing. Some of the needs for testing of these chemicals are now being met by the NTP. The Chemical Industry Institute of Toxicology (CIIT) tests some agents in the general domain; for example, formaldehyde.

Over the last two decades, there has been a major shift in the extent of toxicological testing and in the mechanisms by which toxicological data are developed in the U.S.A. Only a few chemical manufacturers had established substantial toxicological testing laboratories before World War II; e.g., Dupont, Dow, and Union Carbide, and these continue to be active. More recently, a number of other manufacturers also have developed extensive testing facilities, but their course has been variable. With the increased requirement for toxicological test data imposed by stricter regulatory controls, however, that has developed a substantial industry of testing laboratories, which provide test data under contract. More than 280 such contract laboratories are now estimated to exist, with approximately 16,000 employees in total and an annual revenue of more than 450 million dollars.[15]

The Food and Drug Administration (FDA) has, tested chemicals in order to examine the potential toxicity of substances in the public domain or, occasionally, to supplement toxicological data supplied by manufacturers. The National Centre for Toxicological Research (NCTR), which is operated by the FDA and located in Arkansas, is a relatively new entry into the toxicological field. NCTR does some testing, but it is more concerned with fundamental toxicological research.

A more recent entry into the field is the NTP, which was established in 1978. This Programme brought together and integrated a number of pre-existing testing activities at the National Cancer Institute (NCI), NCTR, the National Institute of Environmental Health Sciences (NIEHS), and the National Institute for Occupational Safety and Health (NIOSH). This included the large-scale carcinogenicity bioassay programme under the NCI, which was transferred to the NIEHS in 1981. The NTP also has affiliations with the FDA, the Occupational Safety and Health Administration (OSHA), the EPA, the Consumer Product Safety Commission (CPSC), and other federal agencies. The NTP conducts substantial testing activities, in cooperation with the three participating agencies, particularly NIEHS. The Director of the NTP, who reports directly to the Assistant Secretary of Health, is at present also Director of NIEHS. The latter arrangement is not obligatory, but it is fortunate in providing NTP direct access to the extensive research resources of NIEHS. The NTP undertakes a full range of toxicity studies for end-points of many kinds, including carcinogenic effects, disorders of the liver, lung, and kidney, reproductive disabilities, immunological defects, etc. It also conducts an extensive array of short-term tests.

During the current year the NTP will complete and have peer reviewed some 25 lifetime bioassays (approximately 350 to date, including NCI and NTP) for carcinogenicity. Its bacterial reversion (Ames) tests approximate 200 annually (some 1,300 to date).[16] Typical protocols for 2-year tests are illustrated[17] in Table 3.

Table 3. Representative design features of long-term (two-year) toxicology and carcinogenesis studies as performed by NTP (from reference 17).

Animals	:	Fischer 344N rats and B6C3F$_1$ mice
Sex	:	Males and females
Group size	:	60 animals/group/species/sex/dose
Number of groups	:	Control and 2-4 exposure groups/species/sex
Doses	:	Chosen as proportions (typically 0, 0.25, 0.50, and 1.0) of an estimated maximally tolerated dose (EMTD), based on biochemical, toxicologic, and histopathologic observations from prechronic studies (90-120 day exposures).
Duration	:	104 weeks (-2/3 life span)
Interim sacrifice times	:	at 15 and/or 18 months (10 animals/group/species/sex/dose)
Necropsy	:	All animals.
Diagnoses	:	Three-phase histopathology process.

Normally, the NTP studies were preceded by a series of associated shorter studies which aid in dose selection, particularly the estimated maximally tolerated dose (MTD).[17] However, an important objective of these associated tests is to develop a broad assessment of the general toxicity of the chemicals examined. Included is an array of genetic toxicity studies; for example, gene mutations in bacteria and mammalian cells, chromosome alterations, transformation, DNA damage and repair, fertility and reproductive assessment (sperm morphology and vaginal cytology), teratology tests, systemic toxicity (14, 90 and 120 day exposures), clinical pathology, hematology, biochemistry, and urine-analysis. Also included for most chemicals is a systematic pharmacokinetic study.[16] Thus, in addition to the assessment of carcinogenicity, the program provides a broad, general toxicologic appraisal of the chemicals studied. Multigeneration studies in rodents continue to be used; however, NTP is exploring several alternatives (for example, the fertility assessment by continuous breeding assay system[7]) in searching for more efficient and informative tests.

The NTP thus constitutes a major resource of the federal establishment for the full-scale toxicological evaluation of chemicals. It operates under an Executive Committee made up of the heads of major components of NIH and CDC and the heads of major federal regulatory agencies. General guidance of the scientific programme is under an external Board of Scientific Counsellors, the meetings of

which are open to the public. The Board also is involved in the review of chemicals which are recommended for testing by NTP, and it oversees review of the reports which are produced under NTP auspices.

The Consumer Product Safety Commission (CPSC) has no in-house toxicological resources but supports grants and contracts to examine toxicological issues in its domain of responsibility.

The NIOSH is responsible for substantial research on occupational hazards, including toxicological as well as epidemiological studies. Among its responsibilities is the provision of data underlying the establishment of Permissable Exposure Levels (PEL's) by the OSHA. NIOSH's contribution to this task, however, has been limited thus far. The major source of information leading to PEL's has come from an activity conducted for many years by an independent professional society, the American Conference of Governmental Industrial Hygienists (ACGIH). This committee has been responsible for the establishment of most of the TLV's currently in use in the U.S.A., many of which have been incorporated into regulations as PEL's pending their replacement by new standards proposed to OSHA by NIOSH.

The Department of Defence obtains toxicological information from many of the above-mentioned governmental testing agencies. Also, through the Army, it maintains an environmental health laboratory at Edgewood, Maryland, which has been active in toxicological, work for a number of years.

EPA has a large research programme which is extensive in its coverage; however, it deals primarily with generic issues pertinent to toxicity testing rather than with the testing of specific chemicals themselves. The research conducted in EPA is under the general policy oversight of the Science Advisory Board, which is made up of a number of specialized sub-sections, including health, air pollution, engineering, ecology, etc. Deliberations of the Board's advisory groups are open to the public.

EPA has recently published guidelines for risk assessment relating to the interpretation of a variety of toxicological tests, including tests for carcinogenic effects, tests for studying the effects of mixtures of chemicals, tests for developmental disturbances, methods for the estimation of exposure, etc. The guidelines are intended to provide basic standards for the conduct of toxicological tests used for regulatory purposes.

Substantial research on the basic aspects of chemical toxicology is conducted in NIEHS, which has an extensive intramural programme devoted to research on the basic mechanisms of action of foreign chemicals. In addition, it supports an extensive extramural programme of university-based toxicological research. Included in this programme are some 10 centres of excellence in distinguished universities throughout the nation, which are committed to long-term research on toxicological problems, including relevant epidemiological studies. These centers thus provide a cadre of career-oriented scientists working at the interface between basic science and toxicology, thus advancing our understanding of the basic mechanisms of chemical injury. Proposals for future research directions for NIEHS have recently been published.[18]

7. REVIEW AND EVALUATION OF TOXICOLOGICAL ISSUES

A major resource in the U.S.A. for the review of toxicological problem exists in an organization within the NAS/NRC, which has been known until recently as the Board on Toxicology and Environmental Health Hazards. The Board has recently merged with the Environmental Studies Board and is now identified as the Board on Environmental Studies and Toxicology (BEST). It's reviews, some of which are self-initiated, often arise through specific governmental requests from Congress or federal agencies and are conducted by special or standing committees, which include the Committee on Toxicology. The Board also maintains a Toxicology Information Centre, which is available as a resource, particularly to federal agencies, for consultation on emergency exposure guidelines or for other special studies as required. The Centre can, under special circumstances, provide rapid consultation on possible toxicological problems, which can later be given more deliberate review by the Committee on Toxicology.

Within the Department of Defence, there exists the Armed Forces Epidemiological Board (AFEB), which was organized in World War II. The Board was formed primarily for advice to the military on infectious diseases, but its mission quickly became expanded to cover advice on other environmental hazards, including chemicals. It continues to give such advice at the present time.

8. INFORMATION SOURCES

A number of information sources are available in the field of toxicology. Important sources maintained by the National Library of Medicine are ToxLine and its allied compilations. These are available to any institution having the appropriate computer linkage.[19] In addition, there is an Annual Report on Carcinogens, which is published by NTP.[20]

Finally, there has been a remarkable proliferation of journals published in the field of toxicology, not only in the United States but in other countries as well. These periodicals provide an abundant and growing resource for publication of toxicological research reports and reviews. The numbers of toxicological studies reported in other biomedical journals also has increased substantially and continues to grow.

9. CONCLUSION

It can be said that extensive resources for generating toxicological data exist in the U.S.A. Furthermore, the elaborate resources, that have been developed over the last 20 years are a reflection of public pressures on the U.S. Congress and other agencies in the federal government for closer scrutiny of possible chemical injury to health and the environment. Indeed, public interest in this subject continues unabated, as shown by recent opinion polls. The aggregate expenditure on testing resources nationwide, as noted above, can be estimated now to exceed some 450 million dollars in contract laboratories alone; however, this sum represents only a small fraction of the total value of shipments in the chemical industry in the United States, which is on the order of $160 billion[20], exclusive of drugs, soaps, and toiletries.[21]

The U.S.A. does not stand alone, of course, in the expansion of toxicological activities; most industrialized countries (and growing numbers of developing countries) also now have extensive resources. In parallel with these developments, WHO has responded by establishing the International Programme on Chemical Safety (with UNEP and ILO) and the International Agency for Research on Cancer; UNEP has established the International Register of Potentially Toxic Chemicals.

In short, the widespread demand for the benefits of a flourishing chemical technology have prompted the development of toxicological resources for purposes of assuring that the technology can be operated with prudent safeguards.

ACKNOWLEDGEMENT

The authors are grateful to Ms. Jane Galvin and Ms. Lynda Witte for their assistance in the preparation of this manuscript.

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