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Appendix D: Strategies for Health Effects Research

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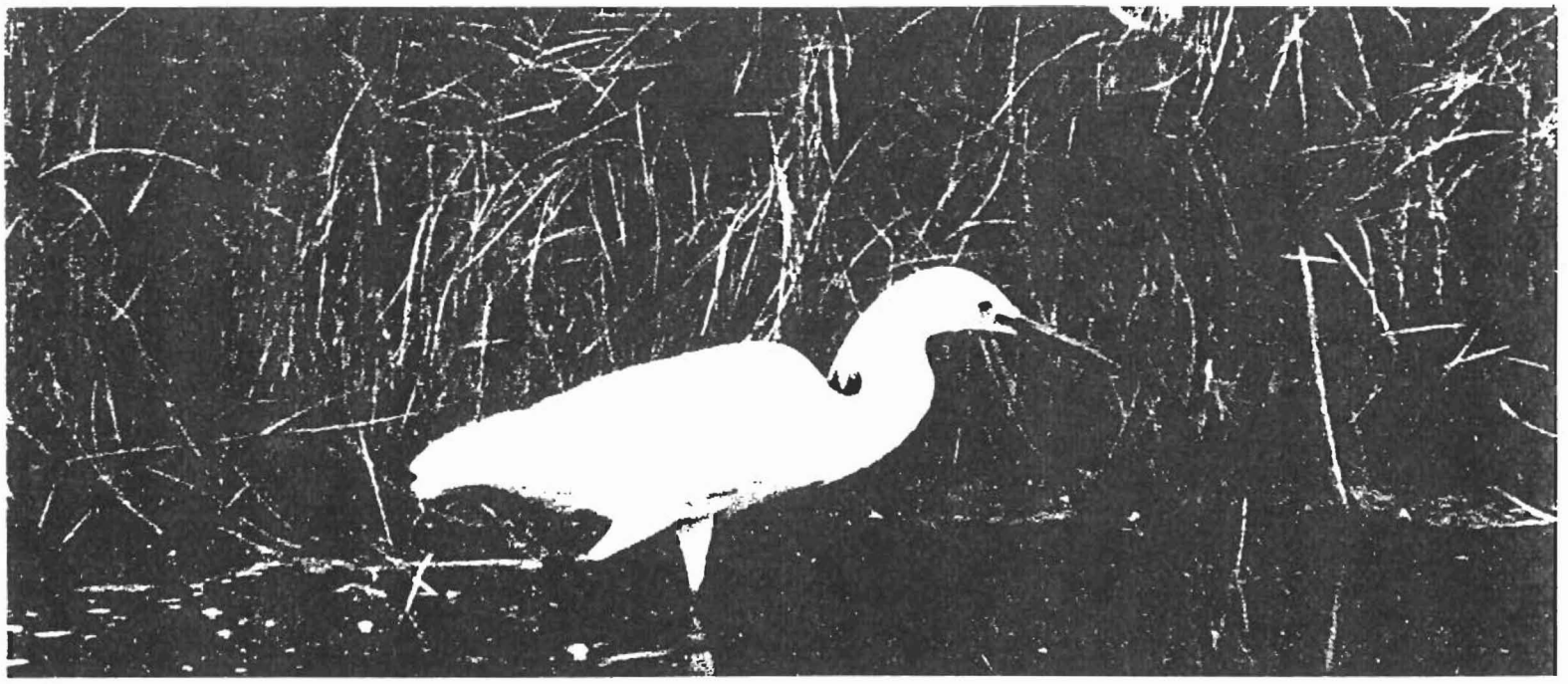

Report of the Subcommittee on Health Effects

Research Strategies Committee

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HEALTH EFFECTS WORK GROUP REPORT OF THE AL ALM COMMITTEE

ABSTRACT

This document attempts to delineate the long-term health effects research needs (both basic and applied) considered most supportive of EPA programs. Chapter 1 provides a historical perspective , describes the nature and sources of environmental determinants of health and disease, touches on the underlying mechanisms of toxicity with implications for risk assessment and disease prevention, and indicates some of the areas where research support is clearly inadequate.

Chapter 2 draws a distinction between the basic and applied long-term health effects research needs of EPA programs by providing specific examples that illustrate the need for research addressing "generic" issues as well as various research activities that have application to specific problems and specific settings but which must be carried on over a period of several years. An attempt has been made to explain how EPA uses/depends on basic research of the type conducted by other Federal Agencies, particularly as it relates to the regulatory mission of the Agency.

In Chapter 3 the toxic metal lead is used as the paradigm to illustrate the place of and necessity for long-sustained, basic research activity in the development of a foundation for constructive action in important problems in environmental health. Continued long-range and basic research investigations on lead toxicity are at one and the same time perhaps among the more justifiable and yet less supportable of such activities in the entire field of environmental health sciences.

A number of leading-edge/long-term basic research activities with potential application to environmental health problems are described in Chapter 4. It attempts to highlight those activities which perhaps have the greatest promise in this area. Many of these include various aspects of the "new molecular biology" research field, such as the study of oncogenes and proto-oncogenes, the development and use of biomarkers to determine internal dose and exposure and for relating exposure to disease. Other newer developments in neurotoxicology, immunotoxicology and reproductive toxicology are described. An important area of basic research includes methods development and validation. Magnetic resonance imaging is discussed as a very promising new technique that should find many useful applications in studies of the internal structures, states, and compositions of various biological systems.

Finally, in Chapter 5 the problem of estimation of population risks is addressed, particularly as it relates to the role of animal data in the quantification of possible human health risks. Factors considered here include choice of mathematical model or extrapolation procedures, primary versus secondary or indirect modes of action and threshold mechanism, problems in species extrapolation and determination of biologically effective dose. Some specific problems in human epidemiologic studies and population risks analysis are also described. Factors affecting the

balance of basic research on cancer and non-cancer endpoints within any Federal organization are also discussed. Long-term, basic research into both cancer and non-cancer endpoints is recognized as being essential if the EPA is to formulate a broad regulatory policy in the most accurate manner possible.

Chapter 1

ENVIRONMENTAL FACTORS AND HUMAN HEALTH

Arthur Upton

HISTORICAL PERSPECTIVE

The past century has seen the conquest of those diseases which have caused the greatest morbidity and mortality in previous generations. In the developed countries of the world, the average life expectancy has doubled, now surpassing the biblical ideal of "three score and ten" years (Figures 1 and 2). This transformation, which would have seemed miraculous to our great grandfathers, has resulted from advances in our understanding of the relationship between health and the environment, broadly speaking. These advances, and the resulting improvements in agriculture, nutrition, sanitation, public health, and medicine, have all but eliminated infectious and parasitic diseases as major causes of death in the industrialized world. Replacing such afflictions as major causes of death in the industrialized world are abnormalities in early growth and development, chronic degenerative diseases, and cancer (Figure 3). These diseases, viewed until recently largely as hereditary or inevitable accompaniments of aging, are now attributed increasingly to environmental causes. Our challenge is to identify the causes and to control them (4).

NATURE AND SOURCES OF ENVIRONMENTAL DETERMINANTS OF HEALTH AN DISEASE

The "environment", defined broadly, encompasses all external factors that may act on the human mind and body. Many of the factors are produced or altered by man himself. They include chemical and physical agents in air, food, water, drugs, cosmetics, consumer products, the home, and the workplace. The "environment" is thus complex and constantly changing. Inevitably, moreover, it contains a myriad of agents in varying combinations and from multiple sources. Furthermore, because the effects of different agents interact in various ways, the ultimate impacts of any given environmental agent may depend on the effect of other agents and the conditions of exposure (4).

Air

Acute episodes of atmospheric pollution, such as those listed in Table 1, have been observed to cause transitory increases in morbidity and mortality. The effects of chronic exposure, however, are less well documented and may vary, depending on the pollutants in question and their concentrations in the atmosphere (4).

On chronic exposure at relatively high concentrations in the workplace, a variety of pollutants are known to cause toxic effects. Examples include various gases (e.g., carbon monoxide, vinyl chloride, coke oven emissions,

radon), metals (e.g., lead, mercury, arsenic, nickel) and dusts (e.g., asbestos, silica, cotton fibers, coal) (5).

Also well documented are the effects of chronic exposure to cigarette smoke. The incidence of lung cancer has risen precipitously, in parallel with the antecedent increase in cigarette consumption (Figure 4). In smokers, furthermore, there is a systematic relationship betweecn the amount of smoke inhaled and mortality from lung cancer (Figure 5), other cancers, heart disease, and respiratory diseases. Lesser effects have been tentatively attributed to passive inhalation of cigarette smoke in chronically exposed nonsmokers.

The ultimate effects of chronic low-level exposure to other widely prevalent combustion products and their derivatives (such as sulfur dioxide, ozone, nitrogen dioxide, benzo(a)pyrene, and various suspended particulates) are less well understood.

Although the air pollution produced as a result of coal combustion is a direct cause of respiratory fatalities, there is no exact measure of their number; however, several estimates have been made of the number of fatalities attributable to the combustion of coal in generating electricity (where about 70% of coal combustion occurs). Inhaber (8), for example, estimated that between 5 and 500 fatalities result per 1000 Mwe of electric power produced each year from pollution generated by coal fired plants. A more recent survey by experts in this area puts the estimate between zero and 1000 fatalities per year per 1000 Mwe of electric power produced (9,10). On the basis of a value of 7×10^5 Mwe of electric power produced in the U. S. by the consumption of coal, the estimates imply that up to 700,000 fatalities per year may result from combustion of coal in the U. S. Within the uncertainties of this estimate, it agrees well with a recent inference by Wilson that "50,000 among the 2 million persons who die each year in the United States may have their lives shortened by air pollution" (11). One may question, therefore, the extent to which current ambient air standards provide adequate protection against the potential long-term health effects of coal combustion products, which cannot be specified with certainty on the basis of existing knowledge (12).

It is noteworthy in the above context that indoor pollution with combustion products may lead to health effects in the chronically exposed, especially children. Of increasing concern is the extent to which elevation of the radon concentrations within houses and buildings, by weather-stripping and other heat-saving measures, may enhance the risk of lung cancer in their occupants (13-15).

Other air-borne pollutants with potential health effects include allergens of various kinds. Although susceptibility to such agents differs widely among individuals, sizable populations are at risk (4). The full significance of air-borne agents as causes of disease is far from established and strongly merits continued study (4).

Water

In the third world microbial contamination of drinking water still

constitutes a major cause of death. Although this type of pollution no longer exists on a significant scale in developed countries, the chemical composition of drinking water has been implicated tentatively in the two leading causes of death in the U. S.: cancer and cardiovascular disease (4,11). It is also noteworthy that water supplies have been found to be polluted in a growing number of areas (Figure 6), owing to contamination by metals, toxic wastes, pesticides, agricultural chemicals, and products of chlorination or ozonization.

The health impacts of small quantities of chemicals in drinking water cannot be assessed precisely on the basis of existing knowledge. Research is needed to elucidate the relevant causal relationships and to clarify the pathways through which compounds affecting human health may enter the water supply (17).

Food

There is some truth to the adage, "you are what you eat". Overall health is undoubtedly influenced by the total intake of calories in the diet, the relative intakes of different types of foods (protein, fat, carbohydrates), the nutritional value of the various foods that are ingested, the presence in food of certain naturally occurring constituents or contaminants, and the presence of man-made additives or pollutants (18). In general, more is known about the nutritional requirements for normal growth, maturation, and reproduction than about the optimal diet for long life and vigor.

In the case of cancer, for example, there appear to be many ways through which the diet may affect the probability of the disease (Table 2); however, the relative contributions of any of these hypothetical mechanisms to the pathogenesis of a particular form of human cancer remains to be established (18). In this connection it is noteworthy that some dietary factors may exert protective effects which are of equal or greater importance than the carcinogenic effects of others. Hence, the net effects of the diet may reflect the balance between the two types of influences.

Because of the importance attributed to the diet in the pathogenesis of cancer, heart disease, and other leading causes of death in the modern world, the role of dietary factors strongly merits further study.

Occupation

As noted above, occupational exposure to diverse physical and chemical agents at relatively high dose levels has been observed to cause various diseases. Collectively, the health impacts of these agents and of work-related stresses may approach those caused by occupationally-related accidents (5).

Occupational diseases are also significant in pointing to risk factors that may affect other populations at lower levels of exposure. In addition, occupational disease represents a category of health effects that is relatively amenable to preventive strategies. To lessen the health

impacts of occupational risk factors, research of several types deserves further emphasis: 1) more systematic and quantitative monitoring of physical and chemical agents in the workplace; 2) more complete surveillance and recording of work-related health effects; and 3) development of clinical and laboratory tests for ascertaining prior exposure to disease causing agents, for identifying high-risk groups, and for detecting work-related diseases at early stages, when they are most readily arrested, or reversed (4).

Toxic Wastes

Love Canal and Times Beach, to mention only two of many recent examples (Tables 3 and 4), testify to the need for more adequate disposal of toxic wastes. Although it is clear that disposal practices have been deficient in many instances, the development of optimally safe and cost-effective techniques will require further research, as will precise assessment of the magnitude of the risks posed by prevailing levels of contamination around existing dump sites (21-23).

The assessment of risks cannot depend on epidemiological approaches alone. This would be tantamount to making guinea pigs of exposed populations. Instead, comparative toxicological methods involving laboratory assays and animal models must be exploited insofar as possible in view of the paucity of toxicological data for most chemicals in the human environment (Figure 7). This will necessitate research to advance the state-of-the-art, in view of existing uncertainties about species differences and the interactive effects of the many chemicals that are characteristically present at dump sites.

MECHANISMS OF TOXICITY: IMPLICATIONS FOR RISK ASSESSMENT AND DISEASE PREVENTION

Toxicological Research

As noted above, many of the impacts of environmental agents result from the combined effects of multiple factors, each of which may contribute differently to the total. Furthermore, the effects of a given agent, or combination of agents, may vary, depending on the conditions of exposure as well as the dose. In addition, although some chemicals exert their effects directly, many act indirectly, through the formation of biological active metabolites or through effects on the metabolism of other substances (4). Because of the complexity of these processes, it is difficult or impossible to assess the effects of a given agent without some understanding of its metabolism and mode of action. Knowledge of the comparative toxicology and mechanisms of action of a substance is also essential in assessing its potential risks for humans on the basis of extrapolation from its observed effects in laboratory animals, since choice of the appropriate extrapolation model cannot be made without assumptions about the relevant dose-effect relationships and mechanisms of action (25-26).

With respect to the dose-effect relationship, it must not be forgotten that for some types of environmental insults no thresholds are known or

presumed to exist. These include the mutagenic, carcinogenic, and some of the teratogenic effects of ionizing radiation (14) and certain chemicals (4). Noteworthy in this connection is the growing evidence that exposure to lead during prenatal life and early infancy may cause permanent impairment in the development of the brain, the dose-effect relationship for which extend down to doses hitherto considered nontoxic and may conceivably have no threshold (27).

In addition, since it is not always feasible to eliminate a toxic agent from the environment, the most practical approach for mitigating its noxious effects may be to arrest or reverse them in exposed individuals. For this purpose knowledge of the mechanisms of such effects may be crucial, as well as the ability to identify affected individuals at early enough stages for effective protective intervention. Methods for monitoring exposed populations, as well as for monitoring the environment, are thus needed.

Social and Behavioral Factors

Any consideration of the role of environmental factors in health should not neglect the influence of social and behavioral influences (28). Among these, socio-economic status is one of the most important since it may affect many, if not all, other environmental influences, directly or indirectly. Mortality from many of the common causes of death tends to vary inversely with socio-economic and educational levels (29). The poor who live in urban ghettos exemplify the problem in their high incidence of malnutrition, congested and stressful living conditions, vermin infestation, chronic exposure to dusts and other air pollutants, and relegation to hazardous working conditions,. Poverty also breeds deviant behavior, including alcoholism, drug addiction, and crime, which have enormous impacts on health.

The importance of wholesome daily living habits in those who are not economically disadvantaged also deserves comment. Such simple hysical exercise, adequate hours of sleep, control of body weight, abstinence from smoking, and avoidance of excessive intake of alcohol are correlated with marked reductions in overall morbidity (30). In Mormons (31) and Seventh Day Adventists (32), who generally practice these habits, mortality from cancer and many other diseases is appreciably lower than in the population at large.

Also noteworthy is the inverse correlation between level of educational attainment and cigarette consumption (33), which points to the importance of education in motivating people not to smoke or to stop smoking. The large numbers of people at all educational levels who continue to smoke, however, attest to the need for further efforts to solve the problem completely. The cigarette problem -- which accounts for more than 300,000 deaths per year in the U. S. from cancer, respiratory ailments, and cardiovascular disease (33) -- exemplifies the importance of behavioral factors, socio-economic influences , and political forces in shaping the environment for better or for worse.

UNDER-RECOGNITION AND UNDER-DIAGNOSIS OF ENVIRONMENTAL DISEASE

As noted above, environmental diseases encompass an extremely broad range of human illnesses. They include, for example, emphysema in persons chronically exposed to acid air pollution, leukemia in persons exposed to benzene, lung cancer and mesothelioma in individuals exposed to asbestos, chronic kidney disease and neurologic impairment in persons exposed to solvents, impairment of brain development in children exposed early in life to lead, heart disease in individuals exposed to carbon monoxide, and impairment of reproductive function in men and women exposed to lead and certain pesticides. Such illnesses afflict millions of persons in the United States.

Because such environmental diseases arise from man-made conditions, they can be prevented through the elimination or reduction of hazardous exposures at the source; i.e., through primary prevention. They are also amenable to secondary prevention -- i.e., early detection in presymptomatic stages when they can still be controlled or cured; this depends, however, on efficiently and effectively identifying populations at high risk. Finally, their impacts may be lessened by tertiary prevention; i.e., the prevention of complications or disability by application of appropriate diagnostic and treatment strategies. Prevention at all three levels requires adequate information about the effects of specific environmental exposures and adequate data on the places and populations affected.

Laws enacted in the past two decades are intended to prevent environmentally-induced disease. These include, for example, the Clean Air Act, the Safe Drinking Water Act, the Resource Conservation and Recovery Act, and the Superfund legislation. In spite of this legislation, however, environmentally-induced disease remains widespread in American society. Given that such illnesses are important and highly preventable, why do they still persist? A series of factors interact to maintain this situation.

1. Despite at least two decades of regulatory and scientific awareness and effort, relatively little is known about the potential health effects of most synthetic chemicals. Most attention and research have been focused on a small number of relatively well known hazards, such as asbestos and lead, and their associated diseases. Virtually no information is available on the toxicity of approximately 80 percent of the 48,000 chemical substances in commercial use (Figure 7). Even for groups of substances which are most closely regulated and about which most is known -- drugs and foods -- reasonably complete information on possible untoward effects is available for only a minority of agents (Figure 7). Premarket evaluation of new chemical products is notably inadequate.

2. Physicians are not trained to suspect the environment as a cause of disease. Most physicians do not routinely obtain histories of environmental exposure for their patients, which would allow them to identify an environmental origin of disease. Recent surveys indicate that environmental histories are recorded on fewer than 10 percent of hospital charts (34). In consequence, many diseases of environmental origin are mistakenly assigned to other causes, such as old age or cigarette smoking, and opportunities for early prevention or treatment

are lost. This problem of inaccuracy in diagnosis is compounded by the fact that disease of environmental origin are typically not clinically or pathologically different from those caused by lifestyle and other factors.

3. Physicians do not receive adequate training in environmental medicine. Very little time is devoted in American medical schools to teaching physicians in training to recognize the symptoms of known toxins, or to understand the known associations between environmental exposures and disease outcomes. The average American medical student receives only four hours of training in environmental and occupational health during the four years of medical school (34).

4. Persons are typically exposed to more than one toxic substance in the environment and often do not realize that they have been exposed at all. Further, the symptoms of many environmental conditions develop only many years after onset of exposure during this long latency (incubation). Persons may change addresses, may be exposed to a variety of environmental exposures, may suffer various environmental exposures, and finally may forget exposures which they had many years ago. All of these issues compound the difficulty that physicians and environmental scientists face in attempting to deduce the etiology of environmentally induced illness.

5. The U. S. Environmental Protection Agency (USEPA) and State environmental agencies are empowered to investigate hazardous and environmental conditions; however, severe resource limitations have reduced the capacity of these agencies to undertake necessary inspection and enforcement actions.

6. Fragmented, unreliable and outdated surveillance systems for environmentally related disease produce significant underestimates of the actual number of cases of environmentally induced illness in our society. As a result, the picture they produce does not convey an appropriate sense of urgency about reducing the burden of environmental disease.

In summary, a profound lack of information on the toxicity of the majority of commercial chemicals, insufficient and inappropriate education of physicians, and inadequate surveillance impede all efforts to reduce the impact of environmentally induced disease in the United States. A coherent plan to improve the surveillance, prevention, diagnosis, and treatment of environmental disease is sorely needed. Models which have recently been developed for the detection, treatment and prevention of occupational disease in states such as New York, New Jersey, and California might serve as useful models for undertaking such an effort (35).

INADEQUACY OF RESEARCH SUPPORT

From the foregoing it is evident that much of the burden of illness in the U. S. today is attributable wholly or in part to environmental risk factors. Thus, of the more than $400 billion annual health expenditures in the U. S., a major part is spent on illnesses that are related directly or

indirectly to environmental causes (36) and that are thus potentially preventable. Although the economic impact of such illnesses cannot be reckoned precisely without more adequate information, it is obviously enormous.

Viewed in the light of the enormous costs of illness to the U. S. population, the sums spent on research to prevent such illness are relatively small. In 1985, for example, only $1,180,370 of the $5,121,557 R&D funds obligated by NIH went specifically to support research on disease prevention (37). This sum amounted to less than 0.25% of the total cost of health care in the U. S. that year (37). The sum spent for the same purpose by all other federal agencies combined was far smaller (37). Hence, in view of EPA's mandate to protect the U. S. population against environmental pollutants, it is clear that the Agency's strategies and budget for the purpose need to be greatly strengthened.

SUMMARY

The major diseases in modern life result in large measure from the influence of environmental causes. Defined broadly, these causes encompass all external influences that may act on the human mind and body. Included, among other influences, are physical and chemical agents in food, water, air, the home, and the workplace, many of which are produced by man and/or subject to his modification. Although some such agents produce adverse effects only at high dose levels, others may cause effects at lower dose levels, conceivably without a threshold. In practice, furthermore, the observed impacts on human health frequently result from the cumulative effects of combinations of agents, in which additive or multiplicative interactions among causal agents are involved. Hence, although environmental factors have been implicated as major causes of disease, the precise role of any one causal factor in the occurrence of a particular disease cannot always be specified. By the same token, it is difficult to predict the potential risks to health that may result from a given agent at any particular dose level. In our present state of knowledge, assessment of such risks is especially uncertain when direct human evidence is lacking and estimates must be based on extrapolation from observations in laboratory animals or other assay systems.

To advance our understanding of the role of environmental factors in health and disease, priority must be given to research on the following: 1) more systematic monitoring and characterization of the human environment; 2) more adequate recording of human morbidity and mortality rates, with record-linkage systems to enable the frequency of specific disease to be related to possible environmental causes; 3) further development of methods for detecting indices of exposure to toxicants and for identifying high-risk subgroups; 4) refinement of laboratory tests for characterizing the biological activity of chemical and physical agents, especially at low doses and in combinations; 5) improvement in techniques for human risk assessment with particular reference to comparative toxicological methods and extrapolation from animal data; and 6) better understanding of the mechanisms of environmentally-related health effects, as needed for improvements in risk assessment and in the primary and secondary prevention of environmentally-related diseases. In addition, more

vigorous efforts should be directed toward the application of existing knowledge, through: 1) public and professional education, 2) standards-setting, 3) implementation of new and existing legislation, 4) law enforcement, and 5) research to evaluate the efficacy of such measures. In pursuit of its mission EPA in coordination with other agencies and institutions must have a long-range research strategy addressing each of the above needs.

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Table 1. Some Selected Acute Air Pollution Episodes

Place	Date	Estimated Nos. of Attributed Excess Deaths
Meuse Valley, Belgium	December 1930	63
Donora, Pennsylvania	October 1948	20
London, England	December 1952	4,000
New York, New York	November 1953	200
London, England	December 1962	700

Table 2. Ways in Which Diet May Affect Incidence of Cancer

1. By providing source of carcinogens or precarcinogens:
   -- Natural components of plants
   -- Products of chemical, bacterial or fungal action during processing or storage
   -- Products of cooking
   -- Contaminants (products of fuel combustion, pesticide residues)

2. By affecting formation of carcinogens:
   -- Provision of substances for formation of nitrosamines (secondary amines, nitrates, nitrites)
   -- Inhibition of formation of nitrosamines as in stomach (Vitamin C)
   -- Alteration of excretion of bile salts and cholesterol into large bowel (fat)
   -- Alteration of metabolism of carcinogens (enzyme induction by meat, fat, indoles in vegetables, antioxidants)
   -- Alteration of enzyme formation (trace elements)
   -- Affect on formation of estrogen (fats, total calories)

3. By modifying effects of carcinogens:
   -- Through transport (alcohol, fiber)
   -- Through effect on concentration in bowel (fiber)
   -- Inhibition of promotion (Vitamin A, beta-carotene)

(From Reference 19)

Table 3. Some Acute Environmental Pollution Episodes

Toxic Pollutant	Location	Year
Mercury	Minimata Bay, Japan	1959
PCBa	Kyushu, Japan	1968
PBBa	St. Louis, Michigan	1973
Lead	Kellogg, Idaho	1976
Dioxina	Seveso, Italy	1976
DBCPa	Lathrop, California	1977
Kepone	Hopewell, Virginia	1978
Multiple Agents	Love Canal, New York	1978
Dioxin	Times Beach, Missouri	1983
Dioxin	Newark, New Jersey	1983

[a]PCB defined as polychlorinated biphenyls, PBB as polybrominated biphenyls, dioxin as 2,3,7,8-tetrachlorodibenzo-p-dioxin, and DBCP as 1,2-dibromo-3-chloropropane.

Table 4. Examples of Outbreaks of Mass Human Poisoning From Toxic Chemicals

Date[a]	Location	Chemical	No. Affected
1930	U.S.A.	Triorthocresylphosphate	16,000
1934	Detroit	Lead	4,000
1952	London	Air pollutants	4,000
1952	Japan	Parathion	1,800
1952	Moringa (Japan)	Arsenic	12,159[b]
1955	Minamata (Japan)	Methylmercury	1,000
1956	Turkey	Hexachlorobenzene	4,000
1958	Kerala (India)	Parathion	828
1959	Morocco	Triorthocresylphosphate	10,000
1960	Iraq	Ethylmercury	1,022
1964	Niggata (Japan)	Methylmercury	646
1967	Qatar	Endrin	691
1968	Japan	Polychlorinated biphenyls	1,665
1971	Iraq	Methylmercury	50,000
1976	Pakistan	Malathion	7,500
1981	Spain	Toxic oil	12,600
1984	Bhopal	Dimethylisocyanate	2,000[c]

[a]Year of onset.

[b]These were the estimated number of exposed babies. It was stated that several thousand were poisoned and 131 died.

[c]Deaths. The full scale of lingering and permanent morbidity remains unknown.

(From Reference 20)

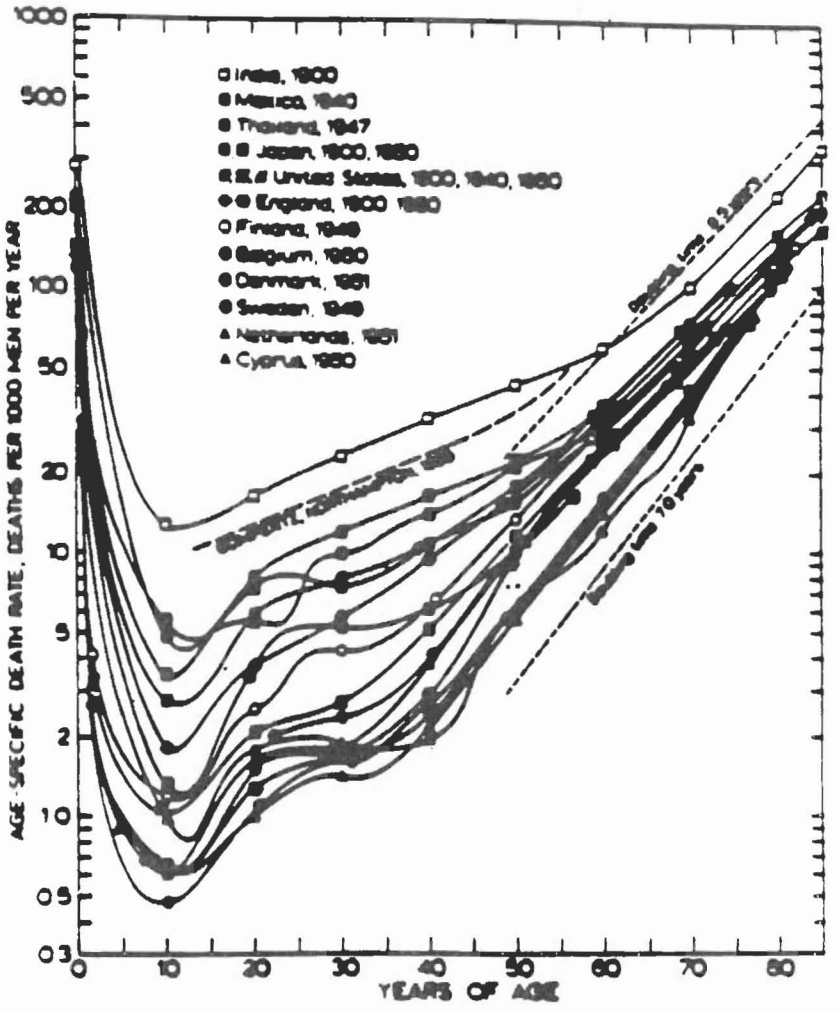


Figure 1 Age-Specific Death Rates in Various Countries and Years (From Reference 1).

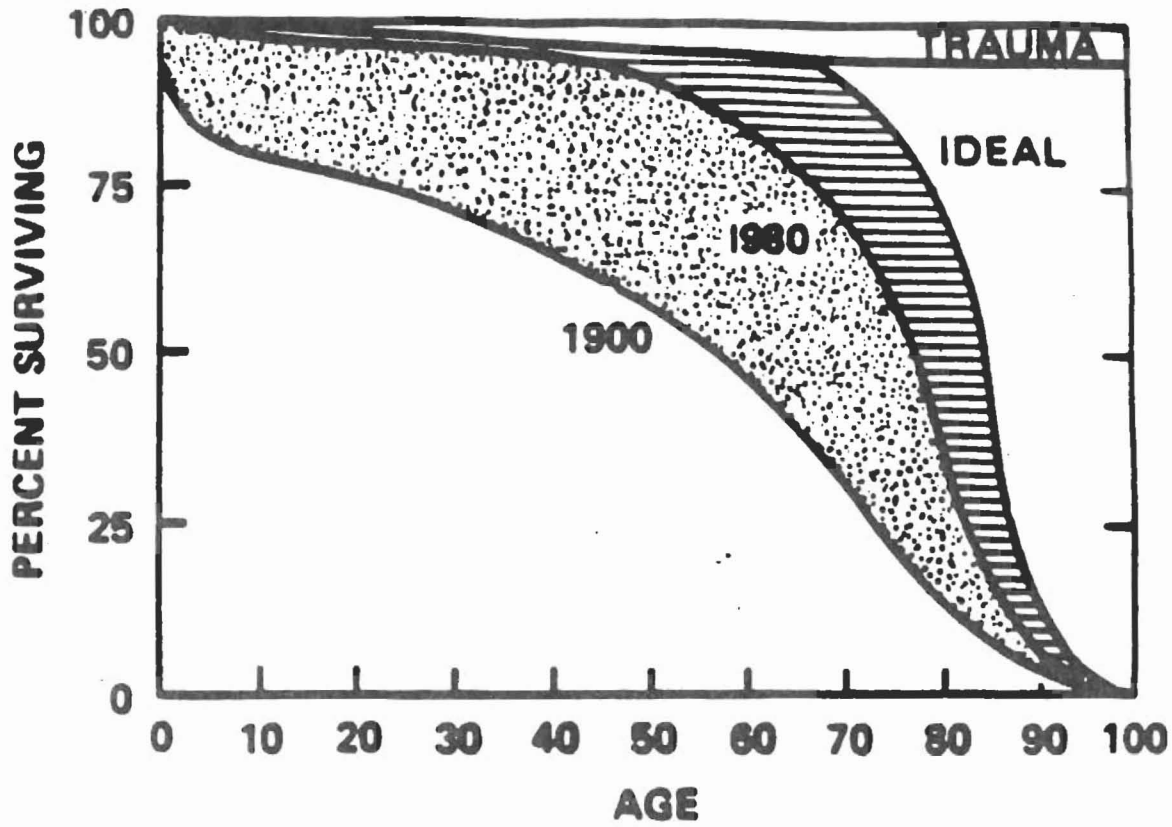


Figure 2

The Increasingly Rectangular Survival Curve in the U.S. About 80 percent (stippled area) of the difference between the 1900 curve and the ideal curve (stippled area plus hatched area) had been eliminated by 1980. Trauma is now the dominant cause of death in early life. (From Reference 2).

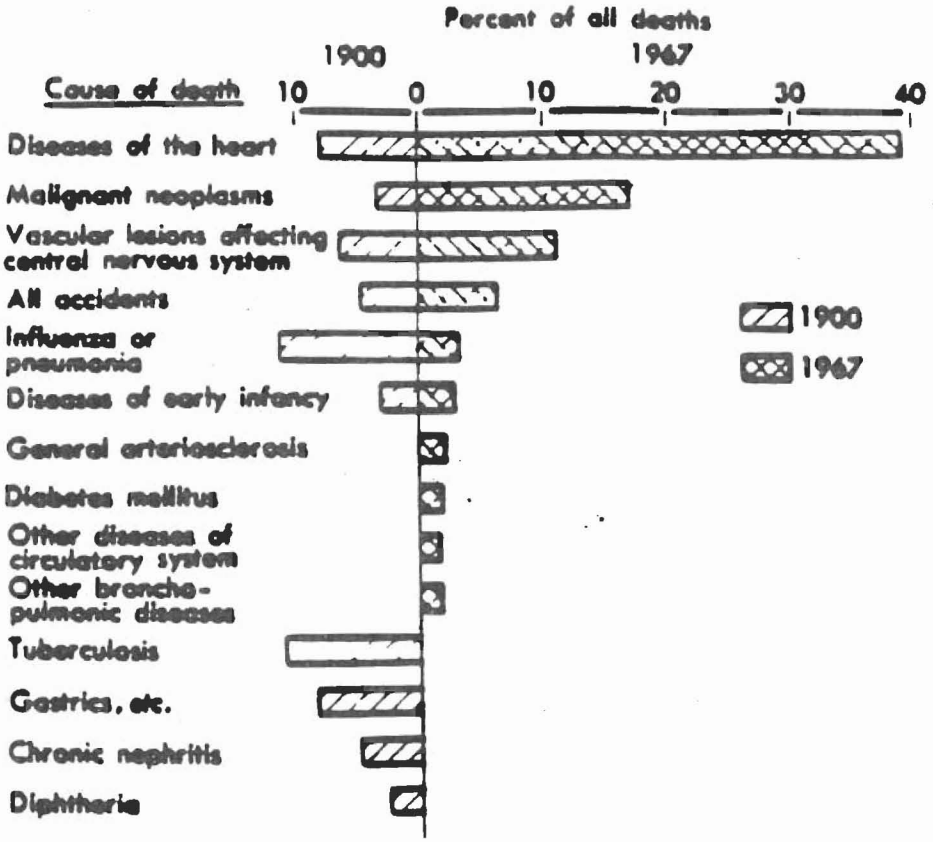


Figure 3 Leading Causes of Death in the United States, 1967, as Compared with 1900. (From Reference 3).

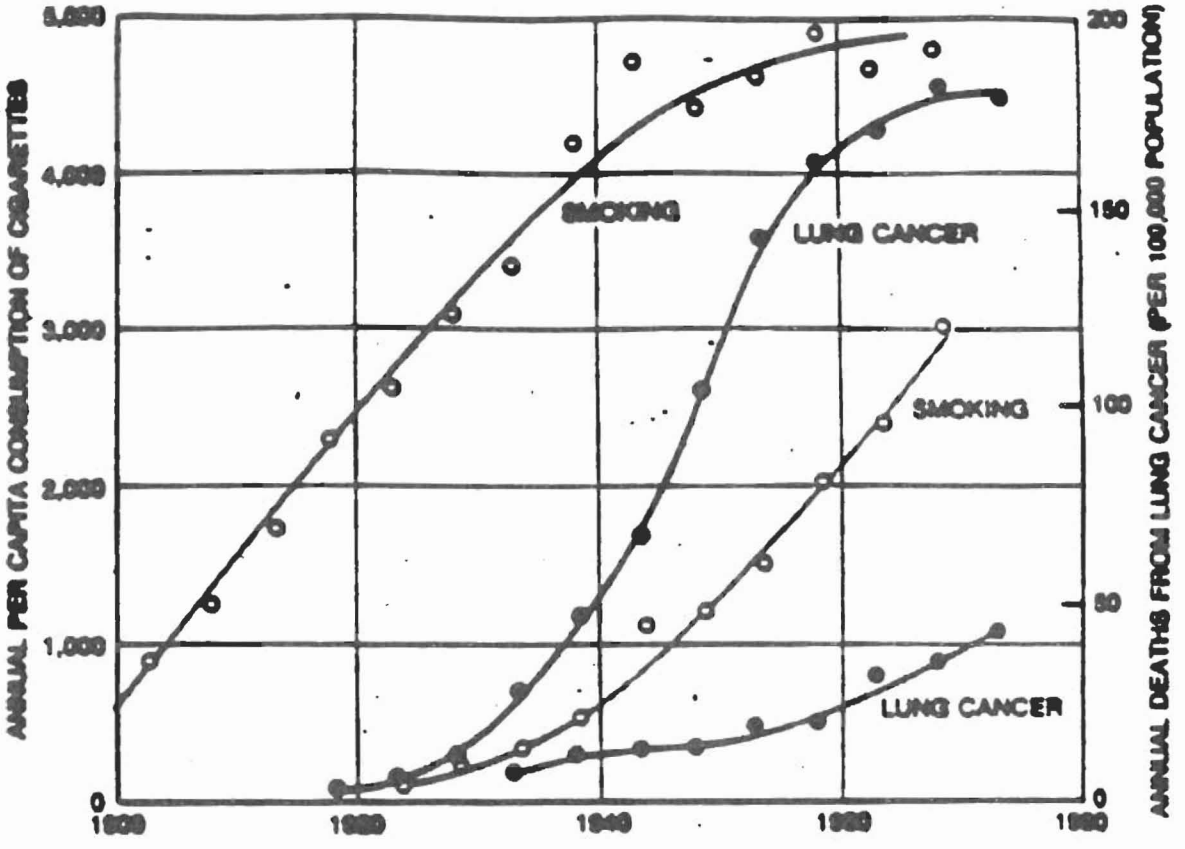


Figure 4 Time-Trends in Lung Cancer Mortality and Cigarette Consumption in England and Wales. (From Reference 6).

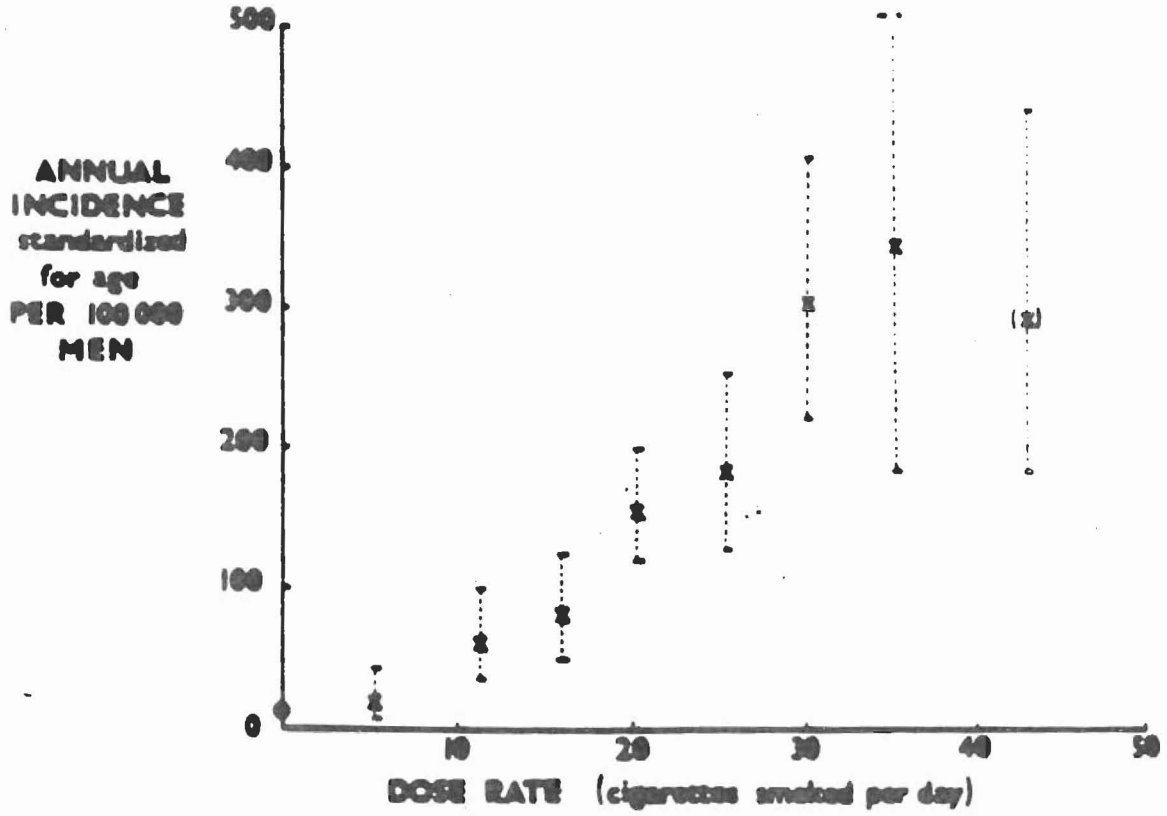
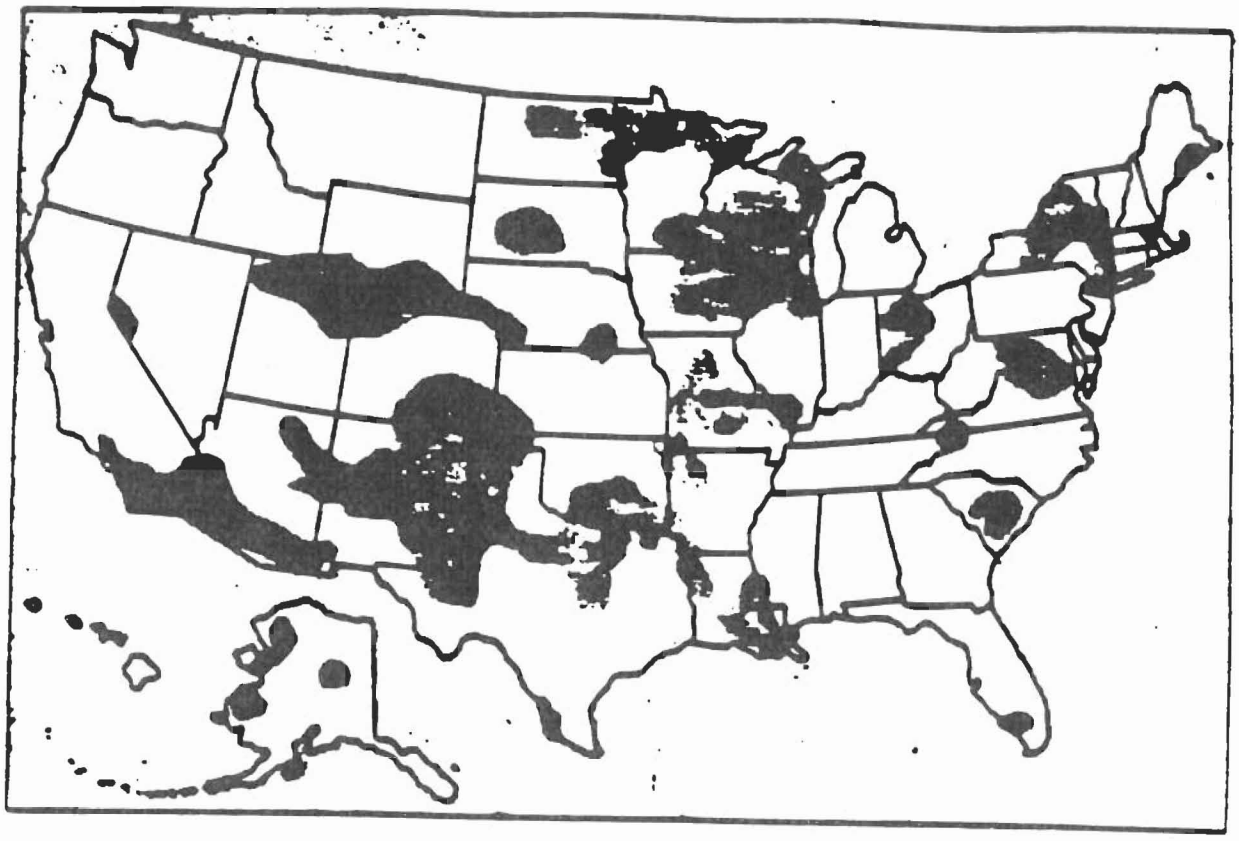


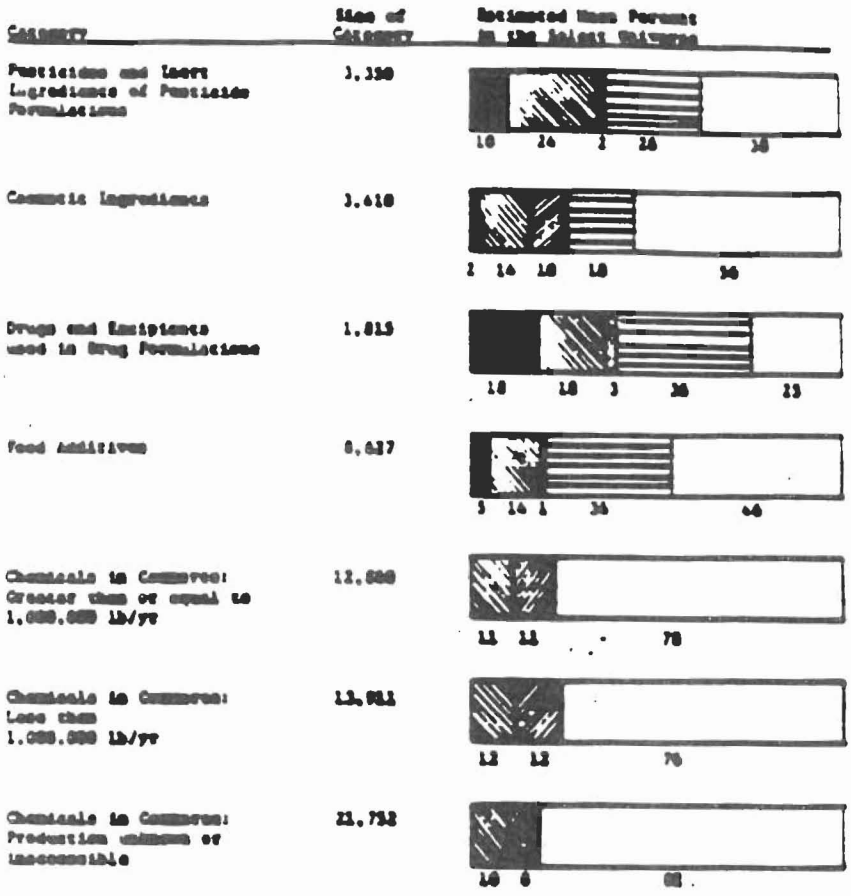
Figure 5 Incidence of Lung Cancer in Regular Cigarette Smokers in Relation to Number of Cigarettes Smoked Per Day. (From Reference 7).



Shaded areas = Reported pollution areas
Open areas = Areas that may not be problem-free, but the problem is not considered major.

O Industrial chemicals other than chlorinated hydrocarbons
Heavy metals, such as mercury, zinc, copper, cadmium and lead
Chlorinated hydrocarbons from treatment processes & energy development
* Coliform and other bacteria
Saline water
General municipal and industrial waste

Figure 6 Drinking Water Problem Areas (As Identified by Federal and State Regional Study Teams). Source: U. S. Water Resource Council. (From Reference 16).



Black bars = Complete health hazard assessment possible
Dotted bars = Partial health hazard assessment possible
Slanted line bars = Minimal toxicity information available
Horizontal line bars = Some toxicity information available (but below minimal)
Open space bars = No toxicity information available

Figure 7 Adequacy of Available Data on Chemicals of Different Categories for Health-Hazard Assessments. (From Reference 24).

Chapter 2

KINDS OF LONG-TERM RESEARCH

James Fouts

LONG-TERM HEALTH EFFECTS RESEARCH SUPPORTIVE OF EPA PROGRAM NEEDS

I. Basic Research

Basic research needed in EPA programs may or may not be directed specifically at support of certain applied research programs. Such basic research may seek only to understand deeper levels of the general universe of problems attacked in the specific, discrete long-term, applied researches (such as described just below). The general basic research philosophy is that understanding more about the ways chemicals cause disease can lead to earlier detection or better tests for adverse health effects (and better designs of epidemiology studies), better analytical methods, etc. All of this can and often does lead to better bases for regulation and, thus, better regulation. (See Section III below)

Some of this basic research can be directed at using some of the "new biology" to advance our ability to assess exposure or to better identify and quantify specific bad effects of (or bad actors in) complex mixtures of chemicals occurring "naturally". Overall though, the distinguishing feature of this basic research is that it addresses more "generic" issues, and that it not necessarily be tied into any one specific problem nor seek "quick" answers. As such, it must be supported for several years to be effective and to give the kinds of findings that will be most useful to many "applied" research programs. It is, however, true that often the most useful facts and new approaches needed in resolving any environmental emergency have come from turning to laboratories doing good basic research.

There are many examples here of the kind of research that probes deeper (and is more risky) than any of the applied programs. Some of these might be:

A. New methods to detect and quantify dioxins

Basic research has identified and characterized an intracellular "receptor" for dioxins and related compounds. Studies carried out over many years resulted in the partial purification of this receptor, and better understanding of the mechanisms, and specificity of several of the biological effects of dioxins. Recently, using the "new biology" techniques, this "dioxin" receptor has been cloned and can now be made available to "methods" (and other) research. Basic research in such areas/uses has pointed to one possible application of these studies--the use of this cloned dioxin receptor to isolate/separate and identify small amounts of dioxins and, at least, some dioxin-like materials in complex mixtures--particularly of the dioxins in soils, waste site effluents, etc.

The research on the "dioxin" receptor is the kind of basic research effort which may now come to "fruition" (e.g., in the new methods

for assaying dioxins in mixtures). But it has stretched over many years, and although never without some merit to the most practical/applied of objectives, has not been of immediate value to most of the EPA needs.

B. New methods for detecting exposure to some toxic chemicals

The cytochrome P-450s are a component of steroid, lipid, and xenobiotic metabolizing enzyme systems found in a variety of living systems (from yeast to humans). Much basic research over at least 35 years has led to some understanding of the diversity and responsiveness of these systems in many species. The "new biology" again has given us some new tools for quantifying and identifying many of these pigments. It is now possible to "fingerprint" the kinds and amounts of many different isozymes of P-450 in tissues of many animals (including humans) and plants. Basic research has described in some detail the responsiveness of these P-450s to various environmental stresses (including chemical exposures). Taken all together then, this long-continuing, basic research program may now be giving us tools for looking at the exposures of plants, animals, and humans to many environmental chemicals--e.g., the amounts and types of P-450s seem to reflect exposures to things like pesticides, smoke, solvents, etc. Further, basic research work (especially in pharmacokinetics) may even give us a tool for assessing both acute and cumulative/chronic toxic exposures (of species ranging from fish to humans) using these monoclonal antibodies for specific P-450s.

II. Applied Research

There are several types of research activity which have application to specific problems and specific settings, but which must be carried on over a period of several years. These can be divided into 3 major categories: 1) research programs with discrete and sequential parts/steps--where one part must usually be done before another can be initiated/planned, 2) research programs that often take a long time, but parts of which can be carried on concurrently, and 3) methods development and validation.

A. Long-term research programs best done in sequential steps

This is usually a series of several, discrete projects, each of which generates data useful/needed in other related projects--either in their design or execution. There are many examples here, but the key feature in each is that this is a long-lasting program with several stages, and each stage feeds into/sets up the next action:

1. The ozone layer and ozone depletion

This is a program which has continued for many years. The human and ecological health effects implications of this are enormous. Human health effects of the ozone-layer depletion include possibly large increases in UV light-induced cancers and other serious skin diseases. Ecological effects on agriculture/crops may be equally human

life-threatening, though less direct. There have been many stages in this overall program:

a. The first studies looked at the issue--Is there any evidence that we are actually losing stratospheric ozone? The answer to this (data supporting this) is still being gathered and debated (at least in some quarters), but the first indications were that evidence existed to suggest a loss; therefore, step 2 was needed.

b. The second step seemed to be: What might be causing this loss of ozone? Is there any human contribution (e.g., chemical) which can destroy ozone and which is likely to get to the ozone layer? Data about the chemistry and interactions of light, ozone, and hydrocarbons had to be generated here first. Some experiments are still being carried out at this stage.

c. The next step was to gather data about the presence of ozone-destroying materials/chemicals (e.g., halogenated hydrocarbons) in the upper layers of the atmosphere. New methods for measurement, collection of samples etc. had to be developed, validated and used.

d. Then real-life sources of these hydrocarbons had to be sorted out and evaluated for their possible contributions to the problem.

e. Then decisions as to which steps would be most effective in changing the amount of hydrocarbons at the ozone layer had to be decided.

Thus, many types of research were/are involved here---chemistry, biochemistry, ecology, climate, stratospheric, marketing, sociological/psychological, and political. However, the steps to be taken next in the overall strategy of dealing with this problem depended on the outcome of those studies made just before and on most of those preceding.

2. The ecologic and health effects of acid rain

A number of issues have been raised here, but they all concern whether acid rain or another source of pollution has caused the effects, and what these effects really are. Acid rain is believed to be formed primarily from industrial sources, though others are also possible and constitute another subset of evolving issues. One example in this problem area is whether the damage to trees (and other flora, here and in Europe) is due to acid rain from factories and electric power generation or is caused by pollutants from cars/traffic, etc. A series of studies has been made and others are continuing. It is becoming obvious from some of the results that the answer is "yes" to both; tree damage (and crop effects/human health effects) may result from acid rain and car exhaust. This answer comes from a series of sequential and evolving researches carried out over several/many years. One of the most recent reports on all this (including some limited assessment of human health effects of acid aerosols) is probably the National Acid Precipitation Assessment Program report issued in September 1987. Human health effects of acid aerosols were recently re-assessed at an EPA-NIEHS sponsored symposium held at NIEHS

in October 1987. The report of this will be published in Environmental Health Perspectives in 1988. This research effort in both ecology and human health effects of acid rain has gone on for years/decades, and some answers are only now becoming barely visible.

B. Long-term studies with concurrent steps

These are studies that just take a long time--the objectives are such that the study just can't be done in short time frames. Many "purely" epidemiology studies fall here--where the questions concern health effects of low level, chronic exposures or seek to determine endpoints resulting only years after exposure or in populations that must "age" to have detectable effects. Most studies on possible causes of cancer or on carcinogenic effects of chemicals are here. So are evaluations of the causes of many other slowly developing effects/diseases (e.g., emphysema, kidney failures, liver damage, and CVS or CNS effects). These evaluations involve multiple studies done at the same time but continuing for a long time on the same populations. Chronic toxicity studies in animals are a subset of this kind of approach. There are many examples here:

1. The "Six Cities Studies" of health effects of air pollution--comparing various indices of health in persons living in 6 cities of widely varying degrees of pollution. This Study has been going on for years now. Some part of the increasing clarity in this Study results from more data--accumulated now over more than 10 years, but some part is the adding of new tests and better data analysis to the screens for health effects. The point is that this Study required/used repeated studies of the same populations/regions over several years to establish effects and to clearly associate these health effects with the changes in air pollution (which occurred during the years of the study) in these 6 "regions". The principal effects now being seen are those on the lung (lung function decrements), but other systems (e.g., kidney, CVS) may be shown to be affected as these studies continue.

2. The effects of maternal polychlorinated biphenyl (PCB) exposures on childhood development. This began with several accidents both in the U.S. and elsewhere (e.g., cooking oil contaminations in Taiwan and Japan and accidents like the dumping of waste oil contaminated with PCBs along highways, and the exposures of persons living near, or walking along these highways). From both short- and long-term animal studies it was known that many serious effects of PCBs were not seen acutely but were instead delayed in onset and subtle. Therefore, several epidemiology studies were begun to follow (for several years) health in populations of PCB-exposed persons and especially in any children they might have. The effects of various levels of maternal exposure to PCBs on childhood development are now being described in some detail but only because these accidentally-exposed populations and a large number of "less-exposed" and "normal"/unexposed women and children were followed for many years.

3. The effects of polybrominated biphenyl (PBB) exposure. Again, this began with an accident--the mixing of PBBs into animal feed and the spread of this chemical/mixture among many farms and into many parts of the food chain in Michigan. Heavily-exposed persons are still being

monitored for effects, since again, animal studies show that these effects are delayed and subtle.

C. Development and validation of test methods

In many cases the methods for detecting and quantifying new environmental toxins/problem chemicals do not exist at the time such "problems" are first discovered. This set of "long-term" research activities is vital in any program seeking to understand and affect environmental health hazards. There are many examples here, but only a few can be given:

1. Dioxins (PCDDs) and dibenzofurans (PCDFs)

Chemical methods for detecting, separating, and quantifying these "families" of toxic materials did not exist when the first "poisoning" episodes in humans occurred. The amounts of these materials present in samples from most accidents is very small, and yet, in animals, these chemicals show toxic effects at extremely low concentrations. We are only now getting the methods needed to detect, quantify and selectively identify and separate the wide variety of these chemicals found in most real life exposures. Some of the newest in analytical techniques were developed to meet this problem/series of problems. The best of separation and analytical methods were required to identify the dibenzofurans as contaminants of the PCBs and dioxin mixtures and also as contributors to some of the toxicological effects/problems associated with these mixtures. This long-term research has stretched over at least twenty years and is not ended yet. Validation of all these methodological advances is still occurring.

2. Lead

With/in several environmental problems we need some measure of the toxic material in "deep" body tissues. Getting at these without painful surgery/biopsy or the use of autopsy material is a must if the amounts of information we need are to be generated--particularly for long-term studies, or for uncovering chronic effects (although this information may also be vital for acute emergencies). Lead, like several other metals, tends to stay only briefly in readily accessible body tissues and fluids. Stores of lead and several other chemicals occur in relatively inaccessible tissues like bone, teeth (or deep fat, etc.). Methods to measure these "deep" stores of toxic chemical are urgently needed. Non-invasive methods are especially useful/attractive for screening/repeated measurements. Newer methods for this in the case of lead may be possible now with X-ray fluoroscopy. Validation of this method is now taking place--total time from concept to use will be about

ten years if all goes well--a long-term effort typical here of several others.

III. How EPA Uses/Depends on Basic Research Conducted by Other Federal Agencies

Health research within the EPA is ultimately directed toward the regulatory mission of the Agency. While such research is often of an "applied" and/or "immediate" nature which answers specific problems that the Agency must deal with in an expeditious manner, sound basic or fundamental research is the only method of improving the scientific rationale underlying regulatory decisions. It is vital that the EPA scientific staff maintain current awareness of relevant basic research by performing such research within the Health Effects Research Laboratory and by closely following the latest developments in toxicological research. The Agency cannot effectively accomplish its research mission without scientists who have competence in and knowledge of the tools of basic research. Without this competence and knowledge health scientists within the Agency would be unable to effectively translate the findings of fundamental research into the applied research areas most supportive of the Agency's regulatory mission. However, since basic research performed by EPA represents only a small fraction of that which is necessary to support its regulatory mission, the Agency must rely heavily on basic research information developed by other Federal agencies particularly by the various Institutes of the Department of Health and Human Services. These organizations have been reponsible for many of the scientific breakthroughs in molecular biology, genetics, biochemistry, immunology, and cancer research that have enabled development of applied methods for exposure monitoring, dosimetry, toxicological testing, and biochemical epidemiology.

Basic research performed through programs developed at the National Institutes of Health has substantially impacted the Agency's regulatory approaches and policies. Research on the molecular basis of mutation, xenobiotic metabolism, pharmacokinetics, and molecular dosimetry performed at the National Institute of Environmental Health Sciences has found applications at EPA in genetic bioassay development and improved metabolic activation systems for in vitro test systems, molecular techniques for exposure monitoring, and advanced methods for human biochemical epidemiology. Fundamental research by the National Cancer Institute on mechanisms of carcinogenesis and immune surveillance has contributed directly to the development of toxicological test methods and guidelines for cancer risk assessment promulgated by the EPA Office of Health and Environmental Assessment. EPA is benefiting directly from widely and federally-funded basic research in the area of neurotoxicology. The discovery of biochemical differences among various cell types within the central nervous system (and their concomitant differential vulnerability) is leading to an improved understanding of mechanisms of neurotoxicity and improved methods for the assessment of adverse neurotoxicologic responses. These methods will undoubtedly contribute to future Agency guidelines for neurotoxicity testing.

In addition to the use which the Agency makes of basic research information generated by other Federal agencies through indirect means

(information appearing in the literature and discussed at scientific forums), the Agency also depends upon active research collaborations which take advantage of basic findings and/or expertise.

EPA scientists frequently engage in collaborative studies with scientists in other governmental agencies as well as their colleagues in academia who may be funded by these agencies. These research efforts often take advantage of expertise in new technologies and new findings that may have applications to the regulatory mission of the Agency. As an example, research on mechanisms involved in the successful fertilization of the oocyte has led to interagency collaborative research to improve methods for the evaluation of male fertility. Other research efforts delineating the fundamental factors involved in dermal absorption have led to joint interagency research projects centered on the development of improved methodologies for the assessment of the kinetics of such exposure.

Clearly, it would be possible to extend this list of relevant examples since much of the scientific information utilized by the Agency for regulatory decision-making and guidelines formulation rests on a foundation of basic research.

Chapter 3

RESEARCH ADVANCES IN THE TOXICOLOGY OF LEAD

Kathryn Mahaffey

PREAMBLE

The place of and necessity for long-sustained basic research activity in the development of a foundation for constructive action in important problems in environmental health could be illustrated by reference to any of several current problem areas. We have chosen the story about lead and its dangers or toxicity to serve this purpose. Lead as a public health problem has been recognized for years (if not centuries). Yet how, what, and when to do something about both preventing its health effects and treating those not prevented have been obvious only recently, and only as a result of long-continuing basic research. For one thing, only long-range, multidisciplinary, continuing basic research has given us the varied tools we need to detect some of the more subtle (yet extremely important) effects of lead. We have moved from counting dead bodies to worrying about things like changed behavior and nerve damages in lead-exposed children--but only because we now have some good tests for such effects of lead. This then is the story of an environmental health research success--made possible only because such slow-moving (and sometimes hard to explain) studies were pursued and supported by far-sighted people who believed that long-range research was and would continue to be extremely cost-benefit positive.

Background

Understanding the range of adverse health effects produced by lead exposure has advanced markedly in this century. Research into the toxic effects of lead provides a paradigm that has guided the entire discipline of clinical and laboratory toxicology for the past five decades. Fundamental multidisciplinary laboratory research in such areas as biochemistry and physiology has been a major key to this progress.

Lead has long been recognized to be acutely toxic at high-dose exposure. In addition, we now recognize, based on reearch findings in the 1970's and 1980's, that lead toxicity reflects two patterns of lead exposure. Adverse neurobehaviorial effects of lead on infants occur at levels within one standard deviation of the mean concentration of the United States population. Superimposed on the general population lead exposure is an additional severe problem of high-level lead exposure concentrated among young children from lower socioeconomic families, particularly those from urban areas.

In children, high-dose exposure to lead, such as results from ingestion of lead-based paint, has been shown to cause a profound neurologic syndrome characterized by coma, convulsions, and in severe cases death. In adults with high-dose exposure to lead, abdominal cramping, a syndrome termed "wrist and ankle drop," and end-stage renal disease are the well-recognized consequences.

The challenge has been to understand that the range of health problems caused by lead was much more extensive than the clinically-obvious disease. What has made this challenge especially difficult is that environmental lead pollution has been at very high levels, producing an elevated body burden of lead in a sizable portion of the population. During the 1970's in metropolitan areas, young children frequently had blood lead concentrations greater than 40 g/dl; a concentration now associated with several neuropsychological impairments. The challenge is to perceive the etiology and severity of health problems that are so common they are considered "normal." In the paradigm of lead public health and preventive medicine have progressed from enumerating mortality and morbidity (i.e., case reports) to understanding the disease process. This progress reflects and has been possible only because of long-range support of environmental research.

Among the most exciting recent findings with respect to understanding of the toxicology of lead is the realization that lead is capable of producing toxic effects in adults and children at relatively low levels of exposure, i.e., levels that are insufficient to produce grossly clinical symptoms. Only a decade ago such levels of lead exposure were considered "safe". Lead is now recognized to produce a syndrome of subclinical toxicity.

Recent research has demonstrated that this subclinical toxicity of lead is a many-faceted syndrome involving multiple organ systems. The developing red blood cells, the nervous system, and the kidneys are the organ systems in which these toxic effects have been more intensively studied.

In the early 1900's lead exposures were so high that occupational records routinely reported lead-induced mortality statistics. For example, Hoffman (1935) reported that the number of deaths attributed to lead poisoning for the United States registration area between 1900 and 1933 was in excess of 3400. The number of deaths among children, who are more susceptible to the effects of lead exposure, remains largely unknown. In the 1940's through the 1960's descriptive reports of clinical aspects of the disease dominated the literature. Prior to the introduction of chelation therapy, severe lead poisoning with encephalopathy had a mortality rate of 65% (NRC, 1972).

Among survivors of lead poisoning profound neurological damage is the predominant, reported effect. For example, Byers and Lord (1943) and other clinicians showed long-term residual sequelae of acute pediatric lead poisoning which included mental retardation, seizures, optic atrophy, sensory motor deficits, and behavioral dysfunctions. Perlstein and Attala (1966) reported such sequelae in 37% of children who suffered lead poisoning without evidence of encephalopathy.

Through screening programs to identify children with lead toxicity before they become symptomatic, and through legal requirements to monitor occupational exposures of workers to lead, severe clinical cases of lead toxicity have been brought under a degree of control; however, they have not been eliminated. These case reports, describing clinical aspects of intoxication, have identified which organ systems are most affected at

high-dose exposures. The limited reversibility or irreversibility has been documented in many of the clinically-reported, neurologic effects. Using these clinical studies as a guide, long-range, multidisciplinary research has extended the understanding of lead toxicity to the current emphasis on biomarkers of exposure, dose-response relationships for specific effects, and identification of susceptible subgroups for these effects.

Research Findings in the 1970's and 1980's

The general picture of adult and pediatric lead poisoning has changed in recent decades. The overall pattern is identification of significant adverse health effects at progressively lower exposures. These can be arbitrarily separated into neurobehavioral, hematopoietic, renal/endocrine, and reproductive effects. As a part of this effort, differential sensitivity of various subpopulations has been revealed. Identification of effects occurring at environmental exposures once considered "normal" has coincided with reducing environmental exposures to lead. Only through reduced exposures can the results given by toxicology and epidemiology research be evaluated in general human populations.

I. Neurobehavioral Effects

Recognition that neurobehavioral effects in children are produced by lead exposures considered "normal" in earlier decades (e.g., blood lead concentrations of 20-50 g/dl) has been among the most significant research findings in the 1970's and 1980's. Longitudinal studies during the past 10-15 years built upon early case reports and cross-sectional studies. The longitudinal prospective designs have permitted gathering improved information on exposure histories. Information on exposure levels and patterns is clearly important in assessing effects of a cumulative toxicant on endpoints such as neurobehavioral function that may reflect changes induced at far earlier, but critical, developmental periods.

The most consistent finding of the prospective studies is that an association exists between low-level lead exposures during developmental periods (especially prenatally) and later deficits in neurobehavioral performance. This latter is reflected by indices such as the Bayley Mental Development Index, a well-standardized test for infant intelligence. Blood lead concentrations of 10-15 g/dl constitute a level of concern for these effects (EPA, 1986). In addition, impaired neurophysiological function has been associated with increasing blood lead concentrations among children. These functional deficits include changes in the auditory brainstem evoked potentials and evidence of lead-related reduced hearing acuity (Robinson et al., 1985, 1987). These subclinical toxic effects of lead on the central nervous system are generally considered to be permanent and irreversible, and they are associated with permanent loss of intelligence and irreversible alteration in patterns of behavior.

Bellinger et al (1987) reported significantly lower post-natal development scores on the Mental Development Index of infants from an upper-middle class population when maternal blood lead levels were in the rnage of 10-25 g/dl. Among adult women ages 20-40 years mean, blood lead levels were between 10 and 12 g/dl based on the NHANES II general

population data for the period 1976-1980 (Mahaffey et al, 1982). Thus, it must be emphasized that these neurobehavioral changes are associated with blood lead levels within one standard deviation of the mean blood lead level of the United States' population reported in the NHANES II data.

The peripheral nervous system is also affected by lead. Typically, adults are likely to demonstrate peripheral rather than central nervous system effects. In the early 1960's investigators began to call attention to "subclinical" neuropathy manifested by changes in peripheral nerve conduction velocity in lead workers not having overt neurological involvement (Sessa et al., 1965). In the 1970's Seppalainen et al. (1972, 1975) reported the slowing of the maximal motor conduction velocity of the median and ulnar nerves and other electromyelographic abnormalities in workers whose blood lead concentrations never exceeded 70 g/dl. Investigations of the behavioral effects of lead uncovered an increased hearing threshold, decreased eye-hand coordination, and other physiological and psychological changes in workers with blood lead concentrations below 80 g/dl (Repko et al., 1975).

II. Hematopoiesis

Anemia has been a symptom of severe clinical lead poisoning in both children and adults. Anemia (increased prevalence of hemotocrit values below 35%) is now recognized to become evident in one-year-old children at blood values of 30 g/dl. Lead interferes with synthesis of heme and the formation of hemoglobin at a number of metabolic steps. In the developing red blood cells lead inhibits the enzyme -aminolevulinic acid dehydratase to increase levels of erythrocyte protoporphyrin in children. The threshold for this effect in children is associated with a blood lead concentration of 15-18 g/dl (Piomelli et al., 1982).

Impaired heme biosynthesis produces effects in addition to anemia. The accumulation of protoporphyrin IX (measured as zinc protoporphyrin or as protoporphyrin in erythrocytes) is not only an indicator of diminished heme biosynthesis but also signals general mitochondrial injury. The final step of heme biosynthesis occurs in the mitochondria. Such injury to the mitochondria can impair a variety of subcellular processes including energy metabolism and homeostasis. Health implications of such impairment include: reduced transport of oxygen to all tissues; impaired cellular energetics; disturbed immunoregulatory role of calcium; disturbed calcium metabolism; disturbed role in hematogenesis control; impaired detoxification of xenobiotics; and impaired metabolism of endogenous agonists (e.g., metabolism of tryptophan).

III. Renal Effects

Acute high-dose lead exposure in children produces a Fanconi-type syndrome with glucosuria, phosphaturia and aminoaciduria secondary to poisoning of the proximal convoluted tubule. High-dose exposure to lead in childhood has been associated with glomerular nephritis and renal disease in adults. Among occupationally-exposed adults, an increased rate for mortality from all causes, from all neoplasms (specifically, cancers of the stomach, liver, and lungs), from chronic nephritis, and from other hypertensive disease (i.e., hypertension due to kidney damage and not heart

disease) were observed in a longitudinal study of workers in lead battery plants and lead smelters (Cooper, 1985).

A statistically-significant relationship has been reported between increases in systolic and diastolic blood presures and increases in blood lead among 40-to-59 year old, white males from the NHANES II survey population (Pirkle et al, 1985).

Impairment of the endocrine functions of the kidney have been reported to occur at much lower lead exposures. Recognition of these effects required development of several areas of research:

A. Understanding the metabolic activation of Vitamin D to 1,25-dihydroxyvitamin D. This metabolite is critical to regulation of calcium metabolism.

B. Recognition that lead impairs various steps in both biosynthesis and function of 1,25-dihydroxyvitamin D.

Currently, the most studied site at which these metabolic pathways converge is the proximal convoluted tubule of the kidney. Here 25-hydroxyvitamin D, formed in liver from Vitamin D, undergoes a second hydroxylation which is catalyzed by the enzyme 1, ,25-hydroxyvitamin D hydroxylase. Research using in vitro techniques (following in vivo exposure of chickens to lead) has demonstrated that lead inhibits the activity of this enzyme. Findings from a clinical investigation among young children indicated that plasma 1,25-dihydroxyvitamin D levels were depressed in proportion to blood lead concentration. Chelation therapy to reduce body burden of lead, resulted in increasing serum concentrations of 1,25-dihydroxyvitamin D up to levels similar to those present in children serving as controls (Rosen et al., 1980). Additional epidemiological research has shown that 1,25-dihydroxyvitamin D concentrations were decreased with increasing blood lead concentration over a broad range of blood lead concentrations, 12 to 120 g/dl (Mahaffey et al., 1982b).

IV. Reproductive Effects

Early in the century a number of adverse effects of lead on reproduction were reported among women with occupational lead exposures. These included increased spontaneous abortion rate, increased still-birth rate, and a higher, post-natal and early childhood mortality rate among children of such exposed women. Exposures associated with these adverse outcomes were very high. However, longitudinal, prospective studies, designed to evaluate neuropsychological effects of lead, have provided important information on reproductive effects at the upper range of current environmental levels. McMichaels et al. (1986) found that the incidence of preterm deliveries (before the 37th week of pregnancy) were significantly related to maternal blood lead at delivery. When late fetal deaths were excluded, the strength of the asociation increased. The relative risk of preterm delivery at exposure levels reflected in blood lead concentrations of 14 g/dl or higher was 8.7 times the risk at blood lead concentrations up to 8 g/dl. Reduction in gestational age at delivery with increasing blood lead concentrations were also reported by Dietrich et al. (1986), Bellinger et al. (1984), Moore et al. (1982), and Bornschein et al. (1987a,

b). The data from Bornschein indicate that for each 10 g/dl increase in blood lead concentrations birth weight decreased between 58 and 601 grams depending on the age of the mother.

The findings of McMichaels et al. (1986) also identified an excess in miscarriages and still births in the high-lead exposure areas. In contrast, data from this study show that average, maternal blood lead concentration was lower for still births than for live births. Placental response to lead remains an unanswered question.

Basic research in the toxic effects of lead at low doses is of profound importance for the fields of preventive medicine and public health. Until recently, blood lead concentrations of 25 g/dl and below were considered safe, and indeed, only five years ago the Centers for Disease Control (CDC) stated that 25 g/dl should constitute a threshold level indicative of increased lead absorption in children. Now, on the basis of recent research it is evident that lead produces toxic effects in children at levels below this guideline. Thus, recent research into the toxicity of lead at low doses is about to force a total re-evaluation of current standards for assessing the exposure of American children to lead.

The importance of these basic research findings stems from the fact that lead exposure remains extremely widespread among children in the United States. Data from the Second National Health and Nutrition Examination Survey (NHANES) indicated that in 1980 9.1% of all preschool children in the United States - 1.5 million children - had blood lead concentrations of 25 g/dl or more (Mahaffey et al., 1982a). Among black preschool children the prevalence of increased lead absorption (high blood lead concentrations) was 25%.

These findings on the high prevalence of increased lead absorption (high blood lead concentrations), when taken in conjunction with the data on subclinical lead toxicity, carry a message of chilling significance. These findings suggest that 9% of all children in this nation, and 25% of minority children, may be suffering irreversible neurologic, intellectual, and behavioral impairment as the result of chronic, low-dose exposure to lead. The implications of these basic research data for public health and environmental medicine are enormous.

This then has been a very condensed story about one of the many pervasive and important environmental health hazards. It is a story that continues beyond the present findings and their implications. It will reach even more successful conclusions only if the kind of studies which brought us to this stage are continued. Continued long-range and basic research investigations on lead toxicity are at one and the same time perhaps among the more justifiable and yet less supportable of such activities in the entire field of environmental health sciences. So much has been done before in lead research that in comparison, no other (few at least) of all the current health hazards has received this much emphasis. Yet it is obvious that this sustained effort in lead research has paid off handsomely and is still needed. It is this "apology" for long-range, basic research that we feel can stand for the entire field of environmental health science, whatever may be the specific stage of development of this research for any one hazard.

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Chapter 4

NEWER BASIC/LONG-TERM RESEARCH WITH APPLICATION TO ENVIRONMENTAL HEALTH PROBLEMS

PREAMBLE

In this Chapter a number of authors discuss some of the newer basic/long-term research with possible applications to current environmental health problems (especially in humans). This does not represent the whole universe of possible basic/long-range research which will or could be of great benefit to such environmental issues. It is, however, an attempt at careful choices of those studies which have required such long-term support for the reaching of this stage where their applications could have great impact on environmental health. As such then, this is a look at the many and more probable benefits of supporting such long-range research more adequately than has been done in the past.

ACTIVATION OF PROTO-ONCOGENES BY CHEMICALS

Marshall Anderson

INTRODUCTION

Proto-oncogenes are cellular genes that are expressed during normal growth and development processes. These genes were initially discovered as the transduced oncogenes of acute transforming retroviruses (1). Recent studies have established that proto-oncogenes can also be activated to cancer causing oncogenes by mechanisms independent of retroviral involvement (2-4). These mechanisms include point mutations or gross DNA rearrangements such as translocations or gene amplifications. The activation of proto-oncogenes by genetic alterations results in altered levels of expression of the normal protein product, or in normal or altered levels of expression of an abnormal protein.

ACTIVATION OF PROTOONCOGENES

The activation of proto-oncogenes in spontaneous and chemically-induced rodent tumors and in human tumors has been studied in great detail during the past several years. Investigations in rodent models for chemical carcinogenesis imply that certain types of oncogenes are activated by carcinogen treatment and that this activation process is an early event in tumor induction (5-6). Alternatively, analysis of some human and rodent tumors suggests that oncogene activation is involved in neoplastic progression (7-9). The number of proto-oncogenes that must be activated in the multistep process of neoplasia is unclear at present. The concerted, low level expression of at least two oncogenes, ras and myc, are needed for the partial transformation of primary rodent cells in vitro (10). Furthermore, in addition to the activation of proto-oncogenes, the loss of specific regulatory functions such as tumor suppressor genes may be a distinct step in neoplastic transformation (11). The implication of activated oncogenes in rodent tumor will be discussed in terms of extrapolation of rodent carcinogenic data to human risk assessment.

The activation of ras proto-oncogenes appears to represent one step in the multistep process of carcinogenesis for a variety of rodent and human tumors (5,6). The activation of ras by point mutations is probably an early event in tumorigenesis and may be the "initiation" event in some cases. Thus, a chemical that induces rodents tumors by activation of ras proto-oncogenes can potentially invoke one step of the neoplastic process in humans exposed to the chemical. Is this property alone enough to classify the chemical as a potential human carcinogen? Dominant transforming oncogenes other than ras have also been detected in chemical-induced rodent tumors (6). The involvement of these oncogenes in the development of human tumors is unclear at present, as well as whether the non-ras genes detected in human tumors can be activated by chemicals or radiation (6).

ONCOGENE ANALYSIS

Most chemicals are classified as potentially hazardous to humans on the basis of long-term carcinogenesis studies in rodents. While these rodent carcinogenesis studies are often designed to mimic the route of human exposure in the environment or workplace, the dose of a given chemical is usually higher than that which actually occurs in human exposure. Coupled with the appearance of species- and strain-specific spontaneously occurring tumors in vehicle-treated rodents, this complicates the extrapolation of rodent carcinogenic data to human risk. Oncogene analysis of tumors from spontaneous origin and from long-term carcinogenesis studies should help determine the mechanisms of tumor formation at a molecular level. For instance, the finding of activating mutations in different codons of the H-ras gene in furan-induced liver tumors versus finding activating mutations in only one codon of the H-ras gene in spontaneous liver tumors suggest that the chemical itself activated the H-ras proto-oncogene by a genotoxic event (12). In general, comparison of patterns of oncogene activation in spontaneous versus chemically-induced rodent tumors, together with cytotoxic information, should be helpful in determining whether the chemical in question is mutagenic, cytotoxic, has a receptor mediated mechanism of promotion, or some combination of these (and other) modes of action. This type of analysis might be of particular importance for compounds such as furan and furfural (12,13) which are negative for mutagenicity in short-term bioassays.

APPLICATION TO STUDY OF CARCINOGENICITY

Another approach which should be helpful in species-to-species extrapolation of risk from carcinogenic data is to examine oncogene activation and expression in tumors from different species induced by the same agent. For example, K-ras oncogenes with the activating lesion in codon 12 were observed in both rat and mouse lung tumors induced by tetranitromethane (14). Even though little is known about the DNA damaging properties of this chemical, these data suggest that this compound is acting in the same manner to induce tumors in both rats and mice. The role of chemicals and radiation in the activation of proto-oncogenes by gene amplification, chromosomal translocation, and other mechanisms which can alter gene expression, is currently being investigated by several groups. Also, as human life span increases, it becomes more important to study chemical-induced enhancement of the progression of benign to malignant tumors. These and similar approaches to explore the mechanisms by which chemicals induce tumors in animal model systems may remove some of the uncertainty in risk analysis of rodent carcinogenic data.

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CARCINOGEN-DNA AND PROTEIN ADDUCTS: RESEARCH PERSPECTIVES

Frederica P. Perera

INTRODUCTION

Advances in basic research in molecular biology and biochemistry have permitted the development of innovative methods applicable to studies of human populations exposed to chemical carcinogens. These highly sensitive techniques can detect and sometimes quantify the internal dose of carcinogens (the amount of the carcinogen or its metabolite in body tissues and fluids) or the biologically effective dose (the amount that has interacted with cellular macromolecules such as DNA, RNA or protein) in target tissue or a surrogate. This latter type of dosimetry data could be especially valuable in studies of cancer etiology by providing a mechanistically relevant link between external exposure data on the one hand and clinical disease on the other. Comparable molecular dosimetry data in rodents and humans have the potential to improve interspecies extrapolation of risk in addition to providing early warning of a carcinogenic hazard to humans. Successful applications of such "adducts research" could directly address major programs/needs at EPA for better estimates of exposure and risk to humans.

Various methods are available to monitor chemical-specific lesions (such as immunoassays for DNA and protein adducts) as well as non-chemical specific biologic alterations (such as cytogenetic effects or somatic cell mutations). Table 1 gives examples of currently available methods for measuring the biologically effective dose of carcinogens. As can readily be seen, all pertain to endpoints associated with carcinogens that exert genetic toxicity. Moreover, almost all available methods depend on readily es for the actual target tissue itself. Despite these limitations, biological markers have significant potential usefulness in cancer etiology and risk assessment.

Table 1. Examples of Human Biologic Monitoring Methods[a]

End Point	Method	Sites & Fluids[b]
Biologically effective dose		
Adducts (DNA)	Immunoassay, postlabeling, fluorescence spectrometry	WBC
Adducts (protein)	Mass spectrometry, ion-exchange amino acid analysis, HPLC, gas chromatography	RBC
Excised adducts	HPLC, fluorescence	Urine
UDS	Cell culture, thymidine incorporation	WBC
SCE	Cytogenetic	WBC
Micronuclei	Cytogenetic	BM,WBC
Chromosomal aberrations	Cytogenetic	WBC
Somatic cell mutation (HGPRT)	Autoradiography, light microscopy	WBC
Somatic cell mutation (glycophorin A)	Immunoassay	RBC
Sperm quality	Analyses of count, morphology, motility	Sperm

(a) Source: See Reference 1 (as modified)

(b) RBC=red blood cells; BM=bone marrow; WBC=white blood cells; UDS=Unscheduled DNA Synthesis; HPLC=High Performance Liquid Chromatography; SCE=Sister Chromatid Exchange; HGPRT=Hypoxanthine Guanine Phosphoribosyl Transferase

ADDUCTS

Carcinogen-DNA and carcinogen-protein adducts have been the focus of considerable research in the past 5 years and illustrate a number of strengths and limitations common to biological markers in general (2,3).

Biological Basis

The biologic rationale for measuring DNA adducts is that these lesions, if unrepaired, can produce a gene mutation. There is considerable evidence that gene mutation in somatic cells "initiates" the multistage process of carcinogenesis (4,5); but it may also result in conversion of tumors to the malignant state (6,7). Carcinogen-DNA adducts resulting in gene mutation may also activate certain oncogenes instrumental in carcinogenesis (8,9,10).

Protein such as hemoglobin can, in theory, act as a more readily available surrogate for DNA. Proportionality between protein and DNA binding has been demonstrated for a number of carcinogens (11,12,13).

Adducts are generally monitored in peripheral blood cells rather than target tissue. However, for only a few carcinogens (e.g., benzo(a)pyrene and cis platinum) is there actual experimental and/or human evidence that comparable levels are formed at both sites (14,15).

METHODS

Techniques to measure carcinogen-DNA adducts include immunoassays using adduct-specific polyclonal or monoclonal antibodies, synchronous fluorescence spectroscopy, HPLC fluorescence spectrophotometry, and ^{32}P-postlabelling. Carcinogen-protein adducts may be determined using antibodies and gas chromatography-mass spectrometry. The sensitivity of the DNA to adduct methods is in the range of one adduct per 10^{6}-10^{10} nucleotides. Those methods aimed at carcinogen-protein adduct quantification also appear to have adequate sensitivity for environmental studies (16). However, unambiguous identification of particular DNA adducts at low levels is difficult with present analytical methods. Moreover, cross-reactivity of antibodies (such as the BPDE-I-DNA antibody which also detects closely related polycyclic aromatic hydrocarbon (PAH-DNA adducts) presents problems in definitive characterization of adducts (17).

ANIMAL AND HUMAN STUDIES

Experimental studies involving acute and/or chronic exposure to diverse carcinogens have shown that the relationship between administered dose and macromolecular binding is generally linear with few exceptions (12,2,18,3). With respect to humans, carcinogen-DNA and -protein adducts have been investigated in human populations with exposures such as cigarette smoke, PAHs, tobacco and betel nut, dietary aflatoxin and N-nitrosamines, cis platinum, psoralen, 4-aminobiphenyl, propylene oxide, vinyl chloride and ethylene oxide (3).

While results thus far support the feasibility and adequate sensitivity of the methods in terms of human studies, they are frequently limited by technical variability in the assays, small sample size, lack of appropriate controls, and inadequate data about exposure. However, they consistently illustrate that there is significant variability in the formation of carcinogen-DNA and -protein adducts between individuals with comparable exposure or administered dose (15,19-25). Another consistent finding in the human studies involving environmental exposure, is that measurable levels of adducts are seen even in so-called "unexposed controls" (19-20,26-29). Both of these observations have obvious implications for risk assessment.

Although still largely in the validation stage, methods to monitor DNA and protein adducts in experimental animals and humans have considerable potential in a number of areas. These include: hazard identification, understanding of mechanisms involved in carcinogenesis, interspecies risk extrapolation and improving the power and timeliness of epidemiology (19,26,30-32).

Research Needs

Research is needed in the following areas:

A. Interlaboratory validation of methods as has been undertaken recently for PAH-DNA immunoassays (33).

B. Research on the stabililty of adducts in stored tissues.

C. Investigation of intra-and inter-individual variation in adduct levels.

D. Research on the persistence of adducts in various cells and tissues.

E. Comparison of adduct levels in DNA versus protein as well as in surrogate versus target tissue for a number of different classes of compounds.

F. Identification of critical sites or "hot spots" on DNA with respect to the carcinogenic effectiveness of adducts.

G. Interspecies comparisons of DNA and protein adduct formation (e.g., humans and rodents with acute and chronic exposure to the same compound(s)).

H. Experimental and human studies on the relationship between adduct formation, gene mutation, and oncogene activation.

I. Longitudinal studies (experimental and human) on the relationship between adduct levels and tumor incidence/cancer risk. Examples would be molecular epidemiological studies in model populations (such as patients exposed to high dose chemotherapy and who experience a high rate of secondary cancer, or heavily-exposed

worker groups). Biologic samples could be drawn at the outset and stored for future analysis.

J. Sample banks to serve as archives of human blood, urine, and tissue for retrospective analysis.

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NEUROTOXICOLOGY

Lawrence Reiter

INTRODUCTION

Epidemiological studies in Europe indicate that long-term exposure to solvents can produce neurobehavioral disorders which, depending on the length and severity of exposure, can range from loss of concentration and memory impairment to mood and personality changes to severe and apparently irreversable dementia. Indeed, cognitive impairment appears to be an early sign of solvent neurotoxicity. These studies have led the international neurotoxicology community to call for improved methods for identifying and characterizing solvent neurotoxicity both in animal models and in human clinical populations.

NEUROBIOLOGY OF LEARNING AND MEMORY

An area of long-term research which promises to produce powerful applications to this problem is the neurobiology of learning and memory. The goal of this field is to understand how normal memory function is carried out by the nervous system as well as how various neuropathological conditions, such as Alzheimer's disease and Korsakoff's syndrome, produce cognitive dysfunction. Interest in this area of neuroscience research is very intense. By some estimates, fully a quarter of all research in the basic neurosciences is concerned with the neurobiology of learning and memory. It is not surprising then that progress in this area is occurring at a very rapid rate. This paper will briefly highlight some specific recent developments in this field which should have a major future impact on neurotoxicological assessment.

Analysis of the neurobiology of learning has been organized around three general areas: (1) key brain regions i.e., which brain regions are essential for different forms of memory; (2) memory "circuits" in the brain, i.e., the delineation of neural pathways through which sensory information results in the production of learned behavioral responses; and (3) synaptic mechanisms, i.e., the nature of the synaptic changes that occur during learning, and the biochemical and cellular processes which underline them. The first of these areas has had, as one of its major concerns, the problem of how to extrapolate from animal models of cognitive dysfunction to human dementia. The latter two areas have been concerned primarily with analyzing the animal model systems at more molecular levels. In the past 5-7 years, dramatic discoveries have been made in all three of these areas.

NEUROTOXICOLOGICAL ASSESSMENT

Attempts in the area of extrapolation have taken two forms. One has been to develop behavioral tests in animals which are more analogous to those which are used to assess cognitive function in humans. The other form, and the one which we will emphasize here, has been to apply

behavioral tests to humans which are analogous to those which are well understood, both behaviorally and neurobiologically, in animals. For example, it has recently been shown that delayed-non-matching-to-sample, a task which is a sensitive indicator of memory impairment associated with limbic system and frontal cortical damage in rats and primates, is also a sensitive indicator of dementia associated with similar neuropathology in human clinical populations. Another example is the successful use of human eyeblink conditioning to detect learning deficits, associated with aging and Alzheimer's disease, which were predicted by neurobiological studies of eyeblink conditioning in rabbits. These recent developments in basic behavioral neuroscience establish a direct neurobehavioral link between the experimental analysis of cognitive dysfunction in animals and its assessment to humans. Neurotoxicological research, aimed at validating the application of these new animal models to the problem of risk assessment will substantially advance progress on the question of how animal studies can be used to characterize risk to human populations, following exposure to solvents and other environmental pollutants.

The second important development which could greatly increase the sophistication of neurotoxicological assessment is the identification of neural circuits subserving learning. The best example of this is the neurobiological study of rabbit eyeblink conditioning. This Pavlovian conditioning preparation has many advantages for neurotoxicological assessments, including: (a) the wealth of knowledge of its behavioral properties, which makes it possible to study anything from simple associative reflexes to complicated cognitive-perceptual processes in a single experimental preparation; (b) the ability to directly compare quantitative measures of both learned and unlearned behavior, on-line and in real time; (c) the ability to directly compare the same type of conditioning in animals and humans; (d) the ease of arranging concurrent electrophysiological recording from discrete brain loci (or, in the human, brain EEG activity recorded from scalp electrodes). However, the most important advantage offered by this recent research development is the wealth of knowledge that we now have about its essential neural circuitry in the brain stem and cerebellum. We also know a good deal about the effect of pharmacological agents on this type of conditioning and this greatly improves our ability to integrate the various aspects of neurotoxicological assessment. If an unknown compound produces a behavioral effect, we have a good idea of where to look for its neurochemical and neuroanatomical effects, and ultimately its mechanism(s) of action. Conversly, if a compound produces an effect on a neurochemical or neuroanatomical system, we know what functional consequences to look for in terms of the types of behavioral or cognitive processes which might be impaired. Some investigators have already begun to use Pavlovian techniques of this kind as animal models in the neurotoxicological assessment process. Just this year (1987), the rabbit eyeblink preparation has been applied to the study of dementia associated with aluminum toxicity.

One final development which is worth mentioning is the use of the in vitro brain slice technique to study neural plasticity. Electrophysiological studies of hippocampal slices have uncovered a phenomen, termed long term potentiation (LTP), which has become very influential as an experimental model for studying the synaptic mechanisms

of learning. In LTP there is, in effect, an increase in synaptic efficacy that occurs with repeated use. Investigations of the cellular and biochemical mechanisms of LTP have revealed a special role of a particular receptor type (the N-methyl-D-aspartate or NMDA receptor). Pharmacological antagonists of the NMDA receptor may prevent the induction of LTP, and may disrupt cognitive function in rats. What is true of drugs may also be true of other compounds with neurotoxic potential (eg., environmental chemicals). It is likely that with continued research in this area, hippocampal slice preparations may be used as a means of screening unknown compounds for their potential ability to produce cognitive dysfunction, and of characterizing the neurobiological mechanisms of any neurotoxic effects which are found.

SUMMARY

In summary, these three general areas of long-term research in behavioral neuroscience create a framework for the analysis of neurobehavioral function which is integrated at both a conceptual and, perhaps more importantly, a practice level. With this framework, it is possible to use information from diverse scientific subdisciplines, including cell biology, neurochemistry, neuroanatomy, neurophysiology, and both animal and human psychology, in a very direct and real way to either (a) identify the risk that compounds with neurotoxic potential may pose to normal cognitive function or (b) characterize the risk of classes of compounds, such as the solvents, which are known to produce memory loss, dementia and other neurobehavioral disorders.

USE OF MONOCLONAL ANTIBODIES IN NEUROTOXICOLOGY

Monoclonal antibodies provide another example of long-term research which has promise for application to a wide variety of environmental problem (See Chapter 2 for some others). This section will describe some new applications in neurotoxicity.

Background

Exposure to a foreign substance often elicits an immune response characterized by production of antibodies. Antibodies are serum proteins that react with antigens (antigens are foreign substances capable of inducing antibody formation). Such antigenic substances can include viruses, bacteria, proteins, or even complex molecules like environmental chemicals. Antigen-antibody reactions are highly specific, indeed, among the most specific known to biology. It is this specificity of the antigen/antibody complex that has been exploited by the biomedical scientist with applications ranging from curing Polio to understanding the molecular basis of enzyme catalysis.

Antibodies are produced in the body by B lymphocytes (B-cells), each of which produces its own unique antibody. In theory, as many as 10 million antibodies can be produced by a mouse in response to a single antigen. Each antibody reacts with a unique antigenic site (termed an epitope) and each antigen contains several epitopes. Because one B-cell can form antibodies against only one epitope but there are many B-cells producing antibodies against each epitope, this is referred to as a polyclonal (many cells) antibody.

The lymphocyte fusion technique of Kohler and Milstein, for which they received the 1984 Nobel Prize, was designed to overcome the limitations associated with the use of polyclonal antibodies (e.g., contamination, heterogeneity, limited supply). The antibodies produced by Kohler and Milstein were referred to as monoclonal because they were produced by a single (mono) B-cell line (clone). Monoclonal antibodies have several advantages including: 1) inherent specificiy (each clone produces only one specific antibody); 2) unlimited supply (clones produce large amounts of antibody and can be kept indefinitely); and 3) purified antigens are not required for the production of pure antibodies (monoclonals by definition recognize only a single antigenic determinant).

Monoclonals have been used to define, localize, purify, quantify, and modify antigens. The main distinction between the use of monoclonals, as opposed to polyclonal antibodies, is that monoclonals confer far greater precision and accuracy and are available as essentially immortal, off the shelf reagents. Thus, it is now possible to define antigens with a greater degree of certainty than ever before. This inherent trait of monoclonals has made it far easier to identify rare antigens both in vivo and in vitro (e.g., nervous tissue cell types and tissue typing in cell culture). One example of the application of monoclonals that is relevant to the EPA is the use of specific monoclonals to identify dioxin congeners in contaminated soils. True purfication of antigens from heterogeneous sources (e.g., serum, tissue) also is now possible with monoclonals. Thus, rare factors or hormones, such as interferon, can now be easily obtained in

bulk pure form. Likewise, quantification of antigens in complex mixtures is also easier to achieve with monoclonals than with polyclonal antibodies, an example being human chorionic gonadatrophin for pregnancy tests. By targeting specific antigens with monoclonals, modification of toxicity or disease states also may be realized. Examples are treatment of digoxin overdose (with antibody to digoxin), and cancer therapy with anticancer agents linked to monoclonals targeted to tumor cell antigens.

Applications of Monoclonals to Neuroscience/neurotoxicology

The years of research on monoclonal antibodies that followed Kohler and Milstein's original report in 1975 are now beginning to revolutionize neurobiology by providing the tools with which to understand the complex cellular and subcellular organization of the nervous system. Thus, the major impact of monoclonal antibody technology on neuroscience has been the unambiguous identification of different cell classes in the nervous system. Indeed, monoclonals have now been produced which identify previously unknown subsets of neurons and glia (the major cell types of nervous tissue) which otherwise would not appear to be different using classical techniques of light or electron microscopy. Monoclonals have also proved crucial for the identification and characterization of unique macromolecules, and have been even shown to reveal important differences within the same molecule. For example, monoclonal antibodies have now been produced that reveal phosphate-containing versus nonphosphate-containing neurofilaments, the major structural (filament) component of all neurons. The significance of this subtle difference, i.e., the absence or presence of a single phosphate, is that this substitution may be related to a variety of neurological disease states, including Alzheimer's disease, and also may represent a general response to injury of the nervous system.

In neurotoxicology, it is known that toxicant-induced injury to the developing or mature nervous system often is manifested by alterations in the cytoarchitecture of specific neuroanatomical regions. Furthermore, within an affected region, the response to injury may encompass several cell types. Because antigens that distinguish the diverse cell types comprising the mammalian nervous system have been revealed by monoclonal antibodies, these same antibodies can be used to detect, localize and characterize cellular responses to neurotoxic exposures. This can be accomplished by a technique known as immunohistochemistry, where antibodies are used as specific probes for microscopically localizing specific antigens within tissue obtained from toxicant-exposed animals. Quantitative data are obtained with the same antibodies by using monoclonal-based radioimmunoassays. Thus, through the use of monoclonal antibodies an integrated morphological/biochemical evaluation of neurotoxicity may eventually be achieved. The possibility also exists that the sensitivity and specificity of monoclonal antibodies can be applied to the detection and measurement of antigens released into the cerebrospinal fluid and blood as a consequence of neurotoxic exposures. Theoretically, it would then become possible to develop inexpensive monoclonal-antibody based test kits for detecting neurotoxicity in the exposed human population.

In summary, it is clear that current advances in the neurosciences will continue to reveal the extensive cellular and subcellular heterogeneity of the nervous system based on the use of monoclonal antibodies. The EPA, by

actively participating in long-range research, will benefit by having the tools with which to assess and predict environmentally-induced neurotoxicity.

MAGNETIC RESONANCE IMAGING

Morrow Thompson

INTRODUCTION

A major problem in environmental health sciences is the non-invasive detection of small adverse effects or adverse effects at early stages. Research applications of magnetic resonance imaging hold promise for just such advances. In the few years since Lauterbur's (1) paper was published, magnetic resonance (MR) imaging has evolved rapidly into an accepted clinical technique and, also, a research tool of enormous potential. Systems with high field, superconducting magnets (1.5 to 4.7 Tesla) are available commercially and are designed for human beings and laboratory animals (separate systems). Sophisticated techniques that modulate the effects of proton density, relaxation times, and motion permit the acquisition of 3-dimensional images that optimize differences between normal tissue types, define pathologic structures of areas, and allow the measurement of blood flow or perfusion (2-5). For reasons of abundance and signal intensity, the hydrogen nucleus (proton) is probed for the production of practically all MR images. The abilities to image alternate nuclei (e.g. ^{23}Na) and chemically shifted nuclei (e.g. ^{1}H in water versus fat) have been demonstrated and show the versatility and undeveloped potential of the technology.

Present day proton MR images of human beings and laboratory animals contain superb anatomic detail that, in some applications (biologic specimens and small animals), approaches microscopic levels. In recent publications (6,7), images of frog eggs and plant stems have been shown with volume elements (voxels) of 0.2 and 12.0 L, respectively. Perhaps more impressive are experiments being conducted at Duke University in which chemically induced hepatic lesions as small as 100 L in volume have been imaged in rats. The ability to detect such small lesions in live animals requires long imaging sessions (as long as 6 hours), strong magnetic fields and gradients, sophisticated pulse sequences, and little or no relative motion. Because respiratory motion is transferred through the diaphram to the liver, the last issue (no motion) is accomplished by intubating the animal, using a gaseous anesthetic, and synchronizing signal acquisition to respiratory motion (8,9).

Some of the advantages of MR imaging are common to those of other techniques, and other advantages are unique. Similar to computerized tomography (CT) scans, MR imaging is non-invasive and may be performed multiple times on the same animal or patient. In toxicology experiments, for example, the incorporation of MR imaging of a group of animals could provide important information concerning target organs, time to lesion (e.g., tumor) development, and response to continued or modified treatment (e.g., progression or regression of lesions). MR imaging uses fewer animals per exepriment compared with conventional means for gathering similar information.

While imaging techniques based on ionizing radiation are well established, rapidly produced (a distinct advantage compared to MR imaging

at its present state of development), and excellent for demonstrating some anatomic structures or abnormalities (e.g., bone lesions containing calcium deposits, recent hemorrhage), MR imaging has some distinct and important advantages. With current and anticipated magnetic fields, gradients, and RF signals, and with the proper precautions MR imaging is considered safe for patients and technicians (10). Additionally, the MR signal, unlike the penetrating beams of ionizing radiation, contains information in addition to that of tissue (in this case, proton) density. The signal is also determined by the rates at which protons relax in relationship to the molecular lattice (T1, spin-lattice, longitudinal relaxation) and to each other (T2, spin-spin, transverse relaxation). Because these time constants are influenced by the chemical composition of the tissue (probably by the amount and motional freedom of water molecules), the resulting image can permit distinction of tissues that are similar in proton density but differ in relaxation times.

Although not a consistent finding, malignant tumors frequently have T1 and T2 relaxation times greater than those of benign tumors or normal tissue. Recent disappointments concerning the apparent inability of MR imaging (relaxation times) to distinguish between pathologic entities have been expressed (11). This may be partially related to the acquisition of the signal from tissue slices that, because of slice thickness, include degenerative and normal areas within and adjacent to the lesion of interest. In animal experiments at Duke University, this possibility is being explored by excising very thin (only 1.25 mm thick) tissue slices in rats. While signals from such thin slices are weak and imaging sessions are relatively long, the thin sections with high resolution greatly improve the selectivity, and, hopefully, the discriminating ability of the method.

CURRENT AND FUTURE APPLICATIONS

In clinical medicine, MR imaging compliments and frequently exceeds the performance of other imaging methods. MR imaging excells in demonstrating neoplastic, demyelinating, and degenerative processes of the central nervous system. Because of the suscetibility of the thyroid and parathyroid glands to ionizing radiation, MR imaging is a preferred method for examination of these tissues. Respiratory and cardiac gating have been used to produce excellent diagnostic images of the heart, thoracic blood vessels, and lungs. MR images of liver, kidney, reproductive organs, and pelvis routinely demonstrate a variety of neoplastic and non-neoplastic processes. Current and future developments will incorporate the use of faster scanning sequences, 3-dimensional imaging, measurement of perfusion and flow, contrast agents, imaging combined with *in vitro* spectroscopy of different nuclei (e.g., ^{31}P, ^{13}C, ^{23}Na, ^{19}F), chemical shift imaging (e.g., permitting separate proton images of ^{1}H in water verses fat), and, possibly, multinuclear imaging (e.g., ^{31}P, ^{23}N). These developments, in addition to improving the sensitivity of detecting lesions, will allow imaging to be combined with *in vivo* metabolic studies that can characterize biochemical activities in a region of interest.

In toxicologic experiments, techniques have been developed that allow prolonged anesthetization of rats (as long as 6 hours) associated with respiratory and cardiac scan synchronization for thoracic and abdominal

imaging. High field systems (300 MHz, 7 Tesla) are being developed and tested that have a theoretical resolution of 10 M. Areas of active research include the improvement of RF coil designs, and the use of stronger field gradients, surface and implanted coils, and contrast agents. Within a few years, increases in resolution should permit, for example, the visualization of renal glomeruli, preneoplastic hepatocellular foci, and nuclei in the brain. With such developments, Lauterbur's closing statement in his 1973 paper would seem remarkably prophetic, "Zeugmatographic techniques should find many useful applications in studies of the internal structures, states, and compositions of microscopic objects."

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IMMUNOTOXICOLOGY

Michael Luster

In a broad sense immunotoxicology can be defined as the study of adverse (inadvertent) effects of environmental chemicals, therapeutics or biologicals on the immune system. The types of effects that may occur include immunomodulation (i.e., suppression or enhancement), hypersensitivity (allergy) and, in rare instances, autoimmunity. A large body of information has developed over the past 10 years that exposure to certain chemicals or therapeutics can produce immune dysfunction and alter host resistance in experimental animals following acute and subchronic exposure. Examples of these are listed in the attached table. The most extensively studied class of environmental chemicals is the polyhalogenated aromatic hydrocarbons (PHAs), including polychlorinated biphenyls, polybrominated biphenyls, chlorinated dibenzofurans and the prototype of this class, chlorinated dibenzo-p-dioxins.

Despite the species variability associated with the toxic manifestation of these compounds, studies in laboratory animals exposed during neonatal or adult life with PAHs and, in particular, dibenzo-p-dioxins have indicated that the immune system is one of the most sensitive targets for toxicity. These effects are characterized by thymic atrophy and severe and persistent suppression of cell-mediated (T cell) immunity and share many features of neonatal thymectomy. Laboratory studies have further indicated that the target cell for immunosuppression by PHAs is the thymic epithelium which is necessary for T cell maturation. Although only a limited number of reports indicate immune dysfunction following human exposure to PHAs, the effects have been found to be remarkedly similar to these which occur in animals. For example, suppression of a delayed hypersensitivity response and increased susceptibility to respiratory infections have been found in patients who accidentally ingested polychlorinated biphenyl/dibenzofuran-contamined rice oil. Another example of this immune dysregulation by PHAs has been reported in Michigan farm residents who inadvertently ingested polybrominated biphenyls. These individuals also demonstrated persistent suppression of cell-mediated immunity with increased numbers of null cells, possible reflecting the presence of immature cells. Although long-term deleterious consequences of polybrominated biphenyls remain to be determined in humans, early data indicate a correlation between immune alterations and increased tumor incidence.

Thus, it appears that early laboratory studies in rodents have provided a very accurate account of the immunological dysfunction that is observed in humans following inadvertent exposure to these compounds.

EXAMPLES OF IMMUNOLOGICAL ABNORMALITIES ASSOCIATED WITH CHEMICAL EXPOSURE IN RODENTS AND HUMANS

Chemical Class	Example	Laboratory Immune Abnormality	Human Immune Abnormality
Polyhalogenated Aromatic Hydrocarbons	TCDD	+	±
	PCB	+	+
	PBB	+	+
	HCB	+	N.S.
Heavy Metals	Lead	+	-
	Cadmium	+	-
	Methyl Mercury	+	-
Aromatic Hydrocarbons (Solvents)	Benzene	+	+
	Toluene	+	N.S
Polycyclic Aromatic Hydrocarbons	DMBA	+	N.S.
	BaP	+	N.S.
	MCA	+	N.S.
Pesticides	Trimethyl Phosphorothioate	+	N.S.
	Carbofuran	+	N.S.
	Chlordane	+	N.S.
Organotins	DOTC	+	N.S.
	DSTC	+	N.S.
Aromatic Amines	Benzidine	+	+
Oxidant Gases (Air Pollutants)	NO_2	+	N.S.
	O_3	+	+
	SO_2	+	N.S.
Others	Asbestos	+	+
	DMN	+	N.S.

N.S. = Not studied; ± = Positive and negative findings have been reported.

HUMAN CHORIONIC GONADOTROPIN (HCG)

Donald Mattison and Alan Wilcox

BACKGROUND

Public health scientists have long been concerned about the many possible reproductive hazards of environmental pollution. One unanswered and troubling question has been whether there are effects of toxins on the earliest stages of pregnancy. This can include environmentally-induced very early abortions/fetal wastage. If there were some way to detect the earliest stages of pregnancy, then perhaps such effects of occupational, environmental, or drug exposures could be more easily defined and addressed. It is known that about 15% of clinically-recognized pregnancies end in recognized loss (spontaneous abortion). The risk of such loss has been found to be higher in some populations with occupational, environmental, etc., exposures. However, clinical losses don't tell the whole story; clinically-recognized losses represent only a portion of all pregnancy losses. There are at least twice as many earlier losses as recognized spontaneous abortions. Thus, a technique which could detect pregnancy very early and define its ending precisely could help pinpoint whether chemical or other environmental exposures might have been involved in such ending. The application of new researches with human chorionic gonadotrophin offers such possibilities.

METHOD

Determination of very early pregnancy loss requires sensitive and specific methods for identifying pregnancy. The recent development of antibodies to one component of the beta subunit of HCG has vastly improved the capacity of HCG assays to detect early pregnancy. HCG is produced by the conceptus starting at about the seventh day after fertilization. HCG is quickly excreted in the mother's urine and is detectable by immunometric assays. For this reason, HCG assays are the mainstay of studies of early pregnancy. This immunoradiometric assay is reactive to the unique carboxyterminal peptide of the HCG molecule. The assay is up to one hundred times more sensitive than any previously available assay. This added sensitivity has proved to be important because up to three-quarters of early pregnancy losses never reach a level of HCG secretion that could have been detected by previous assays.

IMPLICATIONS

Early pregnancy loss may be one of the earliest signs of human exposure to mutagens or other toxins that damage human reproduction. It should be possible to streamline this type of study, collecting urines only on days when early loss is most likely to be detected. This approach could be extended to high-risk groups of women in occupational or other settings where toxic effects on reproduction are suspected. These assays are now able to measure HCG in urine down to the background levels that occur in healthy non-pregnant persons. These assays are just now beginning to be

applied in epidemiologic studies for the detection of very early pregnancy loss. This is an exciting new applied research area in environmental medicine which is the direct result of very basic research in reproductive biology. This may be a model for future research and suggests that basic and clinical studies are essential if we are to make progress in understanding human reproductive vulnerability to environmental chemical exposure.

Further basic and applied research is needed in this area -- as a high priority -- because of existing data which suggest that there are indeed exposures which can increase the rate of clinically-recognized, spontaneous abortion. These may include various segments of the chemical industry and the microelectronics industry.

Chapter 5

ESTIMATION OF POPULATION RISKS

David Hoel/Michael Hogan

ANIMAL MODELS AND RISK ESTIMATION

Since relevant epidemiologic and clinical information are often lacking on the potential health hazards associated with exposure to a specified agent or chemical, laboratory animal data usually constitute the primary basis for both qualitative and quantitative human risk estimation. The majority of animal-based, human risk estimation is qualitative in nature. That is, laboratory or experimental identification of a given exposure source as a potential human health hazard is often sufficient, in and of itself, to control or even prevent future exposure of the general public to the agent or chemical in question, and no determination of the magnitude of the risk involved in the anticipated exposure may be required (e.g., regulation of potentially carcinogenic food additives under the Delaney Amendment). Nevertheless, it is the role of animal data in the quantification of possible human health risks that is of greater scientific interest and debate.

Animal-based, quantitative risk estimation almost always involves two separate issues or problems that must be addressed: low-dose extrapolation, necessitated by the high dose levels typically employed in laboratory animal studies and, of course, species extrapolation, since the ultimate concern is with the risk posed to humans. Perhaps the single most important issue involved in low-dose extrapolation is the choice of the specific mathematical model or extrapolation procedure to be used in determining the low-dose risk or the acceptable exposure level for the agent under consideration. In carcinogenesis, mathematical modeling may have progressed as far as is possible or defensible without further insights into the mechanisms underlying the carcinogenic process. Certainly the need for greater emphasis on the meaningful incorporation of molecular and biochemical data into risk models is well recognized, and it offers an important research opportunity to those interested in the quantification of potential human risk based on animal data. For noncarcinogenic outcomes or endpoints there is definitely a need to reevaluate the "safety factor" approach to risk determination, which has been the regulatory standard since the mid-50's, and, in some instances, to promote the development of quantitative models similar to those used in carcinogenesis.

Regardless of the toxicologic response of interest, however, it is clear that, increasingly, attention will be focused on making the selected model or extrapolation procedure more closely reflect the underlying biological mechanisms. For example, in carcinogenesis the question of "primary" versus "secondary" or "indirect" modes of action and their potential impact on the risk assessment process is sometimes raised with those who assume the latter mechanism often arguing against traditional low-dose extrapolation models (1). On the other hand, those, who out of

convenience or convention, have relied on the safety factor approach for determining permissible exposure levels for noncarcinogenic toxicants, may need to reconsider the biological issues that underlie its use, giving particular attention to the question of thresholds. For example, if one argues that a threshold mechanism is present, does the threshold represent a true (biological),"no effect" level or merely imply a dose or exposure level where the observable effects are minimal? Does it apply to the population as a whole or vary from individual to individual? (In the latter instance the population dose-response may be indistinguishable from one for which no threshold exists. That is, if threshold levels vary among individuals, then the "population" threshold level would correspond to the threshold for the most sensitive individual in that population, which, for all practical purposes, might be indistinguishable from a zero exposure level.) Another issue of concern is whether there is a biological (as opposed to traditional) basis for the selection of any given safety factor to be used with an observed/estimated threshold value in generating estimates of acceptable human exposure levels (2).

The question of species extrapolation may well generate as much scientific debate as the selection of the most appropriate low-dose extrapolation procedure. Certainly, the utility of the laboratory animal model for identifying potential human health risks is broadly recognized within the scientific community [e.g., see the IARC Preamble (3) regarding the interpretation of experimental results with regard to human carcinogenic risk when epidemiologic or clinical data are not available]. However, there is no universally accepted means of quantitatively scaling the results observed in laboratory animals to humans. What is usually done is to assume that animals and humans have equivalent risks when risk is expressed in terms of the appropriate dosage scale. Yet, human risk estimates based, e.g., on mouse data can vary by as much as 40-fold (4) depending on whether they are expressed in terms of an average lifetime daily mg/kg dose or a total acculumated mg dose, standardized (divided) by body weight. Furthermore, even though necessity may force one to rely on nothing more than a common dosage scale as the basis for extrapolating risk estimates across species, such an approach is only an approximate adjustment for the variety of factors that can contribute to interspecies differences in response (e.g., differences in lifespan, body size, kinetic profile, genetic homogeneity, general environment, etc.). Improvements in the quantitative extrapolation of toxicologic responses across species will require greater emphasis on the use of molecular and biochemical data. For example, the use of pharmacokinetics or molecular dosimetry, when scientifically feasible, to estimate the "biologically effective dose" could significantly reduce the uncertainty associated with interspecies extrapolation of observed toxicologic responses.

HUMAN STUDIES

Mathematical dose-response models for quantitative risk estimation have been and are increasingly being applied to epidemiologic data as well as to laboratory animal results, particularly in the area of carcinogenesis. Some of the better known examples include Peto's fitting of the multistage model to Doll's smoking data (6), Day and Brown's use of the same model to assess whether a number of human cancer risk factors such as smoking,

asbestos and radiation affected early, late or both early and late stages of the carcinogenic process (7), BEIR III's (6) use of absolute and relative risk models to characterize the time-related distribution of site-specific tumors among Japanese A-bomb survivors, and their use of linear, linear-quadratic and quadratic models to predict low-dose cancer risk associated with ionizing radiation. While the use of epidemiologic data obviously eliminates the need for species extrapolation, such data may not be sufficiently sensitive to allow one to chose among competing dose-response models or, in some instances, even to determine if any health risk appears to be associated with low or moderate levels of exposure.

A number of procedures may be employed to increase the sensitivity of the available epidemiologic data. For instance, initial attempts at human risk identification and estimation could be focused on sensitive subgroups within the general population under study, such as the very old or young, individuals with insufficient immune response, individuals suffering from concurrent disease or inherited deficiencies, and individuals also exposed to other known risk factors for the toxicologic endpoint or health effect of interest.

Recently, a new speciality has emerged in the field of epidemiology, which is commonly known as molecular or biochemical epidemiology. One of the primary purposes of molecular epidemiology is to adapt laboratory procedures for the identification and characterization of biochemical markers to epidemiologic field studies, so as to clarify the nature of underlying dose-response relationships, i.e., relationships between exposure and disease or toxicologic effect (8). Specifically, biochemical markers may provide quantitative evidence of generalized exposure (e.g., blood lead levels), organ specific exposure (e.g., DNA adduct formation), biologic change, and early or frank disease to replace the more subjective and qualitative measures that have often been used in epidemiologic investigations (e.g., determining exposure histories through questionnaire data and then classifying study subjects as being either "exposed" or "unexposed".)

While interest in and and even application of biochemical markers is increasing rapidly, validation of their use for epidemiology is currently a major research endeavor, and it is likely to continue to be so in the future. [Among the issues that should be considered in any validation exercise are the determination of marker sensitivity, specificity, predictivity, range of normal or baseline values, and whether the marker is reflecting current or cumulative exposures, average or peak exposures, and cumulative or noncumulative biological effects (8).]

POPULATION RISKS

The last step in the quantitative risk assessment process is the determination of the overall risk for the population of interest or, alternatively, the selection of an acceptable exposure level for that population. Some of the uncertainties involved in using experimental animal or epidemiologic data in hazard identification and, particularly, in dose-response modeling and low-dose risk estimation have already been enumerated. If a strong case can be presented for the presence of a

threshold phenomenon and a safety factor approach is elected, then it is important to remember that failure to compensate adequately for the unknown, underlying threshold can result in a proportion of the exposed population having their individual threshold values falling below the estimated acceptable exposure level in some instances (2).

Another significant factor that must be addressed in developing population risk estimates is the determination or estimation of exposure levels within the population under evaluation. There are a number of potential problems or uncertainties typically involved in the estimation of population exposure levels. Exposures may vary considerably among individuals or even for a single individual across time, so that the use of average exposure levels may not be very representative of the exposure histories of individual population members. While use of worst-case exposures may provide an upperbound on the actual levels of exposure encountered, it can also lead to an overestimate of the population's health risks and certainly engenders a great deal of uncertainty about such estimates. The uncertainty is compounded when average or worst-case exposure estimates are multiplied by the estimated average risk per unit dose to obtain an overall estimate of population risk. For example, even though worst-case exposure estimates may overestimate the actual exposure experience of much or possibly all of the population of interest, "average" risk per unit dose estimates may significantly underestimate the risks of the most susceptible subsets of that population.

Some argue that the uncertainties involved in quantitative risk estimation and concern for the health of the exposed population have often led to the overuse of worst-case or upperbound assumptions in quantitative risk estimation--assumptions that result in what they regard as unduly conservative estimates of the population risks. However, there are other investigators (9) who fear that national concern about the assessment of human health risks has tended to be focused almostly exclusively on cancer risk, and that as a result, other (perhaps less quantifiable) forms of human disease or dysfunction may have received insufficient attention: (See Appendix). If this is the case, then, in any specific situation the estimated "acceptable", "virtually safe" or "minimal risk" dose for carcinogenesis may still entail an unreasonable level of risk of other adverse health outcomes, even when the estimation process has been based on conservative assumptions.

The OSTP cancer document (10) and other science policy reports have stressed the need for qualitative and quantitative characterization of the uncertainties of specific risk estimates (e.g., consideration of the impact of model selection, the use of one set of laboratory data over another, the choice of a particular species as being most representative of humans, etc.). Also important are considerations and specification of the assumptions underlying a particular risk assessment (e.g., the construct of an estimated lifetime average daily dose rate so that animals continuously dosed at a constant rate throughout their lifetimes might be used to estimate the risk in humans who may have received intermittent exposures at varying doses for only a portion of their lifespan). The continued attention to/stress on such descriptions of specific uncertainties and assumptions involved in any given risk assessment and to their potential impact on the estimation of risks has been most helpful to those charged

with regulatory responsibilities for more rational and reasonable decisions about the proper fate of the agent/chemical under consideration.

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APPENDIX

BALANCE OF CANCER AND NON-CANCER ENDPOINTS

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The balance of basic research on cancer and non-cancer endpoints within any Federal organization is dependent upon a variety of factors such as Congressional mandates, the given organization's operational policy, public perceptions and concern, ongoing identification of potential data gaps and to some extent, the range of disciplines represented by the organization's scientific staff. All of these forces have an imp°act on scientific managers in their designing and implementing a basic research program to meet their organization's needs now and in the future.

Generally, in enacting legislative authority Congress exhorts EPA to evaluate a broad range of potential health effects associated with exposure to environmental chemicals and insults. Rarely does legislation require specific health endpoints to be addressed over other endpoints. It is, therefore, EPA's policy which directs attention to specific endpoints of concern for environmental exposures. Being a public institution, EPA is influenced by the perceptions and concerns of the public and industrial sectors regarding adverse health effects of chemicals. Consequently, EPA molds its administrative and regulatory policy to balance these concerns.

For many years the primary environmental health concern, as perceived by the public, was the possibility of chemically-induced cancer. The reasons for this concern include the prevalence and general irreversibility of the disease, its potential for debilitation and eventual lethality, and the knowledge that many chemicals to which there is prevalent human exposure can cause cancer in laboratory animals. As a result of these concerns and the body of data that has been generated over the years, the EPA's (as well as other regulatory agencies) regulatory policy has been largely driven by cancer as the health endpoint of greatest severity.

Over the past several decades, however, it has become increasingly apparent that there are many other adverse health endpoints which may be and have been induced by exposure to environmental agents. The methyl mercury-induced epidemic of birth defects in Japan, the incidents of delayed neuropathy in the Middle East, and the occurrence of male sterility in workers occupationally exposed to chemicals in the USA all have served to alert the public that the potential risk of exposure to environmental agents may require consideration of many health endpoints. The outcome of this realization has been a broadening of the areas of concern and a simultaneous commitment of resources to these additional research areas. Along with this commitment there has been an increasing tendency to consider these health endpoints during the formulation of regulatory policy.

Toxicologists in both the public and private sectors have also identified other organ systems and susceptible populations that are at potential risk from exposure to environmental agents. These realizations have lead to considerable support for research in other areas such as

immunotoxicology, heritable disease, and prepubertal and geriatric populations. Additionally, scientists and the public have become increasingly concerned about the toxic potential of lifetime exposure to relatively small amounts of a multitude of xenobiotics. Increasing resources have been allocated to gain a better understanding of the potential risks from these types of exposures as well as the most accurate ways to measure such risk.

Finally, there are two additional concerns of the general public and the scientific community that influence allocation of resources. The translation of laboratory data into human health risk assessments is an extremely difficult process. While a safe environment is desirable, the regulation and removal of chemicals based upon faulty assumptions may lead to undesirable results (including their substitution with potentially more hazardous compounds) entailing a reduction in the quality of life through increased expense, disease prevalence, and/or reductions of food and other material production. Therefore, as the preceding Chapter indicates, considerable research resources are now and in the future will be devoted to increasing the scientific basis and accuracy of risk estimates. The development of a better understanding of the basic mechanisms responsible for cancer and non-cancer responses is ultimately the most rational way in which to formulate regulatory policy. This obviously leads to a continuing requirement for long-term basic research.

The second factor which influences resources allocations concerns the need for simpler, less expensive, and less whole-animal-oriented forms of testing. The number of agents and complex mixtures of potential concern is far in excess of our ability to test for toxic potential by standard methodologies. Concerns raised by the public about the use of laboratory animals in such studies have been a further impetus to the development of alternative test methods.

The EPA research efforts in non-cancer endpoints have greatly increased over the last decade for the reasons listed above. Whether this increase has led to a proper balance between cancer and non-cancer endpoints is impossible to say, since there are so many competing factors that go into the composition of this balance. Certainly, a resource allocation to both cancer and non-cancer endpoints has enabled the Agency to utilize a broad base of health endpoints in the formulation of regulatory policy.

Long-term basic research into both cancer and non-cancer endpoints is recognized as being essential if the Agency is to formulate a broad regulatory policy in the most accurate manner possible. Rather than consider cancer and non-cancer effects separately, research in the future will evaluate multiple toxicological responses from the same exposure. Issues of adversity and severity of effects over time will be given greater attention. Efforts will be made to capture and analyze toxicological data in a more systematic fashion. These data will form the basis of improved structure-activity and pharmacokinetic modelling, test battery design, and dose-response evaluation of cancer as well as non-cancer endpoints.

Chapter 6

SUMMARY

This century has seen the emergence of abnormalities in early growth and development, chronic degenerative diseases, and cancer as the major causes of human morbidity and mortality in the industrialized nations of the world. Initially, these diseases were often viewed as being the result of heredity or the natural consequence of the aging process. More recently, however, there has been a growing recognition that they frequently have important environmental components or risk factors in their etiology.

Many of these environmental risk factors are either produced directly by humans or subject to their manipulation. They include chemical and physical agents in the air, water, food supply, drugs, consumer products, home and workplace. While detailed estimates of the impact of these risk factors are difficult to generate or verify, it has been variously postulated that a significant number of the two million individuals who die each year in the United States may have had their lives shortened to some degree by the effects of air pollution; that pollutants in our drinking water systems may play a role in the onset of cancer and heart disease, which are the two leading causes of death in this country; and that the collective effects of work-related disease and stress may now be approaching a level of impact more typically associated with workplace accidents. Therefore, federal health researchers and regulators are increasingly being challenged to identify these environmental risk factors and reduce or eliminate their deleterious effects.

Attention has been focused on some of the technical problems that can be encountered when one attempts to assess the true impact of environmental exposures on human health. Often, relevant epidemiologic and clinical information on the potential health hazard associated with exposure to a specific chemical or physical agent will not be available. Even when such data is available, however, it may not be sufficiently sensitive or specific to allow an investigator to choose among competing mathematical models that attempt to characterize the unknown, underlying relationship between exposure and dose. In some instances the available human data may not even permit one to determine if any health risk appears to be associated with low or moderate levels of exposure. As a result, laboratory animal data will often constitute the primary basis for both qualitative (i.e., hazard identification) and quantitative human risk estimation.

Because of the high (often maximally tolerated) doses typically employed in laboratory animal screening studies, quantitative risk estimation based on laboratory data involves two separate issues that must be addressed: low-dose extrapolation and species extrapolation. In some instances (e.g., when the agent of concern is a carcinogen or mutagen) mathematical modeling will be employed to generate low-dose risk estimates, and choice of a particular model may have a significant impact on the magnitude of the estimated risk. In other cases a threshold phenomenon may

be assumed and a safety factor approach used to determine acceptable exposure levels. This approach also clearly suffers from a number of methodological uncertainties and problems.

Ideally, the problem of species extrapolation should be addressed by taking into consideration all of the various species-specific factors that could contribute to interspecies differences in response to the exposure of interest. Instead, the conventional approach to this issue is to assume that humans and the test animal in question will have equivalent responses when comparisons are made on an appropriately-chosen dosage scale. Unfortunately, choice of the most appropriate-dosage scale cannot always be justified on biological grounds, and significant differences can result in the projected human risk estimate depending on the decision reached. As our reliance on quantitative risk estimation/assessment continues to increase, more and more importance is being attached to the need to characterize the uncertainties associated with these risk assessments and to reduce these uncertainties by improving the biological basis upon which the risk assessment process is based.

In addition to technical problems related to the risk assessment process itself, which have complicated and on occasion frustrated our efforts to evaluate adequately the potential risks posed by various environmental hazards, there are also a number of additional factors that have hampered our attempts to reduce the impact of environmentally-related disease. Among these are the lack of substantive toxicologic information on the majority of commercial chemicals that have been introduced into the human environment, the insufficient and sometimes inappropriate training of our nation's physicians with respect to environmental issues, and the inadequate surveillance of populations exposed or potentially exposed to environmental hazards.

Priority must be given to research in a number of important areas if we are to resolve these problems and advance our understanding of the role of environmental factors in human health and diseases. For example, more emphasis needs to be given to the development and refinement of procedures (particularly non-invasive procedures) for measuring low levels of human exposure to toxic environmental agents. Similarly, we need to develop a better understanding of the biological mechanisms that underlie environmentally-related health effects to improve both the quantitative assessment of human health risks and the primary/secondary prevention of environmentally-related diseases. A number of examples of long-term, basic research activities in these areas that either have or may utlimately have direct application to the types of environmental health problems that EPA and other regulatory agencies must address on an ongoing basis are cited in this document.

Comparison of patterns of proto-oncogene (i.e., cellular genes expressed during normal growth and development processes) activation in spontaneous and chemically-induced rodent tumors may provide insight into the mechanisms of tumor formation at the molecular level. In addition, some of the uncertainty involved in species-to-species extrapolation of carcinogenic risk estimates may eventually be removed by interspecies comparisons of oncogene activation and expression.

Recent advances in biochemistry and molecular biology have led to the development of highly sensitive techniques which may allow the quantification of the internal dose of carcinogens or in some cases the biologically effective dose in target tissues. This ability to express external exposure or administered dose levels on a more biologically-relevant basis should eventually lead to a clearer understanding of the relationships between exposure and disease or toxicologic effect for many health hazards in the human environment. Recognition of the potential usefulness of these biochemical markers has led to the emergence of a new field of epidemiology, known as molecular or biochemical epidemiology, that has as one of its major goals the adaptation of these laboratory procedures into epidemiologic field studies.

In the fields of neurotoxicology and immunotoxicology new methodologies promise to enable toxicologists to greatly improve our ability to assess both central nervous and immune system deficits. The utilization of novel techniques in molecular biology (e.g., monoclonal antibodies to specific critical chemical components of these systems) promises to allow improved evaluations of potential disfunctions.

In the area of human reproduction one of the most important questions involves the potential of environmental agents to affect pre-implantation loss. Researchers have recently identified an antibody to a subunit of the hormone human chorionic gonadotropin. This advance enables the identification of spontaneous abortions at an earlier stage and with greater accuracy than was previously possible and may significantly improve our monitoring capabilities.

In addition to identifying specific examples of long-term research activities that either are generating or may generate results directly applicable to the environmental health issues that EPA must address from a regulatory viewpoint, this document also attempts to describe the relationship between long-term and short-term (or immediate) "problem-solving" research and to put it in perspective. For example, it is noted that the general philosophy underlying basic health research is that understanding more about the biologic mechanisms by which environmental hazards such as toxic chemicals induce adverse effects will lead, ultimately, to earlier detection of such effects, more sensitive analytical methods for fully characterizing their potential impact on human health, and a better understanding of how to eliminate or, at least, reduce that impact. The distinguishing characteristic of this basic research is that it typically addresses "generic" scientific issues and is not focused on a specific problem or immediate concern. Furthermore, it must usually be supported for a period of several years before it produces results that may have a direct application to regulatory needs or problems.

The environmental health problem with the toxic metal lead is used to illustrate the necessity of and role for long-term research activities in the development of a sound, scientific foundation necessary for constructive actions dealing with public health problems. While lead toxicity resulting from "high" level exposures has long been recognized as an important public health concern, ongoing, long-term basic research has only recently given us the technical tools to detect some of the more subtle yet extremely important effects of low-level lead exposure.

Even when research is more focused on a specific issue or health concern, it may need to be sustained for a considerable length of time before any practical results or applications can be produced. Research is most often sequential with each new phase of the overall effort dependent on the results from the preceding phase(s). Alternatively, even if the research is focused and the required course of action clearly delineated before any effort is expended, a considerable investment of time and effort may be required before the project is completed. Certainly, this is the case with prospective cohort studies in epidemiology and to a lesser extent with laboratory-based, lifetime carcinogenicity screening experiments.

It seems clear, therefore, that while many of the health effects (or possible health effects) issues that confront EPA require an expeditious if not immediate response, the most appropriate and in many cases the only approach to formulating such responses will be to draw on the experience and insights gained from long-term research. This certainly has been the experience in dealing with most environmental crises to date. The only approach that will enable us to engage in such long-term research is to provide stable, consistent support for such a program. With continued support and in-house expertise EPA can directly address applied research issues with which it is particularly concerned and effectively apply both its own long-term findings and those of other public and private institutions to the solution of critical environmental health problems.