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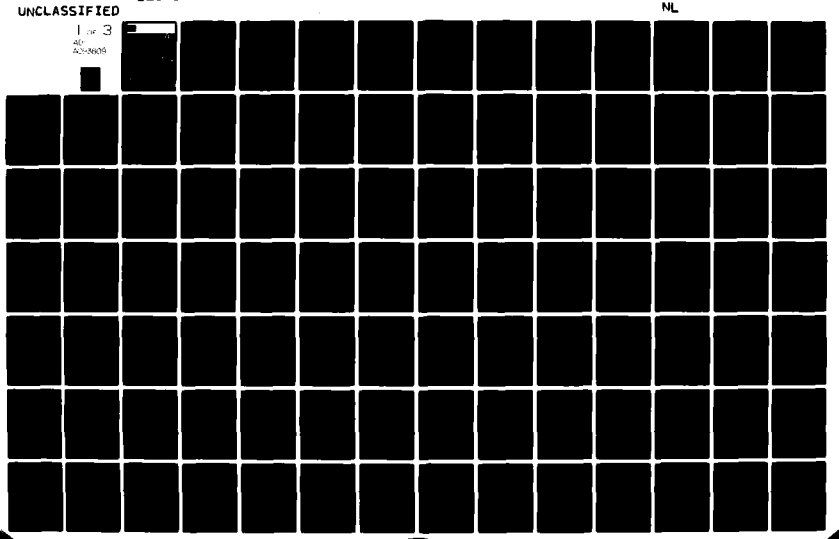

LEVEL II

Principles of Toxicological Interactions Associated with Multiple Chemical Exposures

Panel on Evaluation of Hazards Associated with
Maritime Personnel Exposed to Multiple Cargo Vapors

Board on Toxicology and
Environmental Health Hazards

and

Committee on Maritime Hazardous Materials

National Materials Advisory Board

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2. AD-A093509	3. Recipient's Accession No.
4. Title and Subtitle: Principles of Toxicological Interactions Associated with Multiple Chemical Exposures,			5. Report Date: Date published: Dec 1980
			6.
7. Author(s) Panel on Evaluation of Hazards Associated with Maritime Personnel Exposed to Multiple Cargo Vapors			8. Performing Organization Rept. No.
9. Performing Organization Name and Address: National Research Council, 2101 Constitution Ave., Washington, DC 20418			10. Project/Task/Work Unit No.
			11. Contract(C) or Grant(G) No. (C) DOT-CG-74248-A (G)
12. Sponsoring Organization Name and Address: U.S. Coast Guard, 2100 2nd Street, SW, Washington, DC 20593			13. Type of Report & Period Covered
			14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

407401

18. Availability Statement	19. Security Class (This Report): Unclassified	21. No. of Pages: 204
	20. Security Class (This Page)	22. Price

(See ANSI-Z39.18) See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

Principles of Toxicological Interactions Associated with Multiple Chemical Exposures

Panel on Evaluation of Hazards Associated
with Maritime Personnel Exposed
to Multiple Cargo Vapors

Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences

Committee on Maritime Hazardous Materials
National Materials Advisory Board
Commission on Sociotechnical Systems

National Research Council

National Academy Press
Washington, D.C. 1980

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard to appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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The work on which this project is based was performed pursuant to Contract No. DOT-CG-74248-A with the U.S. Coast Guard.

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CONTENTS

PREFACE

On a typical day, a U.S. Coast Guard inspector may enter confined spaces on as many as five vessels. Because each of these ships may have carried different cargoes, an inspector could be exposed to a mixture of vapors from five different chemicals. One Coast Guard officer, who served 4 years in New Orleans and 2 years in Baton Rouge where such multiple exposures are frequent, stated that inspectors working in the Eighth Coast Guard District may be exposed almost daily to benzene, various nitriles, methanol, caustic soda, carbon tetrachloride, vinyl chloride, and ammonia. The duration of these exposures can vary from a few minutes to 2 hours, the time spent inside a tank during their inspection.

Although permissible levels (threshold limit values) have been established for these vapors and must be attained before marine personnel can enter a confined space, the Coast Guard has become increasingly concerned about the interactions that might accrue from these exposures and possibly result in deleterious health effects.

In 1978, the Coast Guard asked the National Academy of Sciences for guidance, so that it could better meet its commitments for safety and environmental protection in the transportation of hazardous materials. In response, a Committee on Maritime Hazardous Materials was established within the Commission on Sociotechnical Systems.

One portion of this request called for an evaluation of possible synergism among certain chemicals during chronic exposures to low concentrations. To perform this task, a Panel on Evaluation of Hazards Associated with Maritime Personnel Exposed to Multiple Cargo Vapors was formed within the Assembly of Life Sciences. Its main task was to develop a model approach through the identification of principles that might be applied when predicting hazards to personnel exposed to more than one chemical, either simultaneously or in close sequences.

After an extensive search of the literature and various data banks, as well as many conversations with other scientists, the panel concluded that there was insufficient information available to respond to the charge of assessing the potential for interactions of specific chemicals of interest to the marine and shipping industry. The Coast Guard then agreed that a more general overview of possible sites and mechanisms of chemical interaction within the body might be a more satisfactory approach for the panel to follow.

The panel reviewed the potential toxicological interactions that might result from either simultaneous or sequential exposure to different chemicals, and it attempted to establish some basic principles that could be used in future studies of this problem. It paid special attention to the general mechanisms of toxicological interactions and also considered the sites and anticipated mechanisms of reaction at those sites. Moreover, it discussed the importance of the sequence of exposure and other conditions that might alter

toxicological interactions. Methods to analyze the problem quantitatively were also considered by the panel.

The U.S. Coast Guard has an interest in the safety of all personnel exposed to hazardous materials in or on transport vessels. Its attempts to evaluate many of these hazards have been hampered by the lack of data on personnel other than Coast Guard marine inspectors. These inspectors are required to enter cargo tanks, voids, and cofferdams as well as normally manned spaces on ships to ascertain the physical integrity of the hull, machinery, and equipment. Since they may spend some time each day inside cargo tanks, the inspectors are likely to be exposed to many chemicals and other stress factors in varying combinations and sequences. Exposure of maritime personnel to a single substance under carefully controlled conditions is generally unrealistic. Although exposure to multiple chemicals and stresses prevails in many industries and in many environments, the problems associated with toxicological interactions have often been ignored.

This report is a first attempt to assess the added hazards, if any, to marine inspectors who are occupationally exposed to multiple chemicals. The identification of data required to develop a set of principles governing toxicological interactions has broad applications that extend beyond the safety and health of Coast Guard and other marine personnel. Development of such principles should facilitate the prediction of potential hazards associated with the exposure of maritime personnel to multiple cargo vapors.

The panel's major conclusions are presented in Chapter 11 in the form of recommendations for further study. Implementation of these recommendations would result in a better understanding of the science of physiological responses and resultant toxicological interactions following exposure to different chemicals.

The panel appreciates the thoughtful suggestions and information provided by many individuals from universities, government, and industry who gave their time freely in the interest of this endeavor. The document itself is a result of individual contributions and coordinated efforts by members of the panel. Although each member was responsible for a specific section, each reviewed the work of others.

Special thanks are due John J. Gart, Biometry Branch, National Cancer Institute, National Institutes of Health, and Joseph S. Carra, Office of Statistical Analyses, Occupational Safety and Health Administration, who acted as consultants to the panel in a joint effort to prepare Chapter 9, "Mathematical Models for Chemical Interactions." Appendix B, which presents mathematical models and equations, was also written by Dr. Gart. The panel especially wishes to express its gratitude to the staff of the Coast Guard who arranged for the interesting inspection tour of the chemical tank ship, M/T Stolt Sheaf, captained by Per Kjeldstadli.

Finally, the panel thanks the staff of the National Research Council, including James Frazier and Gordon Newell, project staff officers; Frances M. Peter, who served as editor of the manuscript;

Virginia White, Edna Paulson, and Barbara Jaffe for verification and procurement of the many references; and Beulah Bresler for her secretarial assistance.

The panel is hopeful that the Coast Guard will find this report useful in developing procedures for the protection of maritime personnel. It also hopes that the report may motivate scientists to undertake investigations that will develop the knowledge necessary to make scientific predictions of the potential for toxicological interactions among chemicals from multiple sources.

SHELDON D. MURPHY
Chairman
Panel on Evaluation of Hazards Associated with
Maritime Personnel Exposed to Multiple Cargo Vapors

CHAPTER 1

PHILOSOPHY AND OVERVIEW

EVOLUTION OF THE PANEL'S APPROACH

Two important facts became clear early in the panel's deliberations. First, there is little information (other than anecdotal) or data concerning the health of marine inspectors either with or without a relationship to chemical exposures. Second, the air sampling and analyses that have been performed on environments in the tanks that marine inspectors enter are limited in both scope and precision.

These concerns contributed to several important developments directly or indirectly affecting the panel's approach to the charge. In one important development, the Coast Guard initiated a more in-depth analysis of the health status and records of its marine inspectors and associated personnel. This was arranged by one of the panel members with personnel of the Coast Guard medical service and the National Cancer Institute.

Because of the relative lack of data concerning both effects on human health and chemical analyses, the panel began its study by considering a theoretical approach. It believed that the development of a set of general principles or guidelines for acquiring and evaluating data concerning toxicological interactions would greatly facilitate evaluation of specific circumstances of multiple exposures sustained by Coast Guard marine inspectors. Of perhaps greater importance, these guidelines would also probably be of value in many other situations in which exposure to combinations of chemicals complicates the assessment of associated health hazards.

How does the risk to health from exposure to a combination of chemicals compare to the estimated risk from exposure to each chemical by itself? This question may be approached in several ways:

- The literature may be searched for laboratory, clinical, or epidemiological studies that deal specifically with exposures to the combinations of chemicals in question.
- Laboratory and/or epidemiological studies may be initiated to test specifically for interactive effects of certain combinations of chemicals when and if concern for a specific combined exposure arises.
- Knowledge of the toxicokinetic and toxicodynamic characteristics of individual chemicals may be used to judge the potential for altered health risk arising from exposure to specific combinations of chemicals.

All three of these approaches have several limitations when applied to potential exposures to numerous and diverse chemicals. The number of possibly hazardous combinations of exposures multiplies as the list of individual chemicals with potential health effects grows. Furthermore, the numerous types of exposures, i.e., simultaneous, sequential (both close and widely separated in time), repeated, or single exposures to multiple chemicals, greatly complicate the design of studies for assessment of interactions.

Because of these problems, the panel believes that the first approach would probably not provide information of use to the Coast Guard in most cases. However, it did undertake a preliminary literature search for information in accordance with this approach.

The second approach is direct. Obviously, if the numerous combinations of chemicals and the nature of exposure times and concentrations could be reasonably defined and designed into a test program, such a program would remove many areas of doubt that are inherent in the assumption that must be made if the third approach is used. Clearly, the second approach is not suited for an ad-hoc panel. Rather, it should be undertaken by a multidisciplinary testing and research laboratory with essentially unlimited resources.

The third option appeared to the panel to be a useful first approach to address the ultimate charge of assessing altered health risks from multiple chemical exposures. Before the judgments in the third approach could be considered, however, it was necessary to identify the toxicokinetic and toxicodynamic factors that might contribute to altered organismic responses due to multiple chemical exposures as contrasted with single chemical exposures.

This report deals with the basic principles underlying the mechanisms of toxicological interactions in terms of the toxicokinetic and toxicodynamic factors that are involved. The panel recognizes that this is a somewhat idealized approach to the problem and one in which the absence of appropriate data makes conclusive, quantitative, or even qualitative assessments difficult. However, it determined that by studying the sites and mechanisms at which and through which toxicological interactions can occur, the following action could be taken:

(1) A systematic approach to a search of the literature on individual chemicals that may be involved in interactions could be

developed. However, by limiting the search for data specifically to interactions, pertinent data on the interactive potential of compounds may be overlooked.

(2) A set of data points could be identified and pursued by systematic experimentation if a review of the literature indicates the need. This information would be useful in the design of toxicological research on individual chemicals as well as on multiple-chemical exposures.

(3) The essential features of a model can be identified and tested. This information would form the basis for mathematical analyses or predictive modeling. Consequently, the panel concluded that its charge for Phase I of this project would best be met by an in-depth consideration of the sites and mechanisms of toxicological interactions. The results of its deliberations would probably not be limited to the specific exposures encountered by maritime inspectors but should have much broader application.

A prerequisite for the development of any approach, as well as to the understanding of that approach, is a definition of what is meant by toxicological interactions. After considerable discussion, the panel agreed that the term "toxicological interaction" would be defined, and used throughout this report, as follows:

> "A toxicological interaction is a circumstance in which exposure to two or more chemicals results in a qualitatively or quantitatively altered biological response relative to that predicted from the actions of a single chemical. The multiple-chemical exposures may be simultaneous or sequential

in time and the altered response may be greater or smaller in magnitude."

This chapter summarizes the panel's considerations, which are described in detail in subsequent chapters.

GENERAL MECHANISMS OF TOXICOLOGICAL INTERACTIONS

The injury produced by a chemical in a living organism is proportional to the quantity of the biologically active form of the chemical that is available for reaction with critical responsive sites (targets). Thus, toxicological interactions can be perceived, in general, as taking two forms: (1) the quantity of an active form of one or more chemicals available for target-site interaction is altered by the presence (or past presence) of one or more other chemicals, or (2) the reactivity of the target macromolecule with the active form(s) of one or more chemicals is altered by the presence (or past presence) of one or more other chemicals that may or may not be capable of eliciting a response. The first form involves primarily sites of inactivation or loss (i.e., sites of detoxification, excretion, storage, or neutralization) or sites of activation of a chemical. The second involves interaction at sites of action. In the latter case, either affinity for or intrinsic activity at the site of action may be altered. Figure 1-1 illustrates the many sites at which toxicological interactions could occur. Although the complexity is apparent, the figure is not all-inclusive.

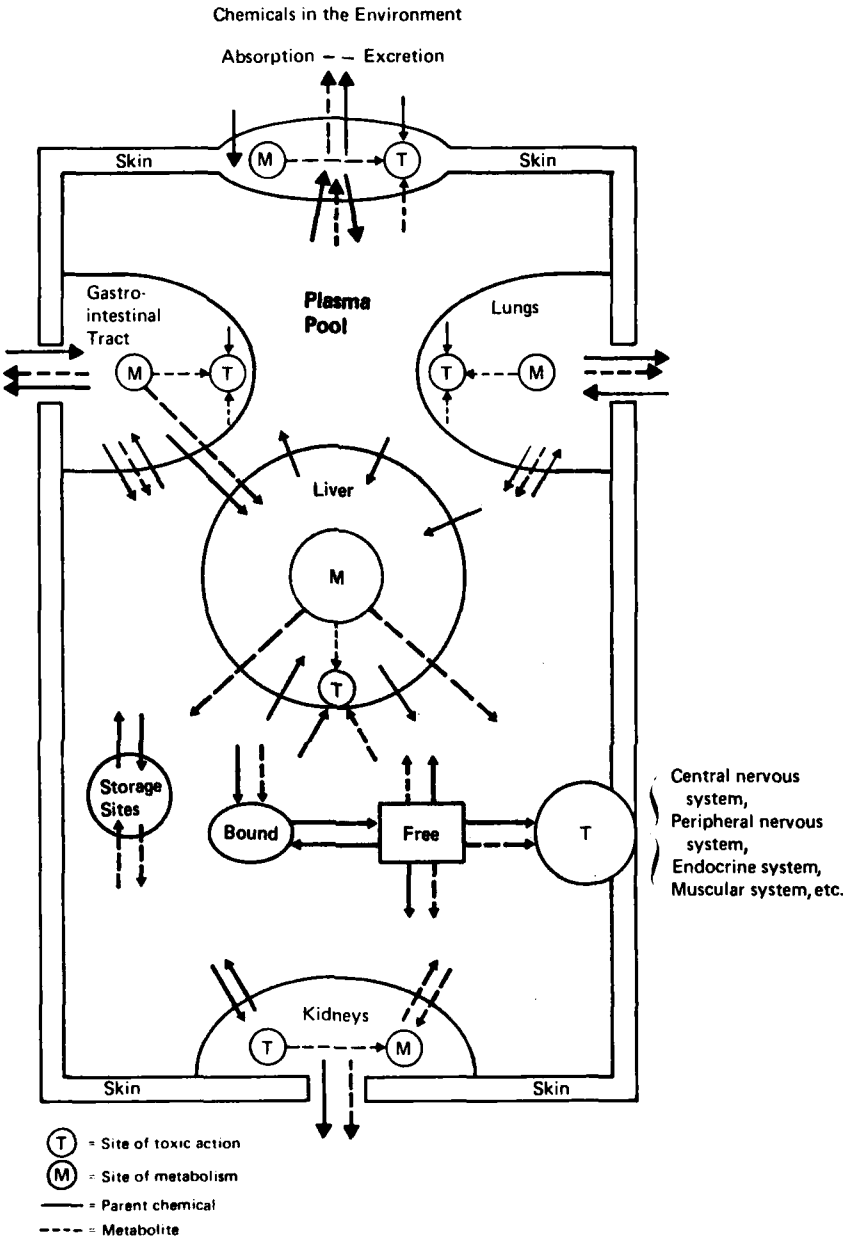

FIGURE 1-1. Sites for potential chemical interactions that may result in toxicological interactions. Each arrow represents a pathway or site for which chemicals may compete and thereby alter each other's biological disposition or intensity of action. Both parent compounds (solid arrows) and metabolites (dashed arrows) may be involved in these interactions. The major processes in which competition for saturable systems may result in toxicological interactions include transmembrane transport systems, storage or binding, biotransformation systems, and target site receptor systems. These can be located in the organs of absorption and excretion or in vital internal organs that do not have a direct interface with the environment.

At least three general mechanisms of reactions among chemicals are involved in toxicological interactions.

Chemical-Chemical Reactions

As a result of a combined exposure, one chemical may react with another in such a way that the potentially injurious chemical(s) never reach target sites in an active form. Numerous examples that might be cited include: neutralization reactions among acids and bases, chelation reactions such as those with heavy metals, and direct reactions between organophosphates and aldoximes.

Many of these reactions are generally considered to be antidotal or beneficial in nature. Thus, reduced injury might be expected if workers were exposed to combinations of such chemicals. However, enhanced injury or an altered form of injury might also arise from such chemical-chemical reactions. One example is the formation of nitrosamines from secondary amines and nitrites in the stomach. Because many nitrosamines are carcinogenic, this chemical-chemical interaction could be classified as one yielding enhanced risk of injury. Reaction between two exogenous chemicals within or outside of the body is a potentially important mechanism for a toxicological interaction, but it does not readily lend itself to analysis by consideration of the biological determinants of chemical injury.

Chemical Competition at Macromolecules

This general mechanism of toxicological interaction is probably the most frequently encountered and the most thoroughly studied. It involves the relative affinities of exogenous chemicals for a limited

number of reaction sites on cellular macromolecules, which may be the molecular sites of absorption, activation, detoxification, injurious action, or excretion. Competition for binding or reaction at these various sites may result in either enhanced or reduced toxicity. Knowledge of the nature of the individual chemicals and the kinetics of their reactions at these sites makes it possible to develop logical models to predict the toxicological consequences of such competition for reactions. This type of mechanism for toxicological interactions generally requires that the interacting chemicals or their reactive derivatives be present in the organism at the same time. However, the form that is present may be only a small residue of the original molecule that is bound to one or more reactive sites.

Altered Cellular Responsiveness or Reactivity

A third general mechanism for toxicological interactions is one in which a cell or tissue is altered by one chemical in such a way that the cell's or tissue's response to a second chemical is altered, even if the first chemical is no longer present. This type of toxicological interaction is more likely to result when exposures are separated in time. Promotion by one compound of chemical carcinogenesis that is initiated by another chemical would be included in this classification. Induction of biotransformation enzymes for one chemical by exposure to another could be another example, and alterations by one chemical of the repair of a cellular lesion induced by another represents still a third subclass of this general mechanism.

TEMPORAL RELATIONSHIPS

When an organism is subjected to multiple-chemical exposures, the nature and degree of toxicological interaction will be dependent in part on the temporal relationships between or among exposures.

Although classical considerations of toxicological interactions have dealt with simultaneous exposures to combinations of two or more chemicals, an equally likely situation would be that exposures to more than one chemical would be sequential. The order in which these exposures occur and the length of time between them determine the likelihood of a toxicological interaction.

When exposures occur simultaneously or very close in time, the occurrence of toxicological interactions very likely depends upon competition for sites of absorption, biotransformation, reaction with target tissue, and excretion. The concentration within the organism of each potentially interacting compound in a combination and the relative binding affinities and/or intrinsic activities of the compounds are the most important factors in these interactions.

When exposure to different chemicals are separate in time, the mechanism of the interaction, the biological half-life of each chemical or its metabolites, the duration of binding to tissue macromolecules, and the rate at which injury is repaired may all assume a greater importance than their relative binding affinities and intrinsic activities. For example, if chemical A potentiates chemical B by blocking its rapid detoxification, it is likely that this toxicological interaction would not occur if exposure to A followed exposure to B. The probability that this toxicological interaction

would occur with the reverse order would depend on the time between exposures and whether or not the inhibition of B's metabolism by A is competitive or noncompetitive. Another classic example of the importance of the temporal order of exposure is two-stage carcinogenesis, when a toxicological interaction is based upon complementary cellular effects and is sequence-dependent. A third example of sequence dependency would be a toxicological interaction that resulted from the interference of one chemical with the repair of injury produced by another.

In many other situations, toxicological interactions develop only when exposures occur in a certain order. When attempting to predict interactions between combinations of chemicals solely on the basis of kinetic data on individual chemicals, one should keep in mind that the nature, mechanism, and duration of the cellular injury are also critical factors.

The frequency of exposure can also determine whether or not toxicological interactions will occur. Obviously, the more often there is exposure to a chemical, the greater the statistical probability that it will occur in the presence of or close in time to the exposure to another, possibly interacting chemical. But beyond mere statistical considerations are the influences of frequency of exposure on the accumulation of a body burden of the chemical, the accumulation of cellular injury with or without accumulated body burden, and the opportunity for reversal of action or repair of injury. All of these factors influence the occurrence of toxicological interactions with different mechanisms.

The applications of these principles to several of the specific sites at which toxicological interactions may occur are discussed in detail in subsequent chapters and are summarized briefly below.

SITES AND MECHANISMS OF TOXICOLOGICAL INTERACTIONS

Absorption

A major factor determining the toxicity of chemicals is the route or routes by which such agents gain entry into the body. The inhalation and dermal routes of absorption are most significant in the work environment of marine personnel. Oral ingestion plays only a minor role, although inhaled particles may be swallowed after being transported out of the lung by mucociliary action. Absorptive processes involve the penetration of a chemical through biological membranes by either diffusion, transport, or pinocytotic mechanisms. Gases are absorbed readily throughout the entire respiratory tract. Respirable particles, depending upon size, can penetrate the alveolar zone where they may be solubilized and absorbed. Larger particles will be deposited in the upper respiratory tract or nasal passages where solution and absorption can occur. Many lipid-soluble chemicals will penetrate readily through the skin. Chemicals may be absorbed from the digestive tract in the mouth, stomach, small intestine, and colon. Simple diffusion is the most common absorptive process although active transport mechanisms for some chemicals do exist.

On either the pulmonary or dermal surface, toxicological interactions may result from a physical irritation of the membranes produced by one chemical with a subsequent change in permeability characteristics and an enhancement of absorption of other chemicals. In the

respiratory tract, chemicals may exert an inhibitory effect on ciliary movement or mucus production, thereby reducing the removal of particulate matter from the airways. Moreover, delivery of toxic inhalants to the lungs may be modified by changes in pulmonary ventilation and perfusion as, for example, during physical exertion. Hypersensitive, allergic, photosensitive, and irritative skin reactions may dramatically alter dermal absorption as will humidity and temperature. Factors affecting smooth muscle motility, blood flow, secretions, etc., will also influence absorption from the gastrointestinal tract.

Elimination

Absorbed chemicals may be eliminated from the body at several sites. The major routes are exhalation via the lungs, fecal excretion via the liver and gastrointestinal tract, and excretion by the kidneys. The skin and saliva serve as minor routes of elimination.

Any chemical that disrupts the structural or functional integrity of the organs involved in elimination may interfere dramatically with the efficient excretion of other toxic agents. Such disruption may be caused by chemical interference with ciliary transport and macrophage activity in the lung, with metabolism in the liver, and with competition for secretive and reabsorbtive mechanisms in the kidney. Fecal elimination of toxic agents is dependent upon either nonabsorption from the intestinal tract, secretion of the agent or metabolites in the bile, or the lack of reabsorption of these compounds from the intestinal tract. Interactions could result from the alteration by one chemical of the disposition of another at any of these sites.

Dermal excretion is restricted primarily to water-soluble ions and chemicals with low molecular weights; however, few studies have been devoted to this route of excretion.

Biotransformation

In recent years it has been well documented experimentally that exposure to a chemical can markedly modify the metabolism of another by a variety of enzymatic reactions, including oxidation, reduction, hydrolysis, or group transfer.

Since the cytochrome P-450-mediated microsomal oxidases play a dominant role in the metabolism of most lipophilic foreign compounds, events that modify the activity of this system in the liver, lung, and other organs are of particular toxicological importance. Enhanced microsomal oxidation by enzyme induction follows exposure to large doses of most lipophilic chemical agents, particularly those with a relatively long biological half-life. Not only can this lead to an increase in oxidative metabolism but it also may result in qualitative changes in the products formed through preferential induction of catalytically different forms of cytochrome P-450.

Major factors affecting the onset and degree of induction relate to the chemical nature, amount, and duration of the inducer that is present in the tissue under consideration. Thus, despite a wealth of information on induction that occurs in response to high dosage levels of various inducers, there is a paucity of data pertaining to the extent of induction attained as a result of repeated low-level chronic exposures to which maritime personnel might be subjected.

Interactions resulting from the inhibition of microsomal oxidation can occur through several mechanisms. Brief inhibitory effects can result when two oxidizable substrates are presented simultaneously to the microsomal system and one competitively inhibits the metabolism of the other (alternative substrate mechanism). This may be of importance whenever one of the chemicals in a combination has a high affinity for the microsomes and a relatively low rate of metabolic turnover. Noncompetitive inhibitory effects of a potentially more serious nature can be expected following exposure to combinations involving chemicals that are metabolized via reactive inhibitory intermediates or that release active moieties (e.g., free radicals, atomic sulfur) during the course of their metabolism.

Chemical interactions may potentiate or antagonize the toxicity of one or more components. Toxicological interactions result when one chemical, A, inhibits or stimulates enzymes that are responsible for the metabolism of another, B. When toxicant B is inactivated (detoxified) by a given enzyme system, compound A may interact to inhibit or stimulate enzyme activity, thus leading either to a potentiation or antagonism of toxicity, respectively. If, on the other hand, B is metabolically activated, the opposite result will be observed. Recent evidence indicates that an increase in the formation of toxic metabolites may be a particularly important interaction after repeated low-level exposures to multiple chemicals.

Storage

Chemicals may be stored for varying lengths of time, usually in inactive form, in various sites of the body such as parenchymal organs, bone, connective tissue, and tissue lipids. This storage decreases the acute toxicity but prolongs the potential toxicological action of the chemical as it is gradually released to the system.

When storage involves binding, the governing factors are the same as those that determine the disposition of chemicals to active tissue receptors. These factors include the specific affinity of the compound and the strength and reversibility of the bonds that form between the chemical and the storage binding site. In many instances, such as when a chemical is lipid-soluble, accumulation in tissues is governed by various nonspecific factors. Affinity for active transport processes may also determine the access of chemicals to tissue storage areas.

Toxicological interactions may occur between two or more chemicals that bind to the same storage sites when one chemical with a high affinity for those sites prevents binding of another with less affinity, thereby increasing its effective concentration in the plasma. Toxicological interactions between two or more chemicals may also result from competition for uptake into storage sites because of differences in affinity for active transport processes.

In general, toxicological interactions resulting from saturation or displacement of chemicals from tissue storage reservoirs could be expected to occur because of frequent or prolonged exposure to high concentrations of mixtures of chemicals that are selectively accumulated at those sites.

Sites of Action

Chemicals produce effects on living organisms by reacting with specific tissue receptors. The ability of some chemicals to alter the manner in which others react with tissue receptors forms one basis for defining the principles of toxicological interactions between two or more chemicals within cells.

In general, toxicological interactions are governed by the law of mass action. They occur when the binding affinity of a chemical for a specific tissue receptor molecule or the strength or reversibility of that bond is different from that of another chemical that normally binds there. The interaction is an antagonism or addition resulting from the ability of one chemical to displace another from its site of action.

A principal biological manifestation of toxicological interactions involving enzymes as tissue receptor sites is the alteration of essential metabolic processes that are regulated by such enzymes. Such alterations may occur when two or more chemicals compete for the same enzyme binding site, thereby altering the intensity of the effect produced by one chemical acting alone. These interactions may also result when two chemicals act noncompetitively to form reactive complexes with two different but functionally related enzymes or with different sites on the same enzyme, thereby producing a combination of the effects of those chemicals.

The formation of covalent bonds between chemicals and tissue elements is an important mechanism of chemical interaction. Such bonds are essentially irreversible and may result in toxicological

consequences of prolonged duration or intensity. Covalent binding of chemicals with enzymes effectively removes the enzyme as a biological receptor site for other chemicals and may therefore enhance or diminish the toxic effects of other chemicals that are normally activated or detoxified by those enzymatic processes.

Toxicological interactions between chemicals involving covalent binding may also occur through the formation of chelate complexes, a process that is especially important in describing interactions involving metals. In such cases, toxicological interactions may be manifested when substitution of nonphysiological metals in chelate complexes that perform essential biological functions diminishes or abolishes those functions. Alternatively, the formation of chelate complexes between exogenously administered chemicals such as ethylenediaminetetraacetic acid (EDTA) and potentially toxic metals may prevent or reverse toxic reactions within tissue components.

Importance of Sequence of Tissue Injury and Repair

Acute interactions between chemicals are usually recognized with comparative ease. They may enhance or prevent acute tissue lesions such as cell death, alter physiological parameters, or modify behavior patterns. Acute interactions are most likely to occur when there is little difference in time between exposure(s) to the offending agents. However, there may also be interactions in which only repeated exposures will produce lesions or when exposure to two agents is separated in time.

Two-stage carcinogenesis is one example. Administration of a sub-carcinogenic amount of a carcinogen will result in tumor formation in mouse skin provided that the skin is subsequently treated with a promoting agent. Tumors result even if the two treatments are separated by weeks. On the other hand, no tumors develop if exposure to a promoting agent precedes exposure to the carcinogen. Some evidence suggests that the principle of two-stage carcinogenesis also applies to epithelial tissue other than the mouse skin, e.g., liver, stomach, colon, lung, and urinary bladder.

Certain forms of toxic lung fibrosis may also be caused by an interaction between two or more agents rather than by one agent alone. The alveolar epithelium may become damaged by a multitude of agents, which are either inhaled or carried into the lung by the bloodstream. Ordinarily, the lesion is repaired. However, fibrosis develops if a second agent affects proliferation and renewal of the epithelial layer during a critical time of epithelial recovery. Again, the timing of the interaction appears to be important since no fibrosis develops if exposure to the second agent occurs only after epithelial recovery is complete.

Both examples document that interactions between chemicals may occur not only when two agents compete for the same target(s) but also when one chemical elicits changes in behavior of a cell or a tissue and a second agent adversely affects the biological response controlled by that behavior.

Conditions Altering Toxicological Interactions

Many endogenous and exogenous variables can alter the biological response of an organism to chemical exposure. This is a serious concern when more than one chemical is presented to an organism simultaneously. Environmental factors, such as cold, heat, noise, vibration, and relative lack of oxygen, water, or food, may increase or decrease biological effects, depending upon the environmental stress and the specific chemical involved. Since many dietary factors are necessary for optimal detoxification processes, altered nutrition can modify the effect of exposure to more than one chemical. Starvation or malnutrition, protein deficiency, deficiency or overabundance of certain vitamins, and the absence of certain essential minerals will significantly alter the biological effect of multiple chemicals. Preexisting disease states in organs that are essential for dealing with foreign chemicals will impair the detoxifying systems and make the host more susceptible to toxic effects. By overloading already weakened organ functions, multiple chemical exposure can effect a pronounced toxic change. It is likely that other conditions also affect chemical interactions, and the potential for such alterations must be realistically identified when evaluating hazards to human health resulting from exposure to two or more foreign compounds.

MATHEMATICAL MODELS

A chapter describing the theory of chemical interactions in the form of mathematical models is included as a part of this document. It attempts to describe the various interactive relationships between chemicals in a manner that is understandable by the

nonstatistician. Discussion of the "one-hit" model, in which a single compound produces a disease, proceeds through independent action and interaction of two compounds and also consideration of parallelism and "dosewise" additivity responses. Appendix B, a paper by Dr. John Gart of the National Cancer Institute, contains a more technical discussion of this subject.

LITERATURE SEARCH

Preliminary attempts to locate articles dealing with toxicological interactions among the exemplary compounds identified by the Coast Guard involved searches in MEDLINE and TOXLINE under such subject headings as drug synergism, drug antagonism, and interactions. Although volumes have been written about potential drug-drug interactions, there are few publications on interactions of commonly or widely used drugs with chemicals of concern to those in maritime occupations. Further search of the literature and communications with other scientists led only to a few additional reports. Finally, all references in an additional computer data base were searched for interactive data for 11 of the compounds on the Coast Guard's list. This literature search revealed that many reports concerning toxicological interactions cannot be retrieved under the key words related to interactions.

CHAPTER 2

ABSORPTION AS A SITE OF INTERACTION

The absorption of foreign chemicals involves transfer of these molecules across various barrier membranes of the body, such as the gastrointestinal epithelium, pulmonary epithelium, renal tubular epithelium, hepatic parenchyma, the skin, and placental membranes. Transfer in one direction produces absorption; in the opposite direction, it results in excretion.

The substances are transferred across biological membranes by four mechanisms: simple diffusion through the membrane and down a concentration gradient; filtration through aqueous pores in the membrane; pinocytosis through microscopic invaginations of the cell wall that engulfs droplets of extracellular fluid; and active transport by which compounds are transported across membranes against a concentration gradient by processes involving carriers and requiring energy. Simple diffusion is generally considered to be the principal mechanism by which foreign compounds are transferred across cell membranes. Active transport occurs with certain compounds, but the roles of filtration and pinocytosis are largely unknown. In the transfer of foreign compounds by simple diffusion, only lipid-soluble un-ionized molecules readily pass through the membranes. Nonelectrolytes are transferred according to their lipid-solubility, and electrolytes move according to their degree of ionization and lipid-solubility of the un-ionized molecules. The degree of ionization of an organic electrolyte is a function of the dissociation constant of the compound and the pH of the medium.

When humans are exposed to a chemical, a biological or toxicological effect can occur only after the substance has been absorbed. As a rule, only that part of a substance that is present in a dissolved, dispersed molecular form can be absorbed. The uptake by an organism of an environmental or applied substance is highly dependent upon the degree and the rate at which the absorbable form of the substance contacts the surfaces of the organism that are capable of absorption. When a person is exposed to toxic substances in industrial or other environments, the form of the substance markedly influences absorption and, therefore, toxicity. The properties of the substance, such as fineness of distribution, lipid or water solubility, the size of the droplets in a fog, or the size of dust particles in an aerosol, will all determine the depth of penetration into the lungs. Absorption of gases and vapors from the lung is also a function of their solubility in blood, lung ventilation, and pulmonary circulation rates. The presence of a certain concentration of a toxin in the air does not always provide the same degree of risk.

In occupational poisoning, absorption occurs primarily through the respiratory tract, although the skin may also be important. Both organ systems are in direct and continuous contact with the environment. Absorption of gases through the respiratory system is directly dependent on the concentration of gases in the inhaled air.

ABSORPTION FROM THE LUNG

Despite extensive toxicological studies of volatile or volatilizable halogenated aliphatic hydrocarbons and aromatic hydrocarbons,

the routes of administration most frequently used have been either oral or intraperitoneal, the agent having been dissolved in a suitable vehicle beforehand. Only in the past 5 to 7 years have scientists become concerned about the respiratory tract as a significant route of absorption.

Pulmonary Dynamics

There is surprisingly little quantitative information concerning the absorption of substances from the lung although most texts infer that it is a rapid process (Fingl and Woodbury, 1975). The lung possesses all of the attributes that are essential for an excellent absorptive organ. The alveoli, of which there are from 300 to 400 million in one adult human, are lined with a single layer of flattened epithelial cells that form an extremely thin barrier (0.5 -1.0 μm thick) between the alveolar air and an interstitium richly supplied with capillaries (Levine, 1973). The total surface area of the lung is approximately 70 m^2, and the surface area of the pulmonary capillaries is estimated to be 90 m^2. The lung exchanges air at a rate of approximately 12-15 liters/min. It receives all of the blood supply from the right side of the heart in a volume equivalent to that received by the entire body from the left ventricle during the same time (Levine, 1973). The organ is functionally structured so that it rapidly removes the material present in the alveolar air.

The respiratory rate is much higher (20-30 liters/min) for individuals who work at an active rather than a sedentary job. Physical activity also increases the blood flow through the lungs, which may

increase the rate of absorption of a volatile chemical with low water solubility.

Absorption Characteristics

The rate of absorption in the lung is dependent upon particle size and solubility of the chemical on the alveolar surface. As a rule, gases (and volatile liquids) are small molecules that readily cross the alveolar epithelium. Inhaled aerosols (suspended liquid droplets or solid particles) will deposit along various segments of the bronchial tree, primarily at points where the airstream changes course and velocity, e.g., in the bronchioles. The point of impaction (or deposition) depends on particle size; particles with a diameter >10 μm are deposited in the nasal passage, those with a 2 μm diameter will reach the alveolar sacs. Particles of pollen, dusts, fumes, and volatilized solvents are smaller than 10 μm. Particles of cigarette smoke are less than 1.0 μm. As a general rule of thumb, the smaller the particle, the deeper into the respiratory tract it will penetrate (Levine, 1973).

Most gases readily cross the alveolar epithelium to enter the bloodstream. As a result of this and the fact that the alveolar epithelium has a large surface, gases are absorbed very rapidly. On the other hand, this also means that gases are excreted rapidly through the lungs if they are not bound to tissue components. For chemical substances in the form of mists (droplets suspended in air), dust (particles suspended in air), and aerosols, the particle size is the decisive factor for determining the degree of penetration of the inhaled substance into the lung.

Retention does not necessarily mean absorption. For instance, in pneumoconiosis the substance is retained locally. Retention can be due to deposition of inhaled particles in different levels of the respiratory system. Large particles deposit in the nose, the trachea, and the bronchi. From there they are transported toward the throat by ciliary epithelia and are usually swallowed. Absorption may then take place from the gastrointestinal tract and not from the respiratory system. Smaller particles penetrate deeper into the respiratory system and deposit in the smaller bronchi and on the alveolar epithelium. Particles of less than 10 μm in diameter are deposited on the surfaces of the respiratory tract. The percentage of deposition decreases as particle size decreases below 1 μm, being minimal at approximately 0.2 μm. But often, the absolute numbers of these fine particles are much greater than for larger particles. Hence, the mass deposited may actually be greater than that for fewer large particles. In addition to particle size, the specific gravity, the charge on the particles, and the hygroscopicity of the particles help determine the tendency of the particles to aggregate into larger particles. The frequency and depth of an individual's respiration, which often depend on age, occupation, working conditions, and environmental temperature and humidity, play a role in determining the amount of substance retained in the lungs.

The mechanisms of absorption from the lung surface are poorly understood. Studies with a number of lipid-insoluble drugs (e.g., urea, mannitol, sucrose, insulin, ouabain, and dextran) in aqueous solution have demonstrated that absorption is mediated by a nonsaturable process of diffusion, which is extremely rapid. The absorption rates are related

to the molecular size: the higher the molecular weight, the slower the rate of absorption (Schanker, 1978). Analysis of absorption rates of known compounds suggests that the pulmonary epithelium contains at least three different types of pores, which allow molecules of a certain size to pass while excluding larger molecules (Enna and Schanker, 1972). The absorption rates of chemicals with low lipid-solubility are similar to those of chemicals absorbed through membrane pores. Highly lipid-soluble chemicals of low molecular weight diffuse very rapidly through the lipoid barrier. Some of them (e.g., salicylic acid, procaine, and digoxin) have absorption half-times of 1 min or less (Burton and Schanker, 1974; Enna and Schanker, 1972; Lanman et al., 1973). There also appears to be a specific carrier-type transport process that is saturable and appears to be shared competitively by a number of organic anions (e.g., phenol red and disodium chromoglycate) (Schanker, 1978).

Anesthetic agents are probably the closest chemical analogs to which marine personnel might be exposed. Therefore, an examination of pulmonary absorption of these agents should be useful. A constant rate of absorption of anesthetics across the alveolar membrane is established by maintaining a constant concentration (particle pressure) of agent in the inhaled air (Goldstein et al., 1974). Thus, inhalation by workers in an "empty" contaminated tank would result in a rapid equilibrium between blood and air concentrations. At a constant rate of absorption, the rate at which a plateau concentration of a material in the blood is approached is dependent upon the rate of elimination and the solubility of the agent. One

does not take into account the quantity of the agent that must be transferred and distributed before equilibrium is attained (Goldstein et al., 1974).

Astrand (1975) and Astrand and Gamberale (1978) have demonstrated that the evaluation of the TLV for water-immiscible solvents, such as methylene chloride, trichloroethylene, styrene, toluene, and white spirit, should be based on actual measurements of uptake into blood rather than on ambient air concentrations. They showed that the percentage of uptake by volunteers (determined by blood levels) varied with the quotient between the alveolar air concentration and the inspiratory air concentration. A linear relationship with a negative slope demonstrated that the percentage of uptake decreased when the alveolar air concentration approached that in the inspiratory air and that the uptake increased when the alveolar concentration was lower than that measured in the inspiratory air. For all solvents studied, the concentrations in alveolar air rose sharply during the first 5-10 min of each exposure period, rising only slightly during the remainder of the test. The concentration of the solvent in arterial blood was found to be a very close linear function of the alveolar air concentration. Therefore, the acute toxicity to such solvents should be related to uptake rather than to ambient air concentration, and a factor estimating pulmonary ventilation should be taken into account.

ABSORPTION FROM THE SKIN

Although exposure to chemical substances via the skin has been studied very little, it is probably a more significant occurrence than is usually suspected. This is especially true for maritime workers

exposed to common cargo chemicals. The skin is an important route by which foreign substances are absorbed into the body. The intact skin with its lipid sebaceous layer is not very permeable to hydrophilic substances. In contrast, lipophilic substances generally penetrate the skin readily by diffusion through the sebaceous layer. The degree to which chemicals are absorbed through the skin is influenced appreciably by the base in which they are applied, e.g., an ointment base (an emulsion of water in oil) or a cream base (an emulsion of oil in water). Local conditions, such as humidity of the skin, temperature, and contact between clothing and skin, also influence absorption, e.g., the higher the temperature of the environment, the greater the absorption through the skin. A damaged skin can be penetrated by hydrophilic as well as by lipophilic substances. Therefore, washing the hands with abrasives, which damage the skin, or with organic solvents such as gasoline and turpentine, which remove the sebaceous layer, increases the chance for penetration by toxic substances. Chemical substances that act as allergens can also penetrate the damaged skin, thereby increasing the risk of developing allergies. In contrast to highly hydrophilic substances, highly lipophilic substances are normally well absorbed through the skin. Phenols, such as phenol and salicylic acid, have special properties. These substances penetrate the skin and cause keratolysis. Concentrated solutions of strong bases, such as sodium hydroxide, and strong acids, such as nitric acid (which colors the skin yellow) and sulfuric acid, also damage the skin.

Dynamics of Skin Absorption

The classic principles of absorption through a lipid-protein series of membranes to the circulatory system, distribution of biotransformation, and eventual excretion via normal biological channels continue to operate in the maritime situations (Goldstein et al., 1974; La Du et al., 1971).

Results of numerous studies have indicated that chemicals penetrate the skin predominantly by passing through a lipidlike barrier. This conclusion is based on many isolated observations that lipid-soluble molecules are absorbed much more rapidly than lipid-insoluble molecules and ions (Calvery et al., 1946; Gemmell and Morrison, 1957; Malkinson, 1956; Rothman, 1954; Wilson, 1961) and on a study of the passage of nonelectrolytes across the excised rabbit skin (Treherne, 1956). Treherne showed that various alcohols and urea derivatives diffuse across whole skin at rates that are roughly proportional to the ether-to-water partition coefficients of the compounds. He concluded that the lipoid barrier of the skin is located within the epidermal layer since the dermis is freely permeable to many solutes and displays the characteristics of a highly porous membrane.

For some time, there was no general agreement among investigators as to the main pathway by which compounds traverse mammalian skin. Some authors stressed the importance of the epidermal route, while others contended that the route through hair follicles, sweat glands, and sebaceous glands was predominant. In later work, Tregear (1961) developed a technique whereby chemical penetration could be assessed using small areas of skin that contained either a desired number of hair follicles or none at all. Studying in this way the absorption of

tri-n-butyl phosphate from the skin of living pigs, he showed that the hair follicle is no more penetrable than an equivalent area of epidermis. In fact, regions of the skin devoid of hair follicles were penetrated slightly more rapidly than regions containing these structures.

Because of the relatively great thickness of the skin, chemicals penetrate this boundary much more slowly than they do most other body membranes. However, the percutaneous absorption of ionized compounds is enhanced by ionophoresis, a method in which a chemical solution in contact with an electrode is placed against the skin and a galvanic current is applied to both the chemical electrode and another electrode placed elsewhere on the body. Absorption through the skin can also be enhanced by dissolving a compound in oil, an ointment base, or other organic solvent and then rubbing it into the skin. Dimethyl sulfoxide (DMSO), an unusual solvent that is miscible with water as well as with many organic solvents, also enhances the percutaneous absorption of certain chemicals (Stoughton and Fritsch, 1964; Weyer, 1967).

The skin efficiently retards the diffusion and evaporation of water except at the sweat glands. The epidermis, although only approximately 0.2-mm thick, is largely responsible. The outer, horny layer (stratum corneum) consists of a continuous sheet of flattened cells that are densely packed with keratin, which constitutes a barrier to the penetration of water-soluble substances. Thus, the intact epidermis behaves qualitatively like cellular membranes in general. Chemicals penetrate at rates determined largely by their lipid/water partition coefficients, except for the very smallest molecules (Griesemer, 1959; Katz and Poulsen, 1971; Scheuplein and Blank, 1971).

Even very soluble substances penetrate slowly in comparison with their rates of penetration of other, thinner cell membranes. The underlying dermis, which consists of loosely arranged connective tissue and is vascularized, is freely permeable.

Toxic effects are often produced by accidental absorption through the skin of highly lipid-soluble substances that are used for various industrial purposes. General experience leads people to suppose that the skin is a reliable protection against the environment, but little thought is given to the possibility of poisoning by this route. Carbon tetrachloride, other organic solvents, and phenolics penetrate the body in this way and can cause serious toxic effects. Organic phosphates, such as diisopropyl fluorophosphate (DFP), parathion, and malathion, and nicotine insecticides have caused deaths in agricultural workers as a result of percutaneous absorption. Chlorovinylarsine dichloride (lewisite), a mustard gas, is readily absorbed through the intact skin.

Some chemical groups are characterized by their property of conferring water solubility on the molecules to which they belong. These hydrophilic, lipophobic, or polar groups are: $-OSO_2ONa$, $-COONa$, $-SO_2Na$, $-OSO_2H$, and $-SO_2H$. The following groups are less efficient: $-OH$, $-SH$, $-O-$, $-CO$, $-CHO$, $-NO_2$, $-NH_2$, $-NHR$, $-NR_2$, $-CN$, $-CNS$, $-COOH$, $-COOR$, $-OPO_3H_2$, $-OS_2O_2H$, $-Cl$, $-Br$, and $-I$. The presence of unsaturated bonds, such as those in $-CH=CH-$ and $-C\equiv C-$, helps to promote hydrophilicity (Hummel, 1962). Other groups (lipophilic, hydrophobic, or nonpolar) increase the liposolubility of the compounds of which they are part. Examples of these groups are aliphatic hydrocarbon

chains, aryl alkyl groups, and polycyclic hydrocarbon groups. Compounds carrying hydrophilic and lipophilic groups in proper equilibrium can modify the characteristics of the boundary surface between two lipids, a lipid and a solid, or a liquid and a gas. Such compounds, called surfactants, are used mainly as detergent, wetting, dispersing, foaming, and emulsifying agents (Moilliet et al., 1961).

Surfactants deeply affect the permeability of cellular membranes by disintegrating or lysing those of high activity through denaturation of the proteins of which the membrane is part or simply by enwrapping it with a layer, which interferes with the absorption of compounds of low activity. Because they disorganize cellular membranes, cause hemolysis, and are easily absorbed by proteins, surfactants are generally not applied to body tissue intentionally.

The sweating of maritime personnel working in a poorly ventilated area may favor absorption of chemicals through the skin. The increased moisture on the skin from perspiration may solublize chemicals that previously could not readily pass through the skin. Sweat is usually acid and provides the opportunity for acid-salts to exist in the lipid-soluble state, thus favoring absorption. Finally, the sweating and associated increased body temperature will cause dilation of the blood vessels in the skin, providing abundant circulatory exposure to any chemical that is capable of moving through the skin barrier.

DERMAL REACTIONS TO TOPICAL CHEMICAL EXPOSURES

Hypersensitivity Reactions

Sensitivity can develop from repeated contact with or repeated ingestion of a certain chemical, even after long intervals. The hyper-

sensitivity reaction is not specific for a particular substance, but it is closely related to the nature of the sensitization process. This process is based upon the formation of specific antibodies for substances that are foreign to the body. Increased sensitivity may be based on increased concentration of an active substance in the plasma or tissues, as a result of a certain dose or degree of exposure. It may also result from enhanced bioactivation caused by inducing agents or decreased elimination or detoxification, e.g., by combining with an enzyme inhibitor that may be involved in a substance's metabolism or with an inhibitor of renal excretion.

Allergic Reactions

Allergic reactions are often observed on the skin. To induce such a reaction, the allergen must enter the organism. Allergic hypersensitivity to a group of chemically related substances is called cross-allergy. Under certain circumstances, practically any substance can cause an allergic reaction. This tendency is dependent partly on the constitution of the individual and partly on the properties of the substance. Some compounds, such as dinitrochlorobenzene, produce hypersensitivity reactions in practically everyone exposed to them. Quinine induces a hypersensitivity reaction in a relatively high percentage of individuals, while other substances, such as carbohydrates, produce hypersensitivity only in exceptional cases.

Photoallergic Reactions

Certain substances, when present in the skin, may undergo photochemical alterations to products that are allergens. Once an allergic sensitization of this nature takes place in an organism, exposure to

sunlight will produce a reaction whenever there is exposure to the substance. This skin reaction has the same characteristics as any allergic reaction, but may be limited to those parts of the skin that were exposed to sunlight. There must be repeated exposure to the causative substance before the photoallergic reaction will occur. These reactions have been seen after exposure to substances such as tetrachlorosalicylanilide, hexachlorophene, and bithionol.

Photosensitization

Photosensitization results from a combined exposure to certain substances and sunlight. The ensuing skin reaction exhibits the characteristics of sunburn erythema, but it is independent of the specific properties of the substance in question. A genuine photosensitization can occur even upon the first contact with a substance and exposure to sunlight. Photosensitization often remains long after exposure to the causative substance. This may be due to binding of the sensitizing substance in the skin.

Phototoxic Reactions

A phototoxic reaction occurs when a substance in the skin is changed into a toxic substance by a photochemical reaction influenced by sunlight. The nature of the symptoms depends on the kind of toxic substance formed and, therefore, on the nature of the substance to which the individual is exposed. Although repeated exposure is usually necessary to produce a photoallergic reaction, photosensitization and phototoxic reactions will occur upon first exposure to the substance if there is simultaneous exposure to sunlight.

Direct Chemical Irritation

Direct chemical irritation is caused by substances that readily react chemically with various tissue components. As a rule, such substances do not reach the general circulation since they have already reacted with tissue at the site of contact with the organism. Direct chemical irritation is sometimes labeled a local irritant action, an etching action, a caustic action, or a necrotic action, depending on the reactivity of the substance. The intact skin offers a certain resistance to the substances, although the resistance is often inadequate.

Chemical Dermatitis

Direct damage to the skin can be caused by contact with chemically reactive substances. These substances include nitrogen mustards and related biological alkylating agents called vesicants (blistering agents) and keratolytic agents, especially phenols, such as salicylic acid. Compounds known for their etching action on the skin are strong alkaline solutions such as sodium hydroxide and potassium hydroxide solutions. Concentrated nitric acid causes not only a strong local alteration of the pH, but it also oxidizes and causes nitration of various components of the skin. Organic solvents remove protective lipid sebaceous layers in the skin, thereby paving the way for the development of allergic dermatoses and chemical dermatitis. Various substances then penetrate the skin through the pores and along the roots of the hair, especially by way of the sebaceous glands. Thus, these are the places where chemical dermatitis is initially manifested.

ABSORPTION FROM THE DIGESTIVE TRACT

Foreign compounds may be absorbed from the mouth or from other areas of the gastrointestinal tract, mainly by simple diffusion. Active transport mechanisms are unlikely to be involved since absorption is proportional to concentration and is unaffected by the simultaneous absorption of compounds of similar structure. When active transport is involved, the transport mechanism has a limited capacity and can be saturated as concentration increases or when similar compounds compete for the same mechanism (Parke, 1968).

In general, absorption takes place along the entire length of the gastrointestinal tract, but the chemical properties of each substance determine whether that material will be absorbed in the strongly acid stomach or in the nearly neutral intestine. Gastric absorption is facilitated by an empty stomach in which the compound will have good access to the mucosal wall. The absorption of some chemicals is aided by the consumption of a fatty meal. Intestinal absorption is favored by the large surface area of the intestinal villi, the presence of bile, and a rich blood supply.

The principles governing the absorption of chemicals from the gastrointestinal lumen are the same as those for passage of chemicals across biological membranes elsewhere. A low degree of ionization, a high lipid/water partition coefficient of the un-ionized form, and a small atomic or molecular radius of water-soluble substances all favor rapid absorption. Water passes readily through the wall of the gastrointestinal lumen in both directions. Magnesium ion is poorly absorbed and acts as a cathartic, retaining an osmotic equivalent

of water as it passes down the intestinal tract. Ionic iron is absorbed as an amino acid complex at a rate usually determined by the body's need for iron. Glucose and amino acids are transported across the intestinal wall by specific carrier systems. Some compounds of high molecular weight, e.g., polysaccharides, neutral fats, cannot be absorbed because they are destroyed by gastrointestinal enzymes, e.g., insulin, epinephrine, and histamine. Substances that form insoluble precipitates in the gastrointestinal lumen or that are not soluble either in water or in lipid obviously cannot be absorbed (Goldstein et al., 1974).

Absorption from the Mouth

Foreign compounds are absorbed from the mouth by diffusing into the mucous membrane of the oral sulci and thence into the bloodstream. They are not exposed to the gastrointestinal digestive juices nor are they transported directly to the liver, as are chemicals absorbed in the stomach and intestines. Since foreign compounds are metabolized principally in the liver, absorption from the mouth delays metabolism and may prolong the activity of a chemical.

Absorption from the Stomach

It has long been believed that absorption of compounds from the stomach is negligible, except for ethanol. This is largely true for nutrient substances, especially macromolecules that require digestion, but it is now known that many foreign compounds, e.g., acidic compounds such as salicylic acid, aspirin, and barbiturates, are readily absorbed from the stomach by simple diffusion of the un-ionized molecules across the gastric mucosa.

Absorption from the Small Intestine

The intestinal epithelium, like the gastric mucosa, allows the passage of undissociated foreign molecules by the process of simple diffusion. Weak acids and bases are absorbed through this route. Alteration of the pH of the intestinal content changes the degree of ionization of the foreign compound and, hence, the extent of absorption.

Certain foreign monosaccharides, amino acids, and pyrimidines are sufficiently similar to natural compounds to be absorbed by the active transport mechanisms that are involved in the absorption of nutrients. Various foreign macromolecules, such as bacterial toxins, are slightly absorbed from the intestine, probably by pinocytosis.

Absorption from the Colon

The pattern of absorption from the colon is similar to that for the small intestine. Weak acids and bases are absorbed readily, whereas highly ionized compounds are absorbed slowly.

Factors Affecting Gastrointestinal Absorption

The absorption of foreign compounds from the gastrointestinal tract may be affected by a number of factors. Accelerated emptying of the stomach's contents into the intestinal tract reduces gastric absorption but may increase intestinal absorption. Increased intestinal peristalsis results in more efficient mixing of the intestinal contents, allowing increased absorption, but it also expedites the emptying process, consequently reducing intestinal absorption. Increased blood flow through the intestines and greater cardiac

output, both of which are associated with digestion and absorption of food, accelerate the absorption of foreign compounds. Gastrointestinal secretions lead to pH changes that alter the degree of ionization and absorption of foreign compounds. Secretion of mucus affects absorption, and enzymes lead to hydrolysis of esters and amides. Calcium, iron, and other metals may form insoluble chelates with certain compounds (e.g., tetracycline) and impair their absorption. The particle size of foreign compounds affects their rate of solubility, especially for those with low solubility. Consequently, it also affects absorption, which occurs only from solution.

SITES OF CHEMICAL INTERACTION WITH ABSORPTION

Chemicals may interact at any phase of their passage through the body. Many interactions occur during absorption, distribution, metabolism, or excretion or at the receptor sites, and can alter the effects of respiratory, topical, or gastrointestinal exposure of maritime workers to chemicals. Specifically, one or more of the following mechanisms are involved: direct effect on the chemical; modification of gastrointestinal absorption; modification of dermatomucosal absorption; alteration of distribution; modification of action at receptor sites; modification of biotransformation; and alteration of excretion (Martin et al., 1971). These mechanisms involve an enormous number of biological, chemical, and physical factors.

DIRECT EFFECT ON THE CHEMICAL

Chemicals may interact directly with each other, either chemically or physically, after they have been administered. Protein

hydrolysates bind barbiturates, digitoxin, digoxin, tetracyclines, and many other drugs. Amino acids, e.g., cysteine, as well as tetracyclines and other potent chelators can interact with calcium-containing medications and, if rapidly infused intravenously, may cause hypocalcemic tetany. Components of a mixture other than the primary chemical may also interact directly with another chemical. Thus, bisulfite and sulfur dioxide, which are used as preservatives for sympathomimetic amines such as epinephrine and phenylephrine, inactivate penicillin G if injected at the same time. A drug may also be affected by chemical interactions when it is topically applied strictly for its local dermal, mucosal, or gastrointestinal effects. It may be adversely affected if some substance hinders its contact with the surface, prevents it from exerting its effects, or destroys or inhibits its activity through some chemical or physical reaction. Thus, soap may inhibit the antifungal activity of acrisorcin on the skin.

PULMONARY ABSORPTION INTERACTIONS

There have been many studies of commonly used industrial solvents, but many were conducted in different laboratories with different inhalation chambers and techniques, most used only single acute administrations, and a number of them had no accurate control over dosage. Very few studies used combinations of agents, and few extensive studies have been conducted in one laboratory.

In one extensive inhalation study, Gage (1970) of Imperial Chemical Industries, Ltd. (ICI) examined the subacute toxicity of 109 volatilizable industrial chemicals in rats for 6 hr/day up to 4 weeks.

He established provisional optimal exposure limits, which he derived from safety factors applied to the highest concentrations producing no adverse effects in the animals. Unfortunately, the coadministration of other chemicals was not investigated. A study of single and repeated exposures to inhaled chloroform (85, 50, and 25 ppm) revealed, not unexpectedly, that repeated exposure (7 hr/day, 5 days/week for 6 months) resulted in more adverse effects than did the single exposures (Torkelson et al., 1976). Rats exposed to 25 ppm of chloroform for 4, 2, or 1 hr/day for 6 months were not adversely affected. The exposure of rats, rabbits, and monkeys to inhaled 1,2,4-trichlorobenzene (25, 50, and 100 ppm) for 26 weeks resulted in pathological changes in the livers and kidneys at 4 and 13 weeks of exposure, but no exposure-related abnormalities were observed after 26 weeks of exposure, suggesting possible physiological adaptation to the chemical insult (Coate et al., 1977).

Few inhalation studies have been conducted with combinations of volatile chemicals. Considerable difficulty is encountered with such research since many industrial solvents have different and quite specific target organs (Hayden et al., 1976). Acute neurotoxic effects of organic solvents may be caused by direct action on the nerve cell membrane or on energy metabolism, whereas chronic neurotoxic effects may be explained by the formation of chemically and biologically reactive intermediates, the development of the neuropathy being dependent upon the stability of the toxic metabolite (Savolainen, 1977). Specific interactions involving biotransformation may involve combinations of organic chemicals. These phenomena are dose-dependent. A recent

inhalation study with animals involving methylene chloride and ethanol demonstrated the complexity of such interactions (Balmer et al., 1976). An antagonism between these two chemicals was suggested by hepatic damage that followed a single 6-hr exposure of rats. However, results of a 5-day, 6-hr/day exposure suggested that ethanol potentiated the effects of methylene chloride.

MODIFICATION OF GASTROINTESTINAL ABSORPTION

Two substances may complex in the gastrointestinal tract, so that both are poorly absorbed. Tetracycline tablets that are formulated with calcium carbonate lead to insoluble calcium salts of the antibiotic, which result in erratic blood levels. Neomycin interferes with the absorption of fats and lipid-soluble drugs from the small intestine. A surprising example of drug interaction operating on absorption is that the presence of phenobarbital reduces the plasma level of the antifungal agent griseofulvin.

Gastrointestinal absorption may be hindered by mechanisms other than direct complexing. For example, intestinal flora can alter many chemicals through hydroxylation, decarboxylation, and ester hydrolysis. Consequently, antibiotics that act upon the flora, abolishing some, can alter the ultimate levels of the chemical in the plasma. Enzymes involved in the transport of essential nutrients across the intestinal wall may also be inhibited by drugs. This, in turn, may result in rather complex interactions.

Alteration of the Functions of the Gastrointestinal Tract

Two major functions of the gastrointestinal tract that affect absorption are the rate at which its contents are transported from the stomach to the rectum and the metabolism that occurs within its bacterial flora. Accordingly, any chemical interaction that markedly influences motility or bacterial balance and growth can have important impacts on rates of absorption. The time required for the stomach to empty varies with the intensity of the gastric motility. Therefore, the length of time a chemical remains in the intestinal tract before it is excreted varies with the intensity of the intestinal peristalsis. The absorption rate of a drug that is absorbed readily from the stomach can be increased by slowing the emptying time, thereby increasing gastric retention. For a drug that is absorbed more readily from the intestines, the absorption rate can be increased by accelerating passage from the stomach into the intestines, i.e., decreasing gastric retention.

Cathartics tend to reduce the absorption of any given medication and, if abused, may also precipitate or aggravate the toxic effects of some drugs by inducing excessive potassium loss. Drugs may also alter gastric motility and emptying time by modifying the contractility of the smooth muscle. Codeine, morphine, and other opiate analgesics decrease motility and depress absorption of drugs that are absorbed more readily from the intestines and increase absorption of those that are absorbed more readily from the stomach. Anticholinergic agents inhibit absorption by decreasing gastrointestinal motility. Cholinergic stimulants accelerate gastric emptying time, thereby

depressing absorption of drugs that are more readily absorbed from the stomach and enhancing absorption of those that are more readily absorbed from the intestine. The gastric emptying time is also modified by exercise, temperature, volume, the nature of the solid and fluid contents, emotional problems, and other factors.

Modifying or eliminating the intestinal flora with antimicrobial agents may alter the susceptibility of patients to a drug. Because antimicrobial action may diminish bacterial synthesis or metabolism of some drugs in the tract, gastrointestinal absorption and systemic toxicity may be either decreased or increased.

Alteration of Physiochemical Characteristics

A chemical interaction may modify gastrointestinal absorption if it alters the physiochemical characteristics of the chemical or the contents of the tract. It can do this by altering the pH, forming a nonabsorbable complex like those formed by certain ions (Al, Ba, Ca, Mg, Sr) with tetracyclines, and modifying rates of deaggregation or dissolution of the drug in the ambient fluid. Moreover, it may modify the diffusion rate of the compound by altering miscibility, viscosity, and other factors exerting an osmotic force, such as magnesium sulfate, a cathartic whose slightly absorbable ions retain water in the intestinal tract. The interaction may also result in the formation of a salt that is either more or less soluble, stable, or absorbable than the original chemical, e.g., soluble iron salts form insoluble carbonates on contact with antacids and other drugs containing the carbonate radical. Furthermore, absorption may be

modified when a chemical is sequestered in a lipoid. Mineral oil does this when given as a cathartic dosed with oil-soluble vitamins A, D, and K, thereby preventing the vitamins from making adequate contact with the intestinal epithelium. Perhaps the most significant of these physiochemical mechanisms are alteration of pH and complexation.

Alteration of Mucosa

The condition of the gastrointestinal mucosa may affect absorption in the tract. The rate at which drugs are transported into the body is usually highest where surface areas are large and vascularization is profuse as in the peritoneal membrane, pulmonary endothelium, and intestinal villi. If the intestinal mucosa is destroyed by toxic doses of an agent such as tannic acid, absorption of a chemical may be as rapid as it is when administered intramuscularly or subcutaneously.

Alteration of Transport Mechanisms

Alteration of active and passive transport from the gastrointestinal tract through its lining into the body fluids may strongly influence chemical absorption by this route. The size of the pores in the absorbing membrane, lipoid solubility, electrochemical, hydrostatic, osmotic, and pH gradients, and many other factors can modify active or passive mechanisms that are involved in gastrointestinal absorption. Chemical interactions may interfere with an active mechanism by competing in the transport cycle.

Amino acids like methyldopa are absorbed slowly from the intestinal tract in the presence of certain natural amino acids that are ingested in the food because primary phenolic amino acids compete

for the same transport sites. The presence of food itself markedly affects the rate of absorption. Either more or very little of a chemical is absorbed if it is rapidly transported through the tract; highly ionized at the ambient pH and its ions are poorly absorbed or nonabsorbable; rendered insoluble or poorly diffusible in gastrointestinal fluid at the absorption site; converted into an insoluble salt, chelate, or other insoluble complex; rendered unstable by the ambient pH; converted into its un-ionized form, which is lipid-insoluble; or sequestered from the absorbing tissues by a nonabsorbable lipoid. The opposite conditions enhance absorbability.

DRUG INTERACTIONS

The likelihood of a drug interaction increases if one medical specialist prescribes a topical drug for the eye while another administers a systemic medication. An adverse drug reaction may occur when a potent, long-acting anticholinesterase agent is applied topically to the eye and, while the drug is actively inhibiting cholinesterase in the body, a muscle relaxant like succinylcholine is adminstered prior to general anesthesia, since there would be continual uncontrolled stimulation of the afferent nerves. A topically applied drug that is absorbed through the skin to membranes of the ear, eye, mouth, nose, rectum, urethra, or vagina may interact with drugs administered perorally or parenterally. Drug interactions that influence rates and sites of dermatomucosal as well as gastrointestinal absorption and function must be avoided.

ALTERATION OF DISTRIBUTION

The most important aspects of distribution that can be modified by chemical interactions are transport, binding, and redistribution.

Alteration of Chemical Transport

The rates and routes of the distribution of a chemical from its site of intake to its sites of action, biotransformation, storage, and excretion may be profoundly influenced by another chemical. Onset, intensity, and duration of action may be affected by changes in fluid flow, physical factors, and transport across membranes. Any physiologically active chemical that alters the flow rate and volume of fluid in the cardiovascular or lymphatic system may also alter the rate at which a drug is moved from one area of the body to another. Therefore, cardiac stimulants, diuretics, hypertensive (pressor) and antihypertensive (hypotensive) agents, and other cardiovascular drugs may influence the distribution of other chemicals. The rate at which an absorbed chemical moves from its site of intake to other areas of the body is influenced appreciably by miscibility, solubility, surface tension, viscosity, and other characteristics of the ambient fluids. Therefore, modification of any of these characteristics may cause the chemical to remain at its site of entry for a prolonged period or diffuse more rapidly than normal. Since transmembranal transport is effected by active transport mechanisms, convective absorption, facilitated or passive diffusion, phagocytosis, and pinocytosis, a chemical interaction that modifies any of these factors also modifies chemical distribution in the body. The rate of transport also varies with the characteristics of the membrane and the forces that drive

the chemical across the membrane. The permeability of some membranes, notably walls of the lymph capillaries, may be increased by histamine and some other chemicals as well as by massage, sunlight, and warmth to such a degree that the walls present no real barrier between the lymph inside and the interstitial fluid outside the vessels.

Displacement from Binding Sites

Displacement of a drug from its binding sites in plasma and tissues may enhance its activity because it is then free to contact receptor sites, initiate its action, and produce physiological effects. The more tenaciously bound chemicals can displace those less firmly bound from binding sites, thereby causing shifts in plasma concentrations and, possibly, major redistribution of the released chemical in the body compartments. Displacement of a substance from its secondary binding sites may activate or potentiate its physiological activity, increase its toxicity, or produce a beneficial effect. Salicylates, sulfonamides, and certain other drugs may precipitate kernicterus in infants by displacing bilirubin from protein binding sites. Displacement of a chemical from its bound state also makes it available for urinary excretion, thereby increasing the rate of its excretion from the body.

MODIFICATION OF ACTION AT RECEPTOR SITES

Interferences with the mechanisms of chemical action at receptor sites may cause hazardous augmentation or reduction of drug effects through activitation or inhibition of mechanisms involving enzymes, neurohumors, and other components. A chemical interaction may enhance

activity at a receptor site if it displaces protein-bound, endogenous, physiologically active chemicals, increases synthesis of active endogenous chemicals, increases release of endogenous stored chemicals, prevents binding to secondary receptors, preserves the active agent at its receptor sites, sensitizes effectors to chemicals, or enhances the affinity between receptors and chemical compounds. An interaction may decrease or destroy activity at a receptor site if it promotes chemical binding to protein and chemical storage, decreases synthesis of active endogenous chemicals, prevents the release of endogenous stored chemicals, prevents drug binding at receptor sites, desensitizes effectors to chemicals, decreases the amount of chemical at receptor sites and its affinity for these sites, or depletes the stores of neurotransmitters and other active chemicals produced in the body.

REFERENCES

Astrand, I. 1975. Uptake of solvents in the blood and tissues of man: a review. Scand. J. Work Environ. Health 1:199-218.

Astrand, I., and F. Gamberale. 1978. Effects on humans of solvents in the inspiratory air: a method for estimation of uptake. Environ. Res. 15:1-4.

Balmer, M. F., F. A. Smith, L. J. Leach, and C. L. Yuile. 1976. Effects in the liver of methylene chloride inhaled alone and with ethyl alcohol. Am. Ind. Hyg. Assoc. J. 37:345-352.

Burton, J. A., and L. S. Schanker. 1974. Absorption of sulphonamides and antitubercular drugs from the rat lung. Xenobiotica 4:291-296.

Calvery, H. O., J. H. Draize, and E. P. Laug. 1946. The metabolism and permeability of normal skin. Physiol. Rev. 26:495-540.

Coate, W. B., T. R. Lewis, and W. M. Busey. 1977. Chronic inhalation exposure of rats, rabbits, and monkeys to 1,2,4-trichlorobenzene. Arch. Environ. Health 249-255.

Enna, S. J., and L. S. Schanker. 1972. Absorption of drugs from the rat lung. Am. J. Physiol. 233:1227-1231.

Fingl, E., and D. M. Woodbury. 1975. Chapter 1, General principles. Pp. 1-46 in L. S. Goodman and A. Gilman, eds. The Pharmacological Basis of Therapeutics, 5th edition. Macmillan, New York.

Gage, J. C. 1970. The subacute inhalation toxicity of 109 industrial chemicals. Br. J. Ind. Med. 27:1-18.

Gemmell, D. H. O., and J. C. Morrison. 1957. The release of medicinal substances from topical applications and their passage through the skin. J. Pharm. Pharmacol. 9:641-656.

Goldstein, A., L. Aronow, and S. M. Kalman. 1974. Principles of Drug Action: The Basis of Pharmacology, 2nd edition. Wiley, New York. 854 pp.

Griesemer, R. D. 1959. Protection against the transfer of matter through the skin. Pp. 25-46 in S. Rothman, ed. The Human Integument, Normal and Abnormal. The American Association for the Advancement of Science, Washington, D.C. Publication No. 54.

Hayden, J. W., E. G. Comstock, and B. S. Comstock. 1976. The clinical toxicology of solvent abuse. Clin. Toxicol. 9:169-184.

Hummel, D. 1962. Identification and Analysis of Surface-Active Agents by Infrared and Chemical Methods. Interscience, New York. 386 pp.

Katz, M., and B. J. Poulsen. 1971. Absorption of drugs through the skin. Pp. 103-174 in B. B. Brodie and J. R. Gillette, eds. Handbook of Experimental Pharmacology, Vol. 28. Springer-Verlag, New York.

La Du, B., H. G. Mandel, and E. L. Way. 1971. Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins, Baltimore. 615 pp.

Lanman, R. C., R. M. Gillilan, and L. S. Schanker. 1973. Absorption of cardiac glycosides from the rat respiratory tract. J. Pharmacol. Exp. Ther. 187:105-111.

Levine, R. R. 1973. Pharmacology: Drug Actions and Reactions. Little, Brown, and Company, Boston. 412 pp.

Malkinson, F. D. 1956. Radioisotope techniques in the study of percutaneous absorption. J. Soc. Cosmet. Chem. 7:109-122.

Martin, E. W., S. F. Alexander, D. J. Farage, and W. E. Hassan, Jr. 1971. Hazards of Medication. Lippincott, Philadelphia. 895 pp.

Moilliet, J. L., B. Collie, and W. Black. 1961. Surface Activity, 2nd edition. Van Nostrand, Princeton, N.J. 518 pp.

Parke, D. V. 1968. The Biochemistry of Foreign Compounds. Pergamon Press, New York. 269 pp.

Rothman, S. 1954. Physiology and Biochemistry of the Skin. University of Chicago Press, Chicago. 741 pp.

Savolainen, H. 1977. Some aspects of the mechanisms by which industrial solvents produce neurotoxic effects. Chem. Biol. Interact. 18:1-10.

Schanker, L. S. 1978. Drug absorption from the lung. Biochem. Pharmacol. 27:381-385.

Scheuplein, R. J., and I. H. Blank. 1971. Permeability of the skin. Physiol. Rev. 51:702-747.

Stoughton, R. B., and W. Fritsch. 1964. Influence of dimethyl-sulfoxide (DMSO) on human percutaneous absorption. Arch. Dermatol. 90:512-517.

Torkelson, T. R., F. Oyen, and V. K. Rowe. 1976. The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Am. Ind. Hyg. Assoc. J. 37:697-705.

Tregear, R. T. 1961. Relative penetrability of hair follicles and epidermis. J. Physiol. 156:307-313.

Treherne, J. E. 1956. The permeability of skin to some non-electrolytes. J. Physiol. 133:171-180.

Weyer, E. M., ed. 1967. Biological actions of dimethyl sulfoxide. Ann. N.Y. Acad. Sci. 141:1-671.

Wilson, K. 1961. New methods for the study of percutaneous absorption. Drug Cosmet. Ind. 88:444-446, 521, 526-529.

CHAPTER 3

ELIMINATION AS A SITE OF INTERACTION

The removal or clearance of volatile toxicants from the body may be facilitated by direct exhalation, which has been observed in studies of dimethylsulfoxide or paraldehyde; translocation from the alveolar air to the bloodstream and subsequent translocation into tissues where biotransformation may occur or the toxicant may be stored; and renal and/or fecal elimination. Despite the volatility of many of these toxicants, a number of them are extensively metabolized in the body rather than merely exhaled.

PULMONARY ELIMINATION

The removal or clearance of toxicants from the alveoli can be facilitated by exhalation, direct translocation from the alveolar air into the bloodstream, removal via bronchial ciliary action to the gastrointestinal tract, and phagocytosis and removal by the lymphatic system. Respiratory impairment or chronic exposure to vapors may markedly incapacitate pulmonary function by altering the pharmacokinetics of absorption and elimination. The inhalation by rats of acidic fumes, paraquat, aerosolized papain, and rock dust (in experiments to induce silicosis) all resulted in irritancy and damage, which appeared to increase the porosity of the pulmonary epithelium, thereby enhancing absorption (Gardiner and Schanker, 1975, 1976a,b,c). Partial collapse of the lung results in a narrowing of the bronchioles, which can easily be obstructed by fluid (Ebert, 1978). In chronic bronchitis, bronchiolar obstruction can result from fibrosis

and inflammation accompanied by marked alterations in secretions and loss of traction on the walls.

Pulmonary function can also be greatly affected by the particle size of a chemical and its concentration in the air. Inhaled agents strongly inhibit the ciliary action of the bronchial epithelium, resulting in reduced efficiency of particle removal and, consequently, an increase in the quantity of particles retained.

In a review of the absorption of drugs via the lung, Schanker (1978) maintained that many fundamental questions need to be answered. What actually happens to a droplet when it is deposited on the bronchiolar or alveolar epithelium? How does the coating of the alveolar surface influence the fate of inhaled agents? How rapidly do dry aerosols (dusts) of chemicals dissolve in the coating of the respiratory tract? Which factors determine the dissolution rates? What is the nature of membrane pores in the pulmonary epithelium? Which physiological and pathological factors influence the size, number, and distribution of pores in the respiratory tract? What are the effects of environmental variables (e.g., heavy smoking) and pulmonary disease on absorption rates of inhaled chemicals? These questions, as relevant for solvents as they are for drugs, are only a few of the many that must be answered. They do not begin to come to grips with the almost predictable, inherent covert toxicities, such as those observed for the aliphatic halogenated anesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), which has been demonstrated to produce birth defects in children of operating room personnel (American Society of Anesthesiologists, 1974).

BILIARY ELIMINATION

Foreign compounds are absorbed from the blood of the hepatic sinusoids into the hepatic parenchymal cells. They are then transferred, as metabolites or conjugates, into the bile or are returned into the blood of the sinusoids, ultimately to be excreted in the urine or feces. Because hepatic parenchymal cells have highly permeable membranes, the boundary between the blood and the bile is extremely porous and permits the passage of most molecules and ions that are smaller than proteins. Therefore, many substances appear in the bile and in plasma in similar concentrations, but highly polar compounds such as the bile salts, bilirubin glucuronide, and conjugates of foreign compounds are excreted in the bile in much higher concentrations by a process of active transport. This active secretion appears to occur with compounds that are present in the blood as anions, have a molecular weight greater than 300, and are bound to plasma proteins. Biliary secretion may also be dependent on the binding of a foreign compound to the proteins of the hepatic cell. The rates of secretion of a number of azo dyes have been shown to be functions of the ratios of binding with liver proteins to the binding with the plasma proteins. Certain organic cations, e.g., the drugs procainamide, ethyl bromide, and mepiperphenidol, are similarly secreted into the bile by an active transport mechanism (Parke, 1968).

Foreign compounds are excreted in the bile mostly as conjugates, which may be hydrolyzed by hydrolytic enzymes (e.g., β-glucuronidase and sulfatase) in the bile or by enzymes of the intestinal secretion

and flora. Many glucuronides (such as those of phenol, estriol, and chloramphenicol) are hydrolyzed in the gut. Ethereal sulfates are more stable. Since conjugates are polar compounds, they are unlikely to be readily reabsorbed from the intestine, but their hydrolysis products, if nonpolar, may well be reabsorbed, transported to the liver, reconjugated, and excreted again in the bile. Such a cycle of biliary excretion, intestinal reabsorption, and reexcretion is known as enterohepatic circulation and occurs with chloramphenicol, stilbestrol, sulfonamides, and many other foreign compounds.

GASTROINTESTINAL ELIMINATION

From the pH-partition hypothesis, it may be predicted that organic bases, largely ionized at the pH of the gastric juice, will be secreted from the blood plasma into the stomach. This route of excretion was largely ignored until Parke (1968) showed that various parenterally administered drugs and other foreign compounds, such as aniline, aminopyrine, quinine, dromoran, and mecamylamine, may be secreted into the gastric juice. The excretion of nicotine into the stomach has been associated with its role in causing peptic ulcer.

Similarly, it may be predicted that weak organic acids and bases that are highly ionized at the pH of the intestinal lumen would be secreted by passive transfer from the blood plasma into the intestine when the concentration gradient is favorable. Likewise, one could expect organic acids to be secreted into the alkaline pancreatic juice.

SALIVARY ELIMINATION

To a minor extent, foreign compounds are excreted in body secretions by passive transfer of the un-ionized molecules. Sulfonamide drugs are excreted in the parotid saliva of humans at lower concentrations than those occurring unbound in the plasma. However, sulfonamides, phenobarbitone, and other acidic drugs are excreted in higher concentrations in the parotid saliva of ruminants, which is alkaline (pH 8.2-8.4). Penicillin is actively secreted by the salivary apparatus (Parke, 1968).

DERMAL ELIMINATION

The elimination of foreign chemicals via the skin has not been studied extensively. There are only a few known cases of elimination of chemicals via this route. The antileprosy drug ditophal (diethyl dithiolisophthalate) is excreted in the sweat of human subjects in amounts that equal or exceed the total amount excreted in the urine and feces. To a minor extent, sulfonamides are also eliminated via the sweat glands and highly volatile liquids such as dimethyl sulfoxide are excreted via the sebaceous glands. Other highly liquid-soluble agents may also be eliminated via the sebaceous glands. With the exceptions noted above, it would be safe to claim that the skin is a relatively poor route for the elimination of exogenous chemicals that are absorbed by a different route.

RENAL ELIMINATION

Excretion by the kidney consists of three distinct processes: glomerular filtration, passive tubular transfer, and active tubular transport. Glomerular filtration produces an ultrafiltrate of the blood plasma, which contains foreign compounds and their metabolites in approximately the same concentration as that in the blood. Passively tubular transfer occurs in the kidney tubular epithelium. Like other biological membranes, the tubular epithelium, particularly in the distal tubule, behaves as a lipoprotein barrier allowing the transfer of lipid-soluble, un-ionized molecules. Therefore, the un-ionized forms of lipid-soluble compounds in the glomerular filtrate are reabsorbed into the bloodstream, whereas compounds of low lipid-solubility (such as barbital) are only partially reabsorbed. Moreover, compounds that are more highly ionized in the urine than in the blood plasma tend to diffuse across the tubular epithelium from the blood into the glomerular filtrate. Thus, when the tubular urine is more alkaline than the plasma, weak acids are readily transferred into the urine. Conversely, weak bases are transferred when the tubular urine is more acid. Therefore, the rate of renal excretion of weak organic electrolytes is largely dependent on the pH of the urine. For example, the rate at which amphetamine is excreted was 20 times greater in human subjects with urine of pH 5 than in others with urine of pH 8 (Parke, 1968).

The renal tubular epithelium also possesses at least two mechanisms of active transport--one for the secretion of strong organic acids and another for strong bases, both mechanisms being associated

with the proximal tubule. Compounds excreted by active transport are highly ionized and may be transferred into the tubular urine against high concentration gradients. It is unlikely that the active transport mechanisms can distinguish between strong and weak organic electrolytes. The lipid-insoluble, ionic forms of both are probably excreted by these mechanisms. Various drugs and metabolic conjugates, such as quinine, thiazide, acetazoleamide, glucuronides, and sulfate esters, are known to be secreted by active transport. Substances secreted by the same active transport mechanisms compete with each other for that mechanism. Consequently, the excretion rate of one compound can be reduced by administration of another. This competition has used pharmacological manipulations to inhibit excretion of a drug, thus preserving therapeutic blood levels. An example of this is the competitive effect of probenecid on the excretion of penicillin.

Endogenous amino acids and sugars are reabsorbed from the tubular urine by active transport, and similar mechanisms have been indicated for the active reabsorption of certain foreign compounds, such as p-aminohippuric acid. Administration of α-methyldopa produces a reversible aminoaciduria, which probably results from competition of the catechol for the kidney tubular reabsorption mechanism for neutral amino acids. In general, therefore, the relatively polar, lipid-insoluble metabolites and conjugates are less readily reabsorbed from the renal tubules and are more readily secreted by the active transport mechanisms than are the original nonpolar, lipid-soluble foreign compounds. This results in a high renal clearance of the polar

metabolites and, consequently, in a rapid elimination of the foreign compound from the body.

Interactions

As stated above, many volatile solvents are not exhaled but are extensively metabolized in vivo, and the products are eliminated via the urine. Following an inhalation exposure to benzene (110 ppm for 2-3 hr), from 30% to 50% was eliminated via the lungs, from 0.1% to 0.2% unchanged benzene was eliminated via the kidneys, and the balance was metabolized prior to excretion in the urine (29% as phenol, 2.9% as pyrocatechol, and 1% as hydroquinone) (Haley, 1977). Similar data can be provided for toluene, the bulk (50%) of which is excreted as hippuric or benzoylglucuronic acids (Dean, 1978; Hayden et al., 1977). Therefore, it is important to consider the factors that may influence the renal excretion of such metabolites. The excellent paper by Gillette and Mitchell (1975) has covered this in great detail. Their discussion is summarized below.

If a large proportion of a toxic agent is excreted via the kidney in a metabolized form (polar metabolites) using the active transport mechanisms available for acids (or bases) in the proximal portion of the tubule, then the biological half-life of this polar metabolite will be prolonged by inhibitors of drug-metabolizing enzymes; by severe morphological damage (necrosis) of the tissue at the site of detoxification and elimination (e.g., with toluene) (Hayden et al., 1977); by inhibitors of the renal transport system; and by the size of the dose, resulting in saturation of the renal transport system, the binding sites on plasma proteins, or the

detoxification processes that are essential for the formation of the metabolites or the depletion or exhaustion of essential cofactors.

One should ascertain what influence the above factors would exert on the ratio of metabolized to unchanged drug. Moreover, one should assess the relative importance of the drug-metabolizing enzymes in various tissues and their contributions toward the biotransformation of the agent. The influence of specific organ damage, such as hepatic necrosis, on the overall formation of polar metabolites and on the rerouting of a drug for excretion, either as a different metabolite or unchanged parent compound via the lungs or kidney, should also be examined.

Considering the biotransformation of the parent molecule by different tissues, is the rate of metabolism limited by the rate of blood flow through the tissues? The answer to this would also apply to renal elimination, during which any damage to blood vessels in the kidney markedly affects blood flow, urinary pH, and clearance of drugs and metabolites. These factors would also alter the toxicity of the parent chemical, perhaps by increasing the acute toxicity in relation to the chronic toxicity that is associated with the reactive metabolites.

FECAL ELIMINATION

The liver, which is responsible for the majority of phase I and II biotransformation reactions in the body, secretes large amounts of metabolites of exogenous and endogenous agents into the biliary tract, which empties into the duodenum. As stated above, most metabolites and many conjugated products are reabsorbed from the gastrointestinal tract

into the bloodstream, eventually to be eliminated via the kidneys. If the drug or metabolite entering the gastrointestinal tract is not readily reabsorbed or becomes incorporated into the food mass, it will be retained in the lumen and eliminated in the feces.

REFERENCES

American Society of Anesthesiologists. 1974. Occupational disease among operating room personnel: a national survey. Anesthesiology 41:321-340.

Dean, B. J. 1978. Genetic toxicology of benzene, toluene, xylenes and phenols. Mutat. Res. 47:75-97.

Ebert, R. V. 1978. Small airways of the lung--the importance of understanding and assessing the function of pulmonary bronchioles. Ann. Intern. Med. 88:98-103.

Gardiner, T. H., and L. S. Schanker. 1975. Effect of papain-induced emphysema on permeability of rat lung to drugs. Proc. Soc. Exp. Biol. Med. 149:972-977.

Gardiner, T. H., and L. S. Schanker. 1976a. Effect of oxygen toxicity and nitric acid-induced lung damage on drug absorption from the rat lung. Res. Commun. Chem. Pathol. Pharmacol. 15:107-120.

Gardiner, T. H., and L. S. Schanker, 1976b. Effect of paraquat-induced lung damage on permeability of rat lung to drugs. Proc. Soc. Exp. Biol. Med. 151:288-292.

Gardiner, T. H., and L. S. Schanker. 1976c. Enhanced pulmonary absorption of drugs in rats with experimental silicosis. Res. Commun. Chem. Pathol. Pharmacol. 13:559-562.

Gillette, J. R., and J. R. Mitchell. 1975. Drug actions and interactions: theoretical considerations. Pp. 359-383 in Handbook of Experimental Pharmacology, Vol. 28, Concepts in Biochemical Pharmacology, Part 3. Springer-Verlag, New York.

Haley, T. J. 1977. Evaluation of the health effects of benzene inhalation. Clin. Toxicol. 11:531-548.

Hayden, J. W., R. G. Peterson, and J. V. Bruckner. 1977. Toxicology of toluene (methylbenzene): review of current literature. Clin. Toxicol. 11:549-559.

Schanker, L. S. 1978. Drug absorption from the lung. Biochem. Pharmacol. 27:381-385.

Parke, D. V. 1968. The Biochemistry of Foreign Compounds. Pergamon Press, New York. 269 pp.

CHAPTER 4

INTERACTIONS INVOLVING BIOTRANSFORMATION REACTIONS

Almost all drugs, insecticides, and other foreign compounds are metabolized by living organisms. The extent of this metabolism often limits the rate of toxicity or biological activity. Therefore, alterations in the metabolism of a given chemical can lead to profound changes in the extent and/or duration of its biological effects.

Clearly, exposure of an animal to combinations of more than one chemical (either simultaneously or in close succession) can result in interactions whereby one chemical can markedly modify the metabolism of another. Such an interaction may synergize (potentiate) or antagonize the toxicity of one or more components of the mixture.

Drug interactions occur primarily through the ability of one chemical (A) to inhibit or to stimulate the enzymes responsible for the metabolism of another (B). When toxicant B is inactivated (detoxified) by a given enzyme system, compound A may interact to inhibit or stimulate enzyme activity, thereby leading to either potentiation or antagonism of toxicity, respectively. If, on the other hand, B is metabolically activated, the opposite result will be observed.

Many potentially hazardous toxic interactions can occur through quantitative and qualitative modifications in biotransformation. Several result from the increased use of multidrug therapy, and others have been demonstrated in the laboratory. There is growing concern over the potential hazard to humans of toxic interactions resulting from the large number of drugs, pesticides, and other chemicals to which they are inadvertently or occupationally exposed.

Discussions of interactions involving biotransformation cover an extremely broad area for which there is a vast amount of literature. Current knowledge comes mainly from in-vivo studies that have been conducted under carefully controlled laboratory conditions and are often combined with basic in-vitro investigations directed towards establishing mechanistic details at the subcellular or enzyme level. The information available at this level is considerable. However, few attempts have yet been made to determine whether the interactions observed under laboratory conditions (acute doses, nonphysiological routes of exposure, etc.) can occur in human populations exposed to "real-life" environmental or occupational conditions (low level, chronic, oral, dermal, or inhalation exposure), and if they can, whether they constitute a real toxic hazard.

This synopsis contains an outline of some basic mechanisms through which the biotransformation of various toxicants can be modified and an assessment of how these can be evaluated in vivo.

The importance of the hepatic microsomal oxidase system as the primary site for biotransformation of lipophilic foreign compounds has been established. Therefore, the interactions involving this system are emphasized below. However, interactions may involve any enzyme that plays a role in toxicant metabolism.

THE MICROSOMAL MONOXYGENASE SYSTEM

Foreign compounds may interact to induce or to inhibit monoxygenase activity.

Induction

Microsomal enzyme induction has been discussed at length in several recent reviews (Bock and Remmer, 1978; Conney, 1967, 1971; Gelboin, 1971; Remmer, 1972; Sher, 1971; Testa and Jenner, 1976). It may lead to a decrease in toxicity (detoxication) by enhancing the rate of metabolism of a toxicant to inactive products or to an increase in toxicity (intoxication) through enhanced formation of active metabolites.

Inducers. Almost all types of lipophilic compounds will cause some degree of induction (Bock and Remmer, 1978; Conney, 1967, 1971; Gelboin, 1971; Remmer, 1972; Sher, 1971; Testa and Jenner, 1976). In general, high levels of inductions result from exposure to lipophilic materials (e.g., many of the chlorinated hydrocarbon insecticides), which have prolonged biological half-lives. Compounds with low lipophilic character or with short half-lives are not effective inducing agents. Except for the generally established importance of lipophilic character, no structure-activity relationships are apparent in inducing agents.

Inducing agents can be divided into two major groups. Thus, phenobarbital and a large number of other drugs and insecticides cause a rather general increase in the oxidative metabolism of a wide variety of substrates. In contrast, polycyclic hydrocarbons, such as 3-methylcholanthrene, cause an increase in metabolic activity in a more limited spectrum of compounds (Conney, 1967, 1971; Testa and Jenner, 1976).

The different metabolic responses to these two groups of inducing agents are generally attributed to their ability to induce intrinsically different forms of cytochrome P-450, the terminal oxidase of the microsomal electron transport pathway and the catalytic center of the system (Conney, 1967, 1971; Mannering, 1971; Testa and Jenner, 1976). A detailed discussion of these differences is beyond the scope of this report.

Conditions Effecting Induction. The major factors relate to the degree, duration, and route of exposure vis-a-vis the tissue under consideration.

For induction of the hepatic monoxygenase system, there seems to be a general requirement for a prolonged hepatic exposure of the inducing agent (Remmer, 1969). In most induction studies, relatively high concentrations of the inducer are used and little attention is given to evaluating dose-response relationships or to defining the threshold levels of exposure that are required to elicit an effect.

The presence of a threshold concentration of the inducing agent in the tissue is presumably also required for monoxygenase induction in extrahepatic organs and tissues such as lung, skin, intestine, etc. In recent years, considerable attention has been focused on metabolism in these extrahepatic tissues. In some cases, they may be of critical importance in toxicological interactions resulting from inhalation or dermal exposures to combinations of chemical agents. Morever, there is some evidence that qualitative and quantitative differences may exist between the enzymes in these tissues and those in the liver, and including, perhaps, differences in response to inducers (Alvares, 1977; Grafström et al., 1977; Wollenberg and Ullrich, 1977).

To evaluate potential hazards from cargo vapors, it is important to obtain information on the rate and level of accumulation of inhaled chemical vapors, degree and duration of exposure, and the threshold concentrations required to elicit an inductive response in the lung.

Another factor to be considered is the duration of the induction process itself. The inducing action of different compounds begins at different rates and continues after cessation of exposure for various lengths of time. The time at which maximum induction is observed varies from approximately 24 hours after exposure (for 3-methylcholanthrene) to approximately 1-2 weeks after exposure (for the insecticide chlordane) (Testa and Jenner, 1976). Following cessation of exposure to phenobarbital, enzyme activity returns to normal levels within several days, but after exposure to more biologically stable inducers, enzyme activity continues for much longer periods. These effects are clearly related to the biological half-lives of different inducers and their continued presence in the tissue.

Several potent inducing agents actually inhibit drug metabolism during the first few hours after administration.

Tests for Enzyme Induction. Although the mechanistic aspects of induction are complex and incompletely understood and the precise conditions under which induction occurs are still not clearly defined, it is possible to predict the likely in-vivo effects of induction on the biological activity or toxicity of various compounds. Therefore,

if in-vivo tests to detect an induced state could be developed, the potential hazards of exposure to other compounds could be approximated.

Since monoxygenase induction leads to changes in the metabolism of several endogenous materials as well as foreign compounds, several noninvasive tests have been studied for possible use as in-vivo indicators of induction in humans. These tests are based largely on observed changes in steroid and glucose metabolism following induction.

- Steroid Metabolism. Under normal (uninduced) conditions, cortisol, a major adrenal-cortical hormone, is excreted in the urine as its 17-hydroxy derivative. Following treatment with several monoxygenase inducers, the rate of cortisol excretion in the urine is enhanced mainly by an increase in 6β-hydroxycortisol, a product of hepatic oxidase activity that is usually of minor significance. Thus, the ratio of 6β- to 17-hydroxycortisol in the urine has been suggested as a potentially useful indicator for detecting enzyme induction (Roots et al., 1977; Testa and Jenner, 1976) and has been shown to increase significantly following treatment of animals with phenobarbital, phenyl butazone, diphenylhydantoin, and several other drugs (Roots et al., 1977; Testa and Jenner, 1976). No increase in 6β-hydroxycortisol was detected in animals treated with 3-methylcholanthrene (Roots et al., 1977; Testa and Jenner, 1976), suggesting that this indicator cannot be of general utility for all types of inducers. On the other hand, this may indicate its potential usefulness in distinguishing between the effects of the phenobarbital or 3-methylcholanthrene type of inducers.

The potential use of the test in humans has been demonstrated by the increased excretion of 6β-hydroxycortisol (Roots et al., 1977; Testa and Jenner, 1976).

● Glucose Metabolism. A further consequence of exposure to monoxygenase inducers is an increase in the activity of the microsomal enzyme uridine-5'-diphosphate (UDP)-glucuronyl transferase and an increased urinary excretion of D-glucaric acid. This has been observed in several species of animals (Aarts, 1965; Marsh and Reid, 1963; Roots et al., 1977; Testa and Jenner, 1976) and humans (Aarts, 1965; Testa and Jenner, 1976) following treatment with drugs. Although it is only indirectly related to monoxygenase activity per se, it might prove to be a useful in-vivo empirical indicator of induction.

Several other indicators of monoxygenase induction have been investigated in laboratory animals but are unsuitable for routine application to humans. These include measurement of ascorbic acid excretion, which occurs in many species but not in humans, administration of a test drug such as aminopyrine and measurement of its half-life, and in-vitro tests for direct measurement of cytochrome P-450 levels in liver biopsy samples.

In summary, some tests for enzyme induction in humans show promise, but as yet there is no single test or combination of tests that can be used to predict all induction effects likely to be encountered. Furthermore, apparent differences between species preclude simple extrapolation to humans of data obtained with laboratory animals.

A major problem in developing satisfactory in-vivo tests for enzyme induction in humans is the apparently substantial interindividual variation in enzyme activity and the difficulty of establishing a common control level for the various test parameters. Although much of this interindividual variation is due to genetic factors (Vesell, 1977), a large number of variables associated with both the external and internal environment have been identified as potential modifiers of drug metabolism. In many cases these factors could obviate any changes resulting from induction.

Inhibition

The monoxygenase system may be inhibited by several different mechanisms. Numerous compounds have been shown to inhibit the metabolism of other systems both in vivo and in vitro (Anders, 1971; Mannering, 1971; Testa and Jenner, 1976). Since the onset of inhibitory effects is usually observed quite rapidly after administration of a compound, interactions occurring through this basic mechanism may be encountered more often than those resulting from induction.

Alternative Substrate Inhibition. The microsomal enzyme system is noted for its low degree of substrate specificity. When two oxidizable substrates are presented simultaneously to the microsomal system, one can competitively inhibit the other by a process termed alternative substrate inhibition (Anders, 1971; Mannering, 1971; Testa and Jenner, 1976). This is probably a rather short-lasting effect in vivo and will become significant only under acute conditions when the combined concentration of the substrates temporarily

overloads the system causing the substrates to compete for binding sites. This type of inhibition is competitive; the K_m (metabolism constant) of each component when considered as a substrate must equal its K_i (inhibition constant) when considered as an inhibitor. The extent to ich this type of inhibition occurs depends on the relative affinities of the two compounds for the binding sites. Compounds with high affinities and low rates of metabolism can be expected to be the most effective inhibitors of this type. Indeed, some of the best alternative substrate inhibitors are those that undergo little or no metabolism, e.g., perfluorinated hydrocarbons, which cannot be hydroxylated.

Noncompetitive Inhibitory Interactions. The action of most monoxygenase inhibitors occurs at least initially through alternative substrate inhibition. However, several types of compounds are able to exert additional, more intense noncompetitive inhibitory interactions through the formation of a variety of active metabolites which form inhibitory complexes with cytochrome P-450. These compounds are well recognized for their potent inhibition of drug oxidation *in vitro* and for their ability to modify the action of many drugs and insecticides *in vivo*. The well-known drug potentiator SKF-525A owes its activity in part to the formation of such a complex (Schenkman *et al.*, 1972). Other compounds, such as 1,3-benzodioxoles (commercially used as insecticide synergists) (Franklin, 1971; Philpot and Hodgson, 1971) and several amphetamines (Franklin, 1977) form similarly active oxidative metabolites, which complex with and reduce the amount of cytochrome P-450 that is available for further drug

oxidation. The formation of active inhibitory metabolites during drug oxidation is currently receiving much attention. It is likely that other examples will be discovered.

Another group of compounds that undergo monoxygenase-catalyzed activation to form reactive intermediates are those containing thiono-sulfur groups (thiourea, thioacetamide, carbon disulfide, thiobarbital, thiouracil, phosphorothionates, etc.). Several of these compounds inhibit both in-vitro and in-vivo drug oxidation mediated by cytochrome P-450 through the oxidase-catalyzed release of atomic sulfur, which binds covalently to available nucleophiles. The loss of cytochrome P-450 is associated with covalent sulfur binding (Neal et al., 1977).

Covalent binding of a metabolically formed radical intermediate ($^{\cdot}Cl_3$) is also thought to inhibit drug oxidation following exposure to carbon tetrachloride (Diaz Gomez et al., 1973).

Other Inhibitors. Another group of potentially important inhibitors are compounds that can undergo direct ligand binding to cytochrome P-450 because of the unhindered nitrogen atom in their structure. These compounds include several groups of imidazoles and other nitrogen-containing heterocyclic compounds, which are potent inhibitors of drug metabolism both in vitro and in vivo (Wilkinson et al., 1974 a,b).

Biphasic Interactions

Although we tend to discuss inducers and inhibitors of monoxygenase activity as distinct classes of compounds, this distinction should be evaluated carefully.

One important factor to be considered in distinguishing the two effects is the length of time after administration before the effect is observed. The effects of SKF-525A and 1,3-benzodioxoles such as piperonyl butoxide are clearly biphasic. Their initial acute inhibitory action on microsomal metabolism is usually followed by a marked stimulation of the metabolism (Testa and Jenner, 1976; Wilkinson, 1976). Thus, a compound classified as an inhibitor when its effect is measured from 0.5 to 12 hours after administration would be termed an inducer if its effect was not measured until 24 to 48 hours afterward. Similarly, as mentioned earlier, several compounds classified as potent inducers of monoxygenase activity actually inhibit enzyme activity during the period immediately following administration.

We must conclude, therefore, that time after exposure is an extremely important factor that is often given little or no attention in interaction studies. This adds to the complexity of the problem by suggesting that we have concerned ourselves mainly with compounds at either extreme of the spectrum. The compounds we believe to be good inducers may simply be those with a relatively short inhibitory phase, while those we believe to be inhibitors may simply be inducers that exhibit a longer inhibitory phase before induction becomes obvious. There may be many shades of gray between these two extremes.

CHANGES IN ACTIVITIES OF OTHER ENZYMES INVOLVED IN BIOTRANSFORMATION

The precise mechanism by which induction of mixed-function oxidase activity occurs is not fully understood. One complication is that the induction process is not specific for the system mediated by cytochrome P-450. Thus, exposure of animals to established inducers

of mixed-function oxidase activity often results in the coinduction of several other enzymes that appear to be unrelated to the microsomal oxidase system. The complete spectrum of enzymes that are induced by various foreign compounds has not yet been studied in detail, and there is currently no way to predict them.

Since some of the other induced enzymes may also play a role in the detoxication of various toxicants, an entirely new area of potentially important interactions remains to be studied. For example, there is a marked induction of serum and liver aliesterase activity in animals exposed to several drugs and insecticides (Cohen and Murphy, 1974) that are known inducers of the hepatic microsomal oxidase system. This leads to several unexpected interactions between such inducers as paraoxon and other organophosphate insecticides that are bound to aliesterase. Exposure of animals to the chlorinated insecticides aldrin or DDT (both known inducers of mixed-function oxidase) causes an increase in aliesterase titer which in turn protects animals from poisoning by paraoxon (Triolo et al., 1970).

Aliesterase is itself an important detoxication enzyme for several foreign compounds containing carboxyester functional groups, and its inhibition by organophosphates is a well-known and well-studied mechanism by which the toxicity of compounds such as malathion can be potentiated (Wilkinson, 1976).

Toxicological interactions could also occur through depletion by one chemical of a cofactor or cosubstrate that is required for the biotransformation of another. Thus, the anesthetic fluroxene ($CF_3CH_2OCH{=}CH_2$), but not several related compounds, caused a

significant dose-dependent reduction in tissue levels of glutathione, which could affect glutathione-dependent metabolism of a variety of other materials, e.g., 1,1-dichloroethylene). However, in view of the high endogenous levels of glutathione in tissue it is unlikely that such interactions will occur except under conditions of unusually high acute exposures.

CONSEQUENCES OF INTERACTIONS INVOLVING BIOTRANSFORMATION

The consequence of chemical interactions may be to potentiate or to antagonize the toxicity of one or more components (Gillette and Mitchell, 1975; Shand et al., 1975). Toxicological interactions occur when one chemical, A, inhibits or stimulates enzymes responsible for the metabolism of another, B. Where toxicant B is inactivated (detoxified) by a given enzyme system, compound A may interact to inhibit or stimulate enzyme activity, thus leading to potentiation or antagonism of toxicity, respectively. If, on the other hand, B is metabolically activated to a toxic species, the opposite will result.

INTERACTIONS WITH FORMATION OF REACTIVE INTERMEDIATES

Reaction Mechanisms

Recent evidence indicates that an increase in the formation of toxic metabolites may be a particularly important interaction after repeated low-level exposures to multiple chemicals (Jerina and Daly, 1974; Magee and Barnes, 1967; Miller, 1970; Mitchell and Corcoran, 1977; Mitchell et al., 1976, 1977). Other endogenous or exogenous compounds that alter the formation of these reactive species or that react with the biologically active form to spare critical tissue sites of action may modify the toxicity of chemicals that form reactive intermediates as the following examples illustrate.

Epoxides are among the reactive intermediates formed from a wide variety of aromatic compounds. Toxicity due to epoxide formation requires the P-450-mediated conversion of an unsaturated compound to a reactive epoxide, followed by reaction of the epoxide with critical cellular components. Normal cellular reactions of an epoxide include nonenzymatic conversion to a phenol (for benzenoid epoxides), enzymatic and perhaps nonenzymatic reactions with glutathione and with water (epoxide hydratase), and possibly nonenzymatic reactions with other thiols. As far as is known, radical mechanisms of reaction are not involved. Toxic nonenzymatic reactions with critical cell components are likely to occur only under specific circumstances. Depletion of glutathione or inhibition of epoxide hydratase should greatly increase the toxic effects of a reactive epoxide.

Many o- and p-quinones and, recently, quinonimines have been shown to react readily with nucleophiles such as thiols and amines to form isolable addition products. Although reactions of this type have been unquestionably valuable to the synthetic organic chemist for many decades, their significance in the disposition of chemicals and drugs by the body has been appreciated only recently.

Numerous drugs and their metabolites contain catechol or 1,4-dihydroxy functions within their structure. Although these reduced forms do not react readily with nucleophiles, cellular oxidation of these agents to reactive quinone structures followed by addition reactions with endogenous nucleophiles has led to the isolation of glutathione, cysteine, and mercapturic acid conjugates in bile or urine. On the other hand, endogenous cellular nucleophiles such as

glutathione should play key roles in the pathogenesis of cellular injury caused by such metabolites. Finally, further oxidation of diol drugs or their metabolites to specific triol configurations may result in striking exacerbation of the toxicity of these agents through mechanisms not involving adduct formation (see discussion of triols below).

The reactivity of free radicals covers a wide spectrum. Individual reactivity is ultimately determined by the structure of the radical and its environment. Alkyl and acyl radicals, which have been suspected as metabolites of certain drugs, have been found to be particularly reactive. When generated under biological conditions, alkyl radicals can undergo numerous reactions, including hydrogen abstraction (to produce alkanes), addition to multiple bonds, and cross-linking reactions. Although newly formed radicals resulting from propagation reactions (e.g., hydrogen abstraction) may react with oxygen to form hydroperoxyl radicals, reaction with neighboring groups, such as unsaturated sites, would be expected to predominate.

Evidence indicates that alkyl and acyl radicals are the important toxic metabolic products of P-450 oxidation of alkyl- and acylhydrazines. The reaction of O_2^- with alkyl halides (e.g., alkyl bromides, carbon tetrachloride, trichlorobromomethane, and certain general anesthetic gases), sulfates, and phosphates may also result in the formation of alkyl radicals. Although vitamin E is recognized as a radical acceptor, it is likely that alkyl radicals would react very rapidly with neighboring molecules and would not necessarily react selectively with vitamin E. Glutathione should react with alkyl radicals and might have some

protective effect, but this may also not be a selective or biologically important reaction. Exogenous compounds that act as radical scavengers may also be expected to modify the toxic action of compounds that act through a free radical mechanism.

The generation of hydroperoxyl radicals within the body is likely to lead to a high degree of toxicity and a variety of products. The initial step is presumably a P-450 oxidation to generate ROO·, which can react with unsaturated compounds to form a variety of products and undergo cross-linking reactions. Secondary reactions of radicals with polyunsaturated fats may produce hydroperoxides, hydroxy acids, and hydroxyepoxy acids. Reactions of these species with membrane phospholipids could be critical to the normal functions of the cell and cellular response to toxic insult by reactive oxygen species.

Vitamin E should protect phospholipids in membranes against oxidation by hydroperoxide. It is doubtful that vitamin E could protect against reactions occurring at sites other than those associated with membranes. Glutathione may have some protective effect through a termination reaction.

Reactive Oxygen Species Produced from Reactive Metabolites

The generation of reactive oxygen species is most likely based upon the reaction of cellular oxygen with a one-electron intermediate that may be acidic, basic, or neutral, but which will transfer an electron to oxygen to form O_2^-. Examples of toxicity of this type include nitrofurantoin, in which the observed toxicity is apparently due to O_2^- formation, certain triols with a 1,2,4 or 1,3,4 relationship,

which may be capable of generating superoxide ions through a one-electron intermediate that corresponds to a semiquinone, and paraquat for which toxicity is presumably related to the one-electron reduction of oxygen to the superoxide anion.

It is apparent that an interdisciplinary, integrated approach is necessary in order to correlate the formation of chemically reactive metabolites with the incidence, types, and severities of toxicities caused by drugs and other foreign compounds. Studies of covalent binding should also be useful in determining whether alterations in the incidence and severity of various toxicities are due to differences in the metabolism of the foregin compound or to changes in the events that follow the formation of the reactive metabolite.

On the other hand, a survey (Table 4-1) of the different types and locations of the tissue lesions produced by reactive drug metabolites indicates that at least four types of reactive species causing tissue lesions can be postulated: electrophilic intermediates showing significant glutathione conjugation in vivo (e.g., bromobenzene, acetaminophen, 2-furamide); electrophilic species not showing significant glutathione conjugation in vivo (e.g., furosemide, dimethylnitrosamine); alkylating radicals (e.g., carbon tetrachloride, alkyl- and acylhydrazines); and nonalkylating reactive intermediates whose toxicities are potentiated by vitamin E-deficient diets. Thus, it is clear that additional tools and approaches are needed for the study of chemically induced tissue lesions caused by the formation of reactive, nonalkylating species.

TABLE 4-1

Nature of Chemically Reactive Metabolites

Electrophiles Alkylators GSH[a] Threshold Sulfhydryl Protection	Electrophiles Alkylators No GSH Threshold No Sulfhydryl Protection	Radicals and Hydroperoxides Alkylators No GSH Threshold Vitamin E Protection[b]	Reactive Oxygen Species Nonalkylators No GSH Threshold Vitamin E Protection
Acetaminophen	Furosemide	Hydrazines	Paraquat
Phenacetin	Dimethylnitrosamine	Haloalkanes	Nitrofurantoin
Acetanilide			Methyldopa[b]
Halobenzenes			Salicylates[b]
Simple Furans			
Simple Thiophenes			

[a]GSH = reduced glutathione.

[b]Protection is questionable.

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PRINCIPLES OF TOXICOLOGICAL INTERACTIONS ASSOCIATED WITH MULTIP--ETC(U)
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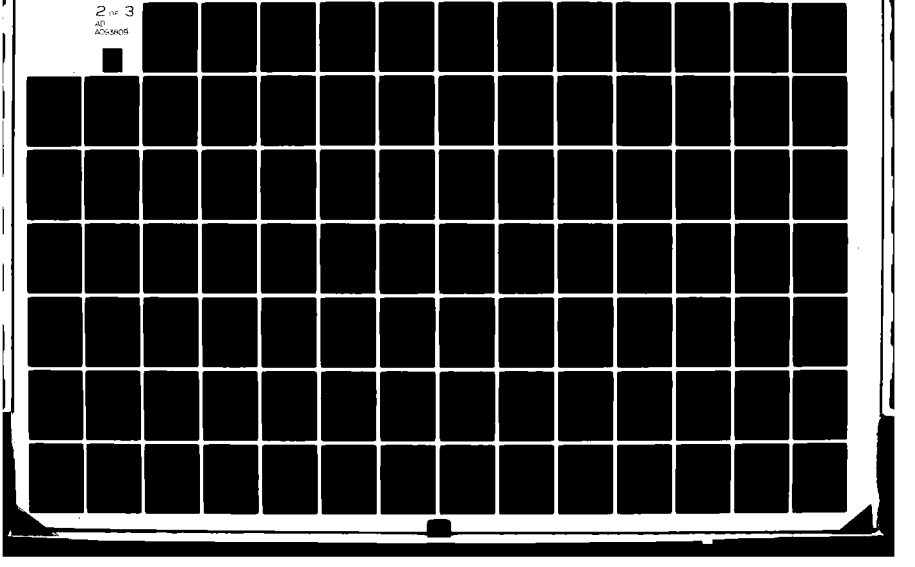

Careful consideration of the types of electrophilic and radical metabolites of chemicals that might be formed, and their likley chemical reaction mechanisms with tissue molecules and other exogenous chemicals, should make it increasingly possible to examine and define the molecular basis for chemically induced tissue injuries. Such knowledge should also make it possible to predict with greater certainty the likelihood of chemical-chemical interactions producing adverse health effects in humans from either simultaneous or sequential exposure.

REFERENCES

Aarts, E. M. 1965. Evidence for the function of D-glucaric acid as an indicator for drug induced enhanced metabolism through the glucuronic acid pathway in man. Biochem. Pharmacol. 14:359-362.

Alvares, A. P. 1977. Stimulatory effects of polychlorinated biphenyls (PCB) on cytochromes P-450 and P-448 mediated microsomal oxidations. Pp. 476-483 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Anders, M. W. 1971. Enhancement and inhibition of drug metabolism. Ann. Rev. Pharmacol. 11:37-56.

Bock, K. W., and H. Remmer. 1978. Introduction to hepatic hemoproteins. Pp. 49-80 in F. DeMatteis and W. N. Aldridge, eds. Handbook of Experimental Pharmacology, Vol. 44. Heme and Hemoproteins. Springer-Verlag, Berlin.

Cohen, S. D., and S. D. Murphy. 1974. A simplified bioassay for organophosphate detoxification and interactions. Toxicol. Appl. Pharmacol. 27:537-550.

Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19:317-366.

Conney, A. H. 1971. Environmental factors influencing drug metabolism. Pp. 253-278 in B. La Du, H. G. Mandel, and E. L. Way, eds. Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins, Baltimore.

Diaz Gomez, M. I., J. A. Castro, E. C. de Ferreyra, N. D'Acosta, and C. R. de Castro. 1973. Irreversible binding of ^{14}C from $^{14}CCl_4$ to liver microsomal lipids and proteins from rats pretreated with compounds altering microsomal mixed-function oxygenase activity. Toxicol. Appl. Pharmacol. 25:534-541.

Franklin, M. R. 1971. The enzymic formation of a methylenedioxyphenyl derivative exhibiting an isocyanide-like spectrum with reduced cytochrome P-450 in hepatic microsomes. Xenobiotica 1:581-591.

Franklin, M. R. 1977. The inhibition of mixed-function oxidation reactions by amphetamines in liver and lung microsomes. Pp. 284-291 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Gelboin, H. V. 1971. Mechanics of induction of drug metabolism enzymes. Pp. 279-307 in B. La Du, H. G. Mandel, and E. L. Way, eds. Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins, Baltimore.

Gillette, J. R., and J. R. Mitchell. 1975. Drug actions and interactions: Theoretical considerations. Pp. 359-382 in J. R. Gillette and J. R. Mitchell, eds. Handbook of Experimental Pharmacology, Vol. XXVIII, Part 3. Springer-Verlag, New York.

Grafström, R., S. J. Stohs, M. D. Burke, P. Moldeus, and S. Orrenius. 1977. Benzo(α)pyrene metabolism by microsomes and isolated epithelial cells from rat small intestine. Pp. 667-674 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Jerina, D. M., and J. W. Daly. 1974. Arene oxides: A new aspect of drug metabolism. Science 185:573-582.

Magee, P. N., and J. M. Barnes. 1967. Carcinogenic nitroso compounds. Adv. Cancer Res. 10:163-246.

Mannering, G. J. 1971. Inhibition of drug metabolism. Pp. 452-476 in B. B. Brodie and J. R. Gillette, eds. Handbook of Experimental Pharmacology, Vol. 28, Concepts in Biochemical Pharmacology, Part 2. Springer-Verlag, New York.

Mannering, G. J. 1971. Properties of cytochrome P-450 as affected by environmental factors: qualitative changes due to administration of polycyclic hydrocarbons. Metabolism 20:228-245.

Marsh, C. A., and L. M. Reid. 1963. Changes in D-glucaric acid excretion induced by stimulators of ascorbic acid biosynthesis. Biochim. Biophys. Acta 78:726-728.

Miller, J. A. 1970. Carcinogenesis by chemicals: An overview-- G. H. A. Clowes Medmorial Lecture. Cancer Res. 30:559-576.

Mitchell, J. R., and G. B. Corcoran. 1977. Macromolecular binding in assessing drug and chemical-induced tissue lesions. Proc. of the Conference on the Status of Predictive Tools in Application to Safety Evaluation: Present and Future (Carcinogenesis and Mutagenesis). J. Environ. Pathol. Toxicol. 1, Special Issue:101-115.

Mitchell, J. R., S. D. Nelson, S. S. Thorgeirsson, R. J. McMurtry, and E. Dybing. 1976. Metabolic activation: Biochemical basis for many drug-induced liver injuries. Pp. 259-279 in H. Popper and F. Schaffner, eds. Progress in Liver Diseases, Vol. V. Grune & Stratton, New York.

Mitchell, J. R., R. J. McMurtry, C. N. Stathan, and S. D. Nelson. 1977. Molecular basis for several drug-induced nephropathies. Am. J. Med. 62:518-526.

Neal, R. A., T. Kamataki, A. L. Hunter, and G. Catignani. 1977. Monooxygenase catalyzed activation of thiono-sulfur containing compounds to reative intermediates. Pp. 467-475 in V. Ullrich, A. Hildebrandt, I. Roots, K. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Philpot, R. M., and E. Hodgson. 1971. A cytochrome P-450-piperonyl butoxide spectrum similar to that produced by ethyl isocyanide. Life Sci. 10, Pt. II:503-512.

Remmer, H. 1969. The induction of hydroxylating enzymes by drugs. Pp. 125-141 in D. Shugar, ed. Biochemical Aspects of Antimetabolites and Drug Hydroxylation. Federation of European Biochemical Societies Symposium Vol. 16. Academic Press, London.

Remmer, H. 1972. Induction of drug metabolizing enzyme system in the liver. Eur. J. Clin. Pharmacol. 5:116-136.

Roots, I., B. Ley, and A. G. Hildebrandt. 1977. *In vivo parameters* of drug metabolism--differences in specificity towards inducing agents. Pp. 581-588 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Schenkman, J. B., B. J. Wilson, and D. L. Cinti. 1972. Diethylaminoethyl 2,2-diphenylvalerate HCl (SKF 525-A)-in vivo and in vitro effects of metabolism by rat liver microsomes--formation of an oxygenated complex. Biochem. Pharmacol. 21:2373-2383.

Shand, D. G., J. R. Mitchell, and J. A. Oates. 1975. Pharmacokinetic drug interactions. Pp. 272-314 in J. R. Gillette and J. R. Mitchell, eds. Handbook of Experimental Pharmacology, Vol. XXVIII, Part 3. Springer-Verlag, New York.

Sher, S. P. 1971. Drug enzyme induction and drug interactions: literature tabulation. Toxicol. Appl. Pharmacol. 18:780-834.

Testa, B., and P. Jenner. 1976. Chapter 2.2 Induction and inhibition of drug-metabolizing enzyme systems. Pp. 329-350 in B. Testa and P. Jenner, eds. Drug Metabolism: Chemical and Biochemical Aspects. Marcel Dekker, New York.

Triolo, A. J., E. Mata, and J. M. Coon. 1970. Effects of organochlorine insecticides on the toxicity and in vitro plasma detoxication of paraoxon. Toxicol. Appl. Pharmacol. 17:174-180.

Vesell, E. S. 1977. Effects of disease states on drug deposition in man. Pp. 628-645 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Wilkinson, C. F. 1976. Chapter 15. Insecticide interactions. Pp. 605-647 in C. F. Wilkinson, ed. Insecticide Biochemistry and Physiology. Plenum Press, New York.

Wilkinson, C. F., K. Hetnarski, G. P. Cantwell, and F. J. Di Carlo. 1974a. Structure-activity relationships in the effects of 1-alkylimidazoles on microsomal oxidation in vitro and in vivo. Biochem. Pharmacol. 23:2377-2386.

Wilkinson, C. F., K. Hetnarski, and L. J. Hicks. 1974b. Substituted imidazoles as inhibitors of microsomal oxidation and insecticide synergists. Pestic. Biochem. Physiol. 4:299-312.

Wollenberg, P., and V. Ullrich. 1977. Characterization of the drug monooxygenase in the mouse small intestine. Pp. 675-679 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

CHAPTER 5

INTERACTIONS AT STORAGE SITES

Various parts of the body serve as sites for the storage of selectively accumulated chemicals. These sites include plasma proteins and other extracellular depots such as connective tissues, bone, intracellular fluids, and fat. During storage, no biological reactions are expressed. Consequently, such reservoirs may be considered "silent receptors" or "sites of loss" (Levine, 1973).

The portion of a chemical that is bound to a silent receptor is in equilibrium with the active portion in the plasma, and the release of the bound chemical occurs as plasma concentrations are reduced through biotransformation or excretion. Thus, the effective plasma level of a chemical may be maintained for a prolonged period, and the physiological and potential toxicological effect may be correspondingly prolonged.

The propensity of a chemical to bind to a silent receptor is governed by the same principles that determine its reaction with active tissue binding sites (Fingl and Woodbury, 1975). These include the affinity of the specific chemical for that receptor as well as the strength and reversibility of the bond formations involved. In addition, such properties as lipid solubility and affinity for active transport processes may determine the amount of chemical found in tissue storage sites. When two or more chemicals with similar properties bind to the same storage site, toxicological

interactions between those chemicals are governed by the extent to which the chemicals compete with each other for that site.

PLASMA PROTEIN AS A STORAGE SITE

When plasma protein, such as albumin, is the site of storage for two or more chemicals, the extent of interaction depends principally upon the strength of the reversible bonds that are formed between the chemicals and the silent receptor and the affinities of the chemicals for that binding site. This point is particularly important if a chemical is strongly bound to a storage site receptor. Thus, if the affinity of a chemical is such that 90% or more is bound to plasma protein, displacement of that chemical by another, producing even a minor percentage change in protein binding, could result in a doubling or tripling of the plasma concentration of the chemical. That increased concentration would be free to exert a toxicological effect. In contrast, a chemical with only a slight affinity for a storage tissue receptor site may be completely displaced by a chemical of higher affinity with relatively minor toxicological consequences.

INTRACELLULAR ACCUMULATION

Many chemicals accumulate in higher concentrations within cells than they do in extracellular fluids. If the intracellular concentration of a chemical is high, the tissue involved may serve as a large storage depot. Accumulation within cells may be brought about by binding of the chemical to intracellular tissue constituents, such

as proteins or phospholipids. It sometimes involves active transport into the cell. Toxicological interactions between chemicals that bind principally to intracellular storage sites may occur when one chemical displaces or prevents another from binding in a manner similar to that involving plasma proteins. They may also occur when a chemical that is normally transported to intracellular storage sites by active processes is prevented from being transported by other chemicals, which block or compete for those processes.

STORAGE IN NEUTRAL BODY FAT

Finally, many chemicals have a high lipid solubility and are stored to a large extent in neutral body fat. Chemicals such as DDT and many nonpolar organic solvents, which have high lipid water partition coefficients, are particularly prone to storage in fatty tissues. In this case, the possibility of toxicological interactions between two or more such chemicals can be measured in terms of their relative lipid solubilities and the extent to which displacement of one chemical by another from fatty tissues increases the availability of unbound chemical, which is free to exert toxicity to the host.

SUMMARY

In general, storage reservoirs within the body permit binding of most chemicals without ensuing toxicity from saturation of silent receptors. Therefore, toxicological interactions are most commonly manifested as a result of exposure to high concentrations of chemical mixtures or during prolonged or continuous exposure to such substances.

REFERENCES

Fingl, E., and D. M. Woodbury. 1975. Chapter 1. General principles. Pp. 1-42 in L. S. Goodman and A. Gilman, eds. The Pharmacological Basis of Therapeutics, 5th edition. Macmillan, New York.

Levine, R. R. 1973. Chapter 5. How drugs reach their site of action. II: Absorption and distribution. Pp. 71-111 in Pharmacology: Drug Actions and Reactions. Little, Brown, and Company, Boston.

CHAPTER 6

INTERACTIONS AT TARGET SITES

This chapter describes the principles governing identification of toxicological interactions involving target enzymes and covalent binding at sites of action.

CHEMICAL BINDING AT TISSUE RECEPTORS: GENERAL CONSIDERATIONS

Chemicals, alone or in combination, produce effects on living organisms primarily through reactions with functionally important receptor molecules that act as target sites for those chemicals. Most chemicals act selectively by combining only with certain receptors, such as enzymes or other macromolecular tissue elements, and showing specific binding characteristics with that receptor. The ability of chemicals to alter either the physicochemical nature of these tissue receptors or the specific binding characteristics of other chemicals forms the basis for defining the principles of toxicological interactions between two or more chemicals within cells.

Before specific toxicological interactions between chemicals can be considered, the nature of the biological interaction between a chemical and its tissue receptor must be defined. A principal characteristic of such an interaction is that it is sufficiently strong to initiate an action-effect sequence. For most chemicals, this would mean a reversible reaction requiring the synchronous operation of various binding forces. The first force to be exerted as a chemical approaches a receptor must overcome the random thermal

agitation of the chemical molecule and draw it to its site of action. The binding forces usually involved in this reaction are ionic bonds formed by electrostatic attraction. Although the formation of one or two ionic bonds may be sufficient to initiate a chemical-receptor combination, the strength of these bonds by themselves is insufficient to hold the molecule in combination long enough to promote an action-effect sequence. Therefore, the additional attraction of other forces, such as hydrogen bonds and Van der Waal's forces, are also required to give the chemical-receptor combination the stability that is essential for chemical action.

The formation of one or even two ionic bonds is also insufficient to confer significant specificity or selectivity upon a chemical-receptor interaction because the receptor requires not only unique physicochemical properties, such as charge, but also a definite structural conformation in order to account for specificity. Thus, the operation of electrostatic attractions of hydrogen bonds and the binding of Van der Waal's forces are also required to maintain these conditions. Although, Van der Waal's forces are the weakest of all the binding attractions, they have the most critical dependence upon the interatomic distance between reactive molecules. Hence, they are the major contributing forces in determining the specificity of chemical-receptor interactions (Levine 1973 a,b).

Infrequently, covalent bonds are also involved in chemical-receptor binding. Because the covalent bond is many times stronger than the ionic bond and the other forces usually involved in chemical-receptor interactions, covalent bonds involve reactions that are essentially irreversible at ordinary body temperatures. Thus, covalent binding

to tissue receptors is characteristic of long-lasting chemical reactions and usually requires synthesis of new tissue receptors before normal biological function can be recovered.

The tendency, or affinity, or any chemical for binding to a tissue receptor is inherent in its molecular structure. In reversible reactions, which involve all but covalent binding forces, that property is governed by the law of mass action, i.e., that the fraction of a chemical that is bound to a receptor site is in equilibrium with the fraction of chemical that is free (Barrow, 1961 a,b). The relationship between the concentration of the chemical and the chemical-receptor complex, which produces its biological effect, may be shown as follows:

$$C + (100 - X) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X$$

where C is the chemical concentration, X is the percentage of the total number of receptors occupied by the chemical, and (100 - X) is the percentage of unoccupied receptors.

The rate at which the chemical combines with unoccupied receptors is proportional to the product of the chemical concentration and the concentration of unoccupied receptors:

$$k_1 C(100 - X)$$

where k_1 is a constant of proportionality.

The rate of dissociation of the chemical receptor complex is proportional to X:

$$k_2X$$

where k_2 is the specific constant for the reverse reaction. At equilibrium, the rate of combination is equal to the rate of dissociation:

$$k_1C(100 - X) = k_2X$$

Thus,

$$C = \frac{k_2}{k_1} X \frac{X}{(100 - X)} \text{ or } C = \frac{X}{K_e (100 - X)} ,$$

where $K_e = \frac{k_1}{k_2}$ = equilibrium (affinity) constant of the particular reaction.

Thus, the greater the affinity constant of an agent for its receptor, the greater its propensity to bind with that receptor and to produce a subsequent biological effect. This principle is essential in order to explain or predict the effects of interaction of two or more chemicals that act at the same tissue receptor (Goldstein et al., 1968). Given equal intrinsic activities, the substance possessing the greatest physicochemical affinity for receptor binding would be expected to elicit the most pronounced biological response at that receptor. Moreover, because of its ability to displace other chemicals from sites of activation or deactivation, the agent possessing the higher binding affinity would also be expected to antagonize or act synergistically with chemicals producing those effects (Fingl and Woodbury, 1970).

ENZYMES AS TARGET SITES OF TOXICOLOGICAL INTERACTIONS

The principles discussed in the previous section are directly applicable to the role of enzymes as cellular target sites for chemical interactions. A principal biological manifestation of chemical interactions at enzymatic receptor sites is the alteration of biological transformation reactions involving either acceleration or deceleration of these processes. The physicochemical forces that regulate the binding of chemicals to enzyme receptors and the ensuing enzymatic reactions dictated by the law of mass action underlie the basic principles by which interactions of chemicals at enzymatic target sites may be understood.

To catalyze a reaction, an enzyme must be able to combine with its substrate. Hence, any agent that interferes with the access of a substrate to active enzyme binding sites will also decrease the rate of metabolism, even if the concentration of the enzyme is normal. The metabolic rate of one chemical may be decreased by another in several ways. In competitive enzyme-substrate interactions, the metabolic rate of one chemical may decrease when another chemical is also a substrate for and successfully competes for the same active site of that enzyme. Competitive inhibition may also occur when one chemical combines reversibly with the active site of the enzyme by virtue of its structural similarity to another chemical, which also acts as substrate. In both cases the extent of inhibiton is dependent upon the concentration of each chemical at the active site of the

enzyme as well as on their respective binding affinities for that site. Such competitive interactions may be represented as follows:

$$E + S_1 + S_2 \rightleftharpoons ES_1 + ES_2 \rightleftharpoons E + P_1 + P_2$$

$$E + S_1 + I \rightleftharpoons ES_1 \pm EI_2 \rightleftharpoons E + P_1 + I$$

where E is enzyme, S_1 and S_2 are chemical substrates, I is a non-substrate, and P_1 and P_2 are end products of the metabolism of E_1 and E_2, respectively.

Inhibition reactions involving enzymes as target sites for chemical interactions may also occur when one chemical is unrelated in structural or physical properties to another but is capable of binding with an enzyme, thereby preventing formation of an enzyme-substrate complex. Such noncompetitive inhibition is typically observed in chemical interactions involving heavy metals or organic phosphate insecticides. It may be either reversible or irreversible and may, therefore, involve covalent binding of chemicals to enzyme target molecules. Since noncompetitive inhibitors do not combine with the enzyme in the same manner as the chemical that acts as substrate, such inhibition depends only on the concentration of the inhibitor. However, neither the binding affinities of the various chemicals nor their respective concentrations in the cell greatly affect the nature of the direct interactions between the chemicals.

Stimulation of the enzymatic metabolism of one chemical by another is an additional mechanism by which enzymes may act as target sites for chemical interactions. This process is usually the

consequence of an increased rate of protein synthesis resulting in new enzyme formation and, hence, an increase in the concentration of enzyme in the cell. However, a change in the structural conformation of an enzyme by one chemical, leading to increased enzymatic activity, may also occur. The former situation generally involves reversible binding of inducing chemicals to nuclear binding sites. Therefore, the extent of induction of new enzyme synthesis would be dependent upon the physicochemical properties of the chemicals that determine their access and affinities for binding to those sites. This manifestation of chemical interaction reflects the properties of both the inducing chemicals and the substrate in interacting with the enzyme. Any direct interaction between inducing and substrate chemicals would also be a determinant of the outcome of an enzymatic process involving more than one chemical substance.

Finally, amplification of biological processes resulting from the action of two or more chemicals may reflect the operation of more than one enzyme as target sites for those chemicals. For example, if two chemicals form reactive complexes with two different enzymes or with different sites on the same enzyme, a combination of the effects of those chemicals should be observed. In this case, the principles governing the binding of chemicals to their respective enzyme binding sites and the law of mass action regarding the ensuing reactions would dictate the extent of the combined effects, as they do with other types of reversible chemical interactions.

COVALENT BINDING AS AN INDEX OF TARGET SITE FOR TOXICOLOGICAL INTERACTIONS

A covalent bond is formed when two atoms share a pair of electrons. It has a typical bond energy of approximately 100 kcal/mol. Because of their high binding energy, covalent bonds are essentially irreversible at ordinary body temperature unless a catalytic agent such as an enzyme intervenes. Such reactions represent chemical interactions that often result in toxic consequences of prolonged duration.

One of the principal mechanisms of chemical interactions involving covalent bonding is observed in irreversible reactions with enzymes. Two types of irreversible enzyme inhibitors operate by formation of a covalent bond (Baker, 1970). The first type reacts with an essential functional group on the enzyme by a bimolecular process:

$$E + IX \xrightarrow{k_a} EI + X$$

where X is a leaving group of the inhibiting chemical, E is the enzyme, and I is the inhibitor.

This process has little specificity since all groups on the surface of all enzymes with the nucleophilic capability to do so will react at varying rates, depending on their rate constant, k_a.

The second type of irreversible inhibition involving covalent bond formation is:

$$E + IX \underset{K_i}{\rightleftharpoons} EIX \xrightarrow{k_b} EI + X$$

In this case, the enzyme, E, forms a reversible complex with the inhibitor, I, which bears the leaving group, X. K_i is the inhibitor

constant, and k_b is the bimolecular rate constant. If a nucleophilic group on the enzyme is closely juxtaposed with the reversible enzyme-inhibitor complex, EIX, then a rapid neighboring group reaction can occur within the complex. Such covalent bond formation can be highly specific since properly positioned neighboring groups can react many orders of magnitude more rapidly than the identical biomolecular reaction (i.e., $k_b > 10^3 k_a$). This highly specific reaction with an enzyme-inhibitor reversible complex has been referred to as "active site-directed irreversible inhibition" or "affinity labeling". Such reactions have an extra dimension of specificity dependent on k_b that does not exist with reversible inhibitors. This is known as the "bridge principle of specificity."

There are two classes of active site-directed irreversible enzyme inhibitors. The first class operates by forming a covalent bond within the active site (endomechanism). The second class forms a covalent bond outside the active site (exomechanism). An example of the first class is the L-glutamine antagonist, L-azaserine, which specifically alkylates a single cysteine in the active site of the enzyme that converts formylglycinamide ribonucleotide to its amidine. The exomechanism is illustrated by the inactivation of glutamate dehydrogenase by 4-(iodoacetamido)salicylic acid. Clearly, two or more chemicals reacting irreversibly with enzyme target sites by either of these covalent binding mechanisms could inibit or inactivate multiple biological processes with subsequent toxic effects throughout their presence in the organism.

Chelate formation is another mechanism by which covalent binding may be important as an index of target site for chemical interactions

in biological systems. This process, which entails the formation of five- or six-membered ring complexes involving coordinate covalent bonds, is especially important in chemical interactions with metals.

Coordinate covalent bond formation occurs when both electrons of the electron pair that forms the bond between two atoms are donated by the same atom. In biological systems, the donor atom is usually nitrogen, oxygen, or sulfur since these elements contain a pair of s-orbital electrons, usually unshared when the valence electrons have participated in bond formation.

The stability of chelate complexes may vary greatly depending on the nature of the chelating agent and the metal. Stability is expressed quantitatively by the stability (equilibrium) constant in the mass action law equation, discussed above, for the equilibrium relationship between the free and complexed reactants. For any given chelating agent, the magnitude of the stability constants is determined largely by the atomic structures of the various metals involved. A metal with a high stability constant would effectively compete with a metal of lower stabililty for the chelating agent. Given sufficient time, it would displace the less tightly bound metal from complexes already formed.

Naturally occurring chelates play an important role in biological systems. Perhaps the primary essentiality of some metals for life rests in their ability to form functional chelate complexes. Such natural chelates as heme and various metalloporphyrins, for example, are well suited to act as bridges to facilitate electron transfer, which must

occur in intermediary metabolism. Therefore, substitution of non-essential metals in chelation complexes that perform essential biological functions may either diminish or abolish that function. Moreover, they may produce biological reactions that are completely different from those that are compatible with the life of the cell.

The covalent binding of metals to tissue receptors as sites of potential interactions between chemicals is of particular importance when considering binding to sulfhydryl groups in proteins and other macromolecules. Bonds between sulfur and metal ions are very strong, as reflected in the essentially irreversible character of metal sulfides such as those of lead, mercury, and silver. When such reactions involve enzymes that require sulfhydryl groups as part of their active centers, profound biological consequences may ensue. Pronounced toxic effects may also result from the covalent binding of metals to sulfhydryl or specific sulfur-sulfur bonds, which are necessary to the maintenance of protein structure and configuration.

Finally, the formation of tightly bound chemical complexes, which affect biological processes without interacting with specific tissue receptors, represents another mechanism by which covalent binding might be important as an index of target site in chemical interactions. The extracellular formation of the complex between edetate (EDTA) and various metals illustrates how the biological effects of individual chemicals can be modified through such an interaction. Underlying this mechanism is the principle that the attractive force, i.e., the binding affinity, of EDTA for a metal

is many orders of magnitude greater than that between the chemical and the biological tissue. A corollary to this principle is that covalent binding may sometimes prevent or reverse the potential toxicity caused by chemical reactions with tissue components.

REFERENCES

Baker, B. R. 1970. Specific irreversible enzyme inhibitors. Ann. Rev. Pharmacol. 10:35-50.

Barrow, G. M. 1961a. Chapter 8. Introduction to the theory of chemical bonding. Pp. 185-221 in Physical Chemistry. McGraw-Hill, New York.

Barrow, G. M. 1961b. Chapter 9. The nature of the chemical bond. Pp. 222-247 in Physical Chemistry. McGraw-Hill, New York.

Fingl, E. and D. M. Woodbury. 1970. Chapter 1. General principles. Pp. 1-42 in L. S. Goodman and A. Gilman, eds. The Pharmacological Basis of Therapeutics, 4th edition. Macmillan, New York.

Goldstein, A., L. Aronow, and S. M. Kalman. 1968. Binding forces in the drug-receptor interaction. Pp. 3-25 in Principles of Drug Action: The Basis of Pharmacology. Harper and Row, New York.

Levine, R. R. 1973a. Chapter 3. How drugs act on the living organism. Pp. 27-47 in Pharmacology: Drug Actions and Reactions. Little, Brown, and Company, Boston.

Levine, R. R. 1973b. Chapter 10. Factors modifying the effects of drugs in individuals. Pp. 261-291 in Pharmacology: Drug Actions and Reactions. Little, Brown, and Company. Boston.

CHAPTER 7

IMPORTANCE OF THE SEQUENCE OF TISSUE INJURY AND RECOVERY

Many aspects of the acute effects produced by combinations of drugs or toxic chemicals are known. The clinical signs and symptoms of such episodes are usually recognized with ease. Anatomopathological lesions are clear-cut and can be related without great difficulty to the offending agents. Acute interactions may involve and may even be quantitated by several end points, such as modification of metabolic pathways, physiologic parameters, appearance of enzymes indicating damage to particular organs in the serum, or defined tissue lesions such as inflammatory changes or acute cell death in target organs. The possible sequelae and significance of acute interactions have been well documented in experiments with both animals and humans. These are discussed more thoroughly in other parts of this report.

Much less is known about the effects of chronic interactions. However, there is no doubt that many of the most prevalent human diseases might not be caused by one agent but by two or even more. For certain diseases, such as liver cirrhosis, coronary heart disease, cardiovascular disease, chronic lung disease, and chronic kidney disease, there is evidence that the pathologic process, developing within months or years rather than within days or weeks, may be caused, aggravated, and modified by chemicals, diet, infectious agents, and genetic background (Lee and Kotin, 1972).

There is very little experimental evidence to permit unequivocal identification of the roles played by suspected etiologic agents in any of these chronic forms of toxicity. Moreover, there is an almost limitless number of agents and combinations thereof to which an individual may be exposed throughout life. The temporal relationship may vary as do levels of exposure in endless numbers of possible combinations. Changes in diet, in endogenous hormonal function, and in exposure to infectious agents may complicate the picture even further. Thus, all these events may produce an altered biological response. Chronic beryllium disease is such an example (Tepper et al., 1961). In people exposed to beryllium dust and fumes, the onset of disease often occurs a year or more after exposure to the offending agent, beryllium. In a few cases, the disease may progress asymptomatically, and in many patients it follows an undramatic course. In some patients, however, additional toxic insults or altered hormonal homeostasis such as pregnancy may cause an acute exacerbation of the disease. Some experimental evidence shows that this is the direct consequence of a translocation of the toxic agent, beryllium, to a new and different target site within the organism (Clary and Stokinger, 1973). Other evidence points to immunologic actions (Reeves, 1977). Nevertheless, it is obvious that the interaction of two offending agents may sometimes dramatically alter the development of the disease.

There are only a limited number of ways in which cells and tissue can react to insults by chemicals. When we imply that a chemical such as carbon tetrachloride, mercury, or silica dust causes specific toxicity, we often find it necessary to define the specific

lesion by its localization in a given target organ or tissue, e.g., the liver, kidney, or lung. In the final analysis, however, the reaction at each of these tissues may be essentially the same: cell death, inflammation, degenerative changes, tissue recovery, or abnormal growth. It is therefore necessary to understand the general pathogenetic mechanisms leading to responses caused by chronic interactions between two or more chemicals. Two possible examples are discussed below.

TWO-STAGE CARCINOGENESIS

Two-stage carcinogenesis may be a special example of interactions between chemicals. The concept of two-stage carcinogenesis was originally developed for mouse skin more than 30 years ago (Berenblum, 1941; Berenblum and Shubik, 1947; Friedewald and Raus, 1944). Since then it has undergone many refinements and has also been one of the thoroughly explored models of carcinogenesis, from the molecular level to the whole animal (Boutwell, 1974).

It is useful to distinguish three possible forms of two-stage carcinogenesis: cocarcinogenesis, initiation-promotion, and enhancement of carcinogenesis (Berenblum, 1978).

Cocarcinogenesis

In cocarcinogenesis, exposure to two weak carcinogens usually results in the formation of more tumors than would occur after exposure to either agent alone. The temporal relationship between the exposures to the two agents does not appear to be critical. Exposure may be simultaneous or sequential, and the order of sequence does not matter. Cocarcinogenesis has been observed in mouse skin, as well as in many other organs and species.

Initiation-Promotion

In classical initiation-promotion, the sequence of events is critical. The initiating agent produces an irreversible and presumably heritable change in skin epithelial cells. If this is followed by repeated topical applications of a second agent, the promoter, tumor development is accelerated and more tumors are formed. The most commonly used promoters are only weakly carcinogenic and, in most systems of two-stage carcinogenesis, are devoid of any carcinogenic action. Two very strict criteria (one related to dose, the other to time) are used to determine a true initiation-promotion phenomenon. True promoters are capable of eliciting tumor formation even when the original dose of the initiator is too small (subcarcinogenic) to produce tumors on its own. The other element is time. Promoters are effective even when they are first applied weeks or months after initiation. Administration of the promoting agent before the initiator never enhances tumor formation. Therefore, interaction between initiator and promoter takes place only if there is a strict temporal sequence of events between exposure to the two agents.

Enhancement

Somewhere in between those two extremes (cocarcinogenesis or true promoting activity) are effects produced by a third class of compounds--those that enhance carcinogenesis. Much evidence indicates that these compounds may act as promoters without fulfilling the strict requirements of tumor-promoting agents. However, these compounds are of particular interest since they provide experimental evidence that the concept of two-stage carcinogenesis

applies to organs other than mouse skin. Among the noncarcinogenic or weakly carcinogenic agents that do not enhance tumor formation are: the commonly used drug phenobarbital, the food additive butylated hydroxytoluene (BHT), the artificial sweeteners saccharin and cyclamates, and endogenous agents such as bile salts.

Peraino and associates (1978) have provided the most complete evidence that liver tumorigenesis most likely proceeds via a two-stage process that is analogous to that of tumor development in mouse skin. They selected the potent carcinogen N-2-fluorenylacetamide (AAF) as an initiating agent and fed it to rats at a level of 0.02% in the diet for various lengths of time. The number of hepatic tumors that were determined 260 days after beginning the exposure was proportional to the lengths of exposure to AAF. Of the animals exposed for 11 days, 2% developed tumors; of the animals exposed for 260 days, 26% developed tumors. However, tumorigenesis was greatly enhanced if the AAF diet was followed by a diet containing 0.05% phenobarbital. If exposure to the phenobarbital was begun immediately after exposure to AAF and continued until termination of the experiment, the incidence of tumors increased by 4 to 8 times. When the duration of exposure to phenobarbital was gradually reduced, the investigators observed that as few as 20 days of exposure to the drug increased the number of liver tumors per tumor-carrying rat although it failed to increase the percentage of rats carrying liver tumors. In further experiments, the animals were maintained on a control diet for various lengths of time after exposure to AAF before being fed phenobarbital. Tumor incidence appeared to be controlled by duration of exposure to

phenobarbital rather than by the length of time after AAF treatment before the exposure to phenobarbital was initiated.

Liver tumorigenesis was also enhanced when rats were exposed to another carcinogen, p-dimethylaminoazobenzene (Peraino et al., 1978) or to the weak carcinogen 2-methyldimethylaminobenzene (Kitagawa et al., 1979). In mice that were susceptible to developing spontaneous liver tumors, phenobarbital greatly enhanced the development of tumors (Peraino et al., 1973; Ponomarkor et al., 1976). Thus, phenobarbital has many characteristics of a promoting agent. However, when control animals were exposed to the initiator without subsequent exposure to phenobarbital, a certain number of animals (between 2% and 10%) always developed tumors (Peraino et al., 1978).

For phenobarbital to be labeled as a true promoting agent, it would have to enhance tumor formation in the livers of animals exposed to an apparently subcarcinogenic amount of AAF. Further studies will be necessary to identify an experimental system in which this criterion, usually applied to two-stage carcinogenesis in mouse skin, can be met.

There has been a large epidemiological study to determine whether the observed enhancement of tumorigenesis in laboratory animals by phenobarbital is potentially harmful to humans. In epileptic patients treated chronically with high doses of phenobarbital, there was no evidence of increased tumor incidence (Clemmesen et al., 1974).

BHT also enhances tumor formation in suitably initiated tissue. Exposure of rodents to dietary AAF followed by a diet containing 0.5% BHT (but not 0.05% BHT) enhanced tumor formation in the liver

(Peraino et al., 1977). Witschi et al. (1977) have shown a similar enhancement of tumor formation in the mouse lung. Certain strains of mice develop pulmonary adenomata (tumors derived from the type II alveolar epithelial cells) within 4 to 6 months when treated with a variety of carcinogens (Shimkin and Stoner, 1975). To examine whether it was possible to enhance tumor formation in the lung, the investigators gave urethan to mice at a dose producing 100% tumor incidence in the lung. In the control animals, an average of four tumors per lung was found 3.5 months later, and 12 tumors per lung, 6 months later. The experimental animals were given 13 injections of BHT beginning 1 week after injection of urethan. The dose of BHT caused acute necrosis of type I alveolar cells followed by a proliferation of type II alveolar cells (Hirai et al., 1977). In animals treated with BHT, an average of 12 tumors per lung was observed 3.5 months after exposure to urethan, and 19 tumors per lung after 6 months. The difference between the treated and control groups was statistically significant (Witschi and Lock, 1979). Thus, BHT appears to increase the number of tumors formed and to accelerate their growth to some extent. The minimum number of BHT treatments that increased the number of lung tumors was four. Tumor formation was also enhanced by BHT if the interval between exposure to urethan and the first BHT treatment was extended up to 6 weeks. When the treatment was reversed, i.e., the 13 weekly BHT injections were given before exposure to urethan, tumor incidence was the same in controls as it was in the BHT-treated animals (Witschi and Lock, 1979).

Although BHT enhances formation of tumors in both liver and lung, like phenobarbital it may not be labelled a promoting agent

in the truest sense of the word. When lower concentrations of urethan are given, the number of tumors formed after BHT is proportional to the urethan dose. Moreover, at the lowest level of urethan tested, enhanced tumor formation can no longer be found (Witschi and Lock, 1979).

Nonetheless, the experiments with BHT highlight an important aspect of the possible interaction between carcinogens and an agent that has never shown to be a carcinogen per se but one that is capable of enhancing carcinogenesis under certain conditions. Several studies have shown that BHT can provide protection against chemical carcinogens, if it is administered before the carcinogen (Wattenberg, 1978). On the other hand, some data (Peraino et al., 1977; Witschi et al., 1977) suggest that BHT, if present after administration of the carcinogen, no longer protects but rather enhances and accelerates tumor formation.

The temporal sequence of exposure appears to be the most critical factor in determining the quantitative nature of the interaction. This is even more evident from comparisons of two studies in which the same species (rats) and comparable levels of the same carcinogen (AAF) were used and the same tumor (hepatocarcinoma) was studied. In animals treated with BHT prior to exposure to the carcinogen, tumor incidence was reduced (Ulland et al., 1973). In animals exposed to BHT after the carcinogen, tumor incidence was increased (Peraino et al., 1977). In chronic interactions, such as those in two-stage carcinogenesis, timing of exposure appears to be a very critical factor and should be considered when assessing risk.

Two-stage carcinogenesis has also been demonstrated in the bladder epithelium. Hicks et al. (1978) found that saccharin and sodium cyclamate were capable of greatly increasing formation of tumors following a single administration via urinary catheter of the potent alkylating agent N-methyl-N-nitrosourea (MNU). Of particular importance was their observation that both sweeteners produced bladder tumors in animals that had received an initiating noncarcinogenic dose of MNU. This system appears to satisfy at least one criteria of two-stage carcinogenesis: that a promoter also produces tumor formation following a subcarcinogenic dose of the initiator. It has not been shown that either sweetener is capable of enhancing tumor formation if the interval between exposure to the carcinogen and exposure to the promoting agent is prolonged nor has it been shown that reversal of the procedure, i.e., exposure to saccharin or cyclamate followed by the carcinogen, is without effect. Nevertheless, the findings are not only of considerable theoretical importance but are also significant for estimating the risk of using these artificial sweeteners in food and drink for humans. This has been discussed in greater detail in report of the National Academy of Sciences Panel on Saccharin and Its Impurities (1978).

Finally, there is some evidence to suggest that the formation of colon tumors is slightly enhanced by dietary constituents and by bile salts. Many aspects of this mechanism suggest that this might be yet another example of two-stage carcinogenesis (Reddy et al., 1978).

In summary, there is now compelling experimental evidence to suggest that two-stage carcinogenesis applies to epithelial tissue other than mouse skin. Two-stage carcinogenesis is a particular form of interaction between two chemicals. Perhaps the most important aspect of this interaction is that the temporal sequence of exposure is the determining factor in the eventual outcome.

LUNG FIBROSIS

Acute interaction between two chemicals or between a chemical and a physical agent may also play a role in the development of another form of chronic tissue injury, lung fibrosis, which is quite common in humans. Etiologic agents responsible for its development are infectious agents, inhaled toxic dusts and fumes (e.g., metals such as cadmium, beryllium, and aluminum or fibrogenic dusts such as silica and asbestos), physical agents (e.g., irradiation of the thorax), and oxygen in abnormal concentrations (Morgan and Seaton, 1975). Lung fibrosis might also follow exposure to bloodborne toxicants such as paraquat (Smith et al., 1974) as well as to a number of drugs, especially several antineoplastic agents (e.g., bleomycin and methotrexate) (Sostman et al., 1977). Alterations in the cell population of the lung and the arrangement of interstitial collagen resulting from the loss of coordinated control of collagen synthesis and degradation within the wall of the pulmonary alveoli are common features of lung fibrosis. They result in impairment of gas exchange across the alveolar capillary barrier and reduced ventilation due to decreased compliance and changes of the elastic properties of the lung (Crystal et al., 1978). The biochemical events accompanying

these changes have been studied extensively in slices of lung tissue exposed to an offending agent in vitro, in whole animals, and in specimens of lung tissue obtained via biopsy (Fulmer and Crystal, 1976). Lung fibrosis progresses slowly: the disease may develop and cause death within a few years or it may run a more prolonged course, resulting in crippling pulmonary functions.

Recent work suggests that some forms of lung fibrosis could be caused by an interaction between two chemicals or between a chemical and a physical agent in the alveolar zone of the lung. The experimental evidence, which is still far from complete, may be summarized as follows: in the mouse lung (Hirai et al., 1977) and, to much lesser extent, in the lung of female rats (Larsen and Tarding, 1978) the antioxidant BHT causes acute damage and necrosis of the type I epithelial cells, which line 95% of the alveolar surface. In male rats and in other species, BHT has not yet been found to produce a similar sequence of events. However, necrosis of alveolar type I cells is a common form of acute toxic lung injury and may be induced in various species, including humans, by a large number of toxic inhalants as well as by agents carried into the lungs via the bloodstream (Witschi and Côté, 1977b).

Once the epithelial layer of the alveolus has become damaged, the necrotic cells eventually disintegrate and detach from the basement membrane. As a general rule, these defects are quite often repaired quickly and efficiently (Witschi, 1976). It is now well established that recovery of the tissue is caused by a proliferation of the type II alveolar cells, which are the stem cells of the alveolar epithelium. In a normal lung, this cell population represents

approximately 14% of the total pulmonary parenchymal cells (Weibel et al., 1976). The area that they cover is even smaller since the cells are of cuboidal shape and usually sit in the corners of the alveoli. Following injury to the type I epithelial cells, the type II cells begin to proliferate and to divide. During the next few days portions of the cytoplasm display signs of active movement under the electron microscope. Thin sheets of cytoplasm begin to extend from the body of the cells and to creep over the denuded areas of the basement membrane. Several days after the insult the cells have assumed a shape that is no longer morphologically distinct from type I alveolar cells. This process restores an essentially normal air-blood barrier.

While type II cells are dividing, they appear to be vulnerable to toxic and physical agents. In a resting lung, cell damage usually becomes apparent only after several days of exposure to oxygen concentrations of 90% or more (Adamson et al., 1970; Gould et al., 1972). On the other hand, proliferating epithelial cells may be prevented from carrying out DNA synthesis and, presumably, from dividing by exposure to between 40% and 60% oxygen for as short a time as 16 hours (Witschi and Côté, 1977a). In a normal lung, acute cell damage may be caused by X-rays, usually after administration of 3,000 to 5,000 rads (Phillips and Margolis, 1972). Dividing cells may be affected by as little as 100 rads from X-rays or 50 rads from neutrons. Higher doses of irradiation (400 to 800 rads from X-rays) might prevent recovery and proliferation of lung epithelial cells within 2 to 3 weeks (Meyer et al., 1980).

Physical and chemical agents that are present during the recovery of alveolar wall tissue following a primary insult can affect

an effective repair mechanism. One consequence of this is an increase in total lung collagen content (Haschek and Witschi, 1979). Animals given a dose of BHT that produces uniform and widespread alveolar type I cell damage and then exposed to between 70% and 90% oxygen for 4 to 6 days develop extensive and uniform interstitial fibrosis within 2 to 3 weeks. This has been verified histopathologically and quantitated by measuring total lung hydroxyproline. More than twice the amount of hydroxyproline found in a normal lung may accumulate under these conditions. Calculations of the amount of collagen added to a normal lung by exposure to BHT alone, oxygen alone, or the combination of the two agents show that the combined effect of BHT and oxygen adds substantially more collagen to the lung than does either exposure by itself. Thus, the effect of the combined treatment is not only additive but also synergistic. Similar observations have been made by killing the dividing epithelial cells with X-rays 1 day after exposure to BHT (Haschek et al., 1980).

The synergistic interaction between BHT and oxygen occurs only if there is a critical timing between administration of the two agents. For example, if animals are exposed to 70% oxygen for 6 days immediately following exposure to BHT, fibrosis is apparent 2 weeks after the exposure. If oxygen exposure is begun 6 days after administration of BHT only, no fibrosis develops. Exposure to 70% oxygen for 7 days followed by administration of BHT does not result in a synergistic interaction between the two agents (Haschek and Witschi, 1979).

Similar results are obtained with X-rays. Fibrosis is produced only if the thorax is irradiated 1 day before exposure to BHT, immediately after exposure, or 1 day after exposure. Irradiation 2 or 3 days before or 2 to 6 days after exposure to BHT will not result in fibrosis (Haschek et al., 1980). However, if fibrosis develops following the interaction between BHT and X-rays, it will persist up to 6 months and possibly longer after one single episode (Witschi et al., 1980).

The data summarized above show that exposure to oxygen results only in fibrosis if exposure to oxygen begins soon after exposure to BHT, but not if exposure is delayed until 6 days later. Similarly, X-rays may also induce fibrosis only if the lung parenchyma is irradiated either shortly before or shortly after exposure to BHT. Previous studies have shown that following BHT-induced lung injury there is initially a wave of epithelial cell proliferation followed only later by division of fibroblasts and capillary endothelial cells (Adamson et al., 1977). One possible explanation of these findings is that the second insult following injury by BHT must occur within a very limited time, presumably when there is an increased susceptibility of the type II epithelial cells preparing to divide. If this critical period is missed, an apparently normal recovery of the tissue occurs.

The implications of these observations are potentially far reaching. They seem to establish a broad general principle: in the lung, fibrosis develops if one agent damages the alveolar epithelium and if another toxic agent, which must be present during a critical phase of the recovery period, subsequently inhibits normal reconstitution of the alveolar epithelium. It remains to be established whether this

principle in the pathogenesis of fibrosis following lung damage can be produced by agents other than BHT. For example, we can speculate that lung fibrosis develops if the primary damage to the alveolar epithelium is caused by toxic inhalants such as cadmium fumes (Palmer et al., 1975) or by inhalation of other metallic compounds such as nickel (National Academy of Sciences, 1975) or vanadium (U.S. Department of Health, Education, and Welfare, 1977). Interference with the recovery phase might be brought about by such inhalants as nitrogen oxide, ozone, or even tobacco smoke. The latter two agents have already been found to inhibit cell division in the alveolar zone (Penha et al., 1972) or to give rise to abnormal developments of fibrotic tissue (Frasca et al., 1974).

The pathogenetic principle outlined above might also play a role in the development of lung fibrosis during the course of therapy combining certain antineoplastic drugs and irradiation of the thorax. Thorax irradiation alone has been known for some time to trigger the development of fibrotic changes in the lung (Gross, 1977). The process usually takes several months if not years to develop fully. In most cases, radiation pneumonitis appears months after irradiation of the thorax with high doses of X-rays (3,000 rads and more), and fibrotic changes occur after only months (Rubin and Casarett, 1968). However, excessive fibrosis develops in certain patients within weeks rather than within months during the course of a treatment with both anticancer drugs and X-rays. This has been observed during treatment with actinomycin D, adriamycin, cyclophosphamide, methotrexate, or vincristine (Aisenberg, 1978; Einhorn et al., 1976; Littman et al.,

1974; Nickson, 1978; Rosen et al., 1974; Wara et al., 1976). In laboratory animals, adriamycin, actinomycin D, and cyclophosphamide in combination with thorax X-irradiation lead to accelerated necrosis and, eventually, to fibrosis (Phillips et al., 1975). In these instances the anticancer drug may cause lung damage similar to that induced by BHT. Data on bleomycin support this (Adamson, 1976; Adamson and Bowden, 1977). Therefore, thorax irradiation, if applied shortly before or during epithelial recovery, might interfere with the recovery process and fibrosis would develop.

If this hypothesis can be substantiated in further experiments, it will be important to establish precise dose-effect relationships of the two agents in the lung. It will be even more critical to know their relationships to the type of effect that is produced. If irradiation can cause fibrosis when administered during a critical phase to an animal model exposed to both BHT and X-rays, it should become possible to avoid a similar complication in humans by carefully timing the administration of drugs and irradiation of the thorax.

A similar type of interaction may cause disease in yet another region of the lung, the small airways. Small airway disease is one of the most prevalent forms of chronic lung disease (Bates, 1972; Cosio et al., 1977; Ebert, 1978). The epithelial lining of the small airways is composed essentially of two types of epithelial cells, the ciliated cells and the unciliated (Clara) cells (Clara, 1937). Ciliated cells are readily damaged by toxic inhalants, particularly by the ubiquitous air pollutants nitrogen oxide and ozone. If ciliated cells die, repair is accomplished by division of the stem cells of the bronchiolar and the Clara cells (Evans et al., 1978).

Recent work has shown that Clara cells are vulnerable to the toxic effects of several agents. These cells appear to be rich in mixed-function oxidases and are therefore capable of activating certain agents to highly reactive and toxic metabolites, an event resulting in necrosis of the Clara cells. Among such agents are 4-ipomeanol (Boyd, 1977), 3-methylfuran (Boyd et al., 1978), carbon tetrachloride (Longo et al., 1978), 3-methylindole (Huang et al., 1977), and 4-nitroquinoline-1-oxide (Terao and Otsu, 1973). Tobacco smoke can also damage Clara cells (Kilburn et al., 1974). If Clara cells are damaged when they are supposed to take part in the recovery of the bronchiolar ciliated epithelium, fibrosis might develop as it does when type II alveolar cells are damaged at the time they are supposed to repair damaged type I alveolar cells. Whether this potential interaction applies to the development of small airway disease remains to be established.

REFERENCES

Adamson, I. Y. R. 1976. Pulmonary toxicity of bleomycin. Environ. Health Perspect. 16:119-126.

Adamson, I. Y. R., and D. H. Bowden. 1977. Origin of ciliated alveolar epithelial cells in bleomycin-induced lung injury. Am. J. Pathol. 87:569-575.

Adamson, I. Y. R., D. H. Bowden, and J. P. Wyatt. 1970. Oxygen poisoning in mice: ultrastructure and surfactant studies during exposure and recovery. Arch. Pathol. 90:463-472.

Adamson, I. Y. R., D. H. Bowden, M. G. Cote, and H. Witschi. 1977. Lung injury induced by butylated hydroxytoluene. Lab. Invest. 36:26-32.

Aisenberg, A. C. 1978. The staging and treatment of Hodgkin's disease. N. Engl. J. Med. 299:1228-1232.

Bates, D. V. 1972. Air pollutants and the human lung. Am. Rev. Respir. Dis. 105:1-13.

Berenblum, I. 1941. The mechanism of carcinogenesis. Cancer Res. 1:807-814.

Berenblum, I. 1978. Historical perspective. Pp. 1-10 in T. J. Slaga, A. Sivak, and R. K. Boutwell, eds. Carcinogenesis--A Comprehensive Survey, Vol. 2. Mechanisms of Tumor Promotion and Cocarcinogenesis. Raven Press, New York.

Berenblum, I., and P. Shubik. 1947. A new, quantitative approach to the study of the stages of chemical carcinogenesis in the mouse's skin. Br. J. Cancer 1:383-391.

Boutwell, R. K. 1974. The function and mechanism of promoters of carcinogenesis. CRC Crit. Rev. Toxicol. 2:419-443.

Boyd, M. R. 1977. Evidence for the Clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in lung. Nature 269:713-715.

Boyd, M. R., C. N. Statham, R. B. Franklin, and J. R. Mitchell. 1978. Pulmonary bronchiolar alkylation and necrosis by 3-methylfuran, a naturally occurring potential atmospheric contaminant. Nature 272:270-271.

Clara, M. 1937. Zür Histobiologie des Bronchalepethels. Z. Mikrosk. Anat. Forsch. 41:321-347.

Clary, J. J., and H. E. Stokinger. 1973. The mechanism of delayed biologic response following beryllium exposure. J. Occup. Med. 15:255-259.

Clemmesen, J., V. Fuglsang-Frederiksen, and C. M. Plum. 1974. Are anticonvulsants oncogenic? Lancet 1:705-707.

Cosio, M., H. Ghezzo, J. C. Hogg, R. Corbin, M. Loveland, J. Dosman, and P. T. Macklem. 1977. The relations between structural changes in small airways and pulmonary-function tests. N. Engl. J. Med. 298:1277-1281.

Crystal, R. G., J. D. Fulmer, B. J. Baum, J. Bernardo, et al. 1978. Cells, collagen an idiopathic pulmonary fibrosis. Lung 155:199-224.

Ebert, R. V. 1978. Small airways of the lung--the importance of understanding and assessing the function of pulmonary bronchioles. Ann. Intern. Med. 88:98-103.

Einhorn, L., M. Krause, N. Hornback, and B. Furnas. 1976. Enhanced pulmonary toxicity with bleomycin and radiotherapy in oat cell lung cancer. Cancer 37:2414-2416.

Evans, M. J., L. J. Cabral-Anderson, and G. Freeman. 1978. Role of the Clara cell in renewal of the bronchiolar epithelium. Lab. Invest. 38:648-655.

Frasca, J. M., O. Auerbach, V. R. Parks, and J. D. Jamieson. 1974. Alveolar cell hyperplasia in the lungs of smoking dogs. Exp. Mol. Pathol. 21:300-312.

Friedewald, W. F., and P. Raus. 1944. The initiating and promoting elements in tumor production. J. Exp. Med. 80:101-126.

Fulmer, J. D., and R. G. Crystal. 1976. The biochemical basis of pulmonary function. Pp. 419-466 in R. G. Crystal, ed. Lung Biology in Health and Disease, Vol. 2. The Biochemical Basis of Pulmonary Function. Marcel Dekker, New York.

Gould, V. E., R. Tosco, R. F. Wheelis, N. S. Gould, and Y. Kapanci. 1972. Oxygen pneumonitis in man: ultrastructure observations on the development of alveolar lesions. Lab. Invest. 26:499-508.

Gross, N. J. 1977. Pulmonary effects of radiation therapy. Ann. Intern. Med. 86:81-92.

Haschek, W. M., and H. P. Witschi. 1979. Pulmonary fibrosis--A possible mechanism. Toxicol. Appl. Pharmacol. 51:475-487.

Haschek, W. M., K. L. Meyer, R. L. Ullrich, and H. P. Witschi. 1980. Potentiation of chemically induced lung fibrosis by thorax irradiation. Ind. J. Radiat. Oncol. Biol. Phys. 6:449-455.

Hicks, R. M., J. Chowaniec, and J. St. J. Wakefield. 1978. Experimental induction of bladder tumors by a two-stage system. Pp. 475-489 in T. J. Slaga, A. Sivak, and R. K. Boutwell, eds. Carcinogenesis--A Comprehensive Survey, Vol. 2. Mechanisms of Tumor Promotion and Cocarcinogenesis. Raven Press, New York.

Hirai, K., H. Witschi, and M. G. Cote. 1977. Electron microscopy of butylated hydroxytoluene-induced lung damage in mice. Exp. Mol. Pathol. 27:295-308.

Huang, T. W., J. R. Carson, T. M. Bray, and B. J. Bradley. 1977. 3-Methylindole-induced pulmonary injury in goats. Am. J. Pathol. 87:647-666.

Kilburn, K. H., W. N. McKenzie, and R. J. Thurston. 1974. Cellular effects of cigarette smoke on hamster airways. Chest 67:54S-55S.

Kitagawa, T., H. C. Pitot, E. C. Miller, and J. A. Miller. 1979. Promotion by dietary phenobarbital of hepatocarcinogenesis by 2-methyl-N, N-dimethyl-4-aminoazobenzene in the rat. Cancer Res. 39:112-115.

Larsen, J. C., and F. Tarding. 1978. Stimulation of DNA synthesis in mouse and rat lung following administration of butylated hydroxytoluene. Arch. Toxicol., Suppl. 1:147-150.

Lee, D. H. K., and P. Kotin. 1972. Multiple factors in the causation of environmentally induced disease. Academic Press, New York. 225 pp. (Fogarty Proceedings No. 12.)

Littman, P., L. W. Davis, J. Nash, M. Tefft, P. Borns, and P. Lepanto. 1974. The hazard of acute radiation pneumonitis in children receiving mediastinal radiation. Cancer 33:1520-1525.

Longo, N., C. Statham, H. Sasame, and M. Boyd. 1978. Pulmonary clara-cell damage by carbon tetrachloride. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37(3):505, abst. no. 1536.

Meyer, K. R., H. P. Witschi, and R. L. Ullrich. 1980. Proliferative response of type 2 lung epithelial cells after X-rays and fission neutrons. Radiation Res. 82(3):559-569.

Morgan, W. K. C., and A. Seaton. 1975. Occupational Lung Diseases. Saunders, Philadelphia. 391 pp.

National Academy of Sciences. 1975. Nickel. Committee on Medical and Biologic Effects of Environmental Pollutants, National Academy of Sciences, Washington, D.C. 277 pp.

National Academy of Sciences. 1978. Saccharin: Technical Assessment of Risks and Benefits. Panel 1: Saccharin and Its Impurities, Committee for a Study on Saccharin and Food Safety Policy, National Academy of Sciences, Washington, D.C. [200] pp.

Nickson, J. J. 1978. Survival and complication of radiotherapy following involved and extended field therapy of Hodgkin's disease, stages I and II. Cancer 38:288-305.

Palmer, K. C., G. L. Snider, and J. A. Hayes. 1975. Cellular proliferation induced in the lung by cadmium aerosol. Am. Rev. Respir. Dis. 112:173-179.

Penha, P. D., L. Amaral, and S. Werthamer. 1972. Ultrastructure and biochemical alteration in mouse lung exposed to ozone. Am. J. Pathol. 66:57a-58a.

Peraino, C., R. J. M. Fry, and E. Staffeldt. 1973. Enhancement of spontaneous hepatic tumorigenesis in C3H mice by dietary phenobarbital. J. Nat. Cancer Inst. 51:1349-1350.

Peraino, C., R. J. M. Fry, E. Staffeldt, and J. P. Christopher. 1977. Enhancing effects of phenobarbitone and butylated hydroxy-toluene on 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. Food Cosmet. Toxicol. 15:93-96.

Peraino, C., R. J. M. Fry, and D. D. Grube. 1978. Drug-induced enhancement of hepatic tumorigenesis. Pp. 421-432 in T. J. Slaga, A. Sivak, and R. K. Boutwell, eds. Carcinogenesis--A Comprehensive Survey, Vol. 2. Mechanisms of Tumor Promotion and Cocarcinogenesis. Raven Press, New York.

Phillips, T. L., and L. Margolis. 1972. Radiation pathology and the clinical response of lung and esophagus. Front. Radiat. Ther. Oncol. 6:254-273.

Phillips, T. L., M. D. Wharam, and L. W. Margolis. 1975. Modification of radiation injury to normal tissues by chemotherapeutic agents. Cancer 35:1678-1684.

Ponomarkor, V., L. Tomatis, and V. Turusov. 1976. The effect of long-term administration of phenobarbitone in CF-1 mice. Cancer Lett. 1:165-172.

Reddy, B. S., J. H. Weisburger, and E. L. Wynder. 1978. Colon cancer bile salts as tumor promoters. Pp. 453-464 in T. J. Slaga, A. Sivak, and R. K. Boutwell, eds. Carcinogenesis--A Comprehensive Survey Vol. 2. Mechanisms of Tumor Promotion and Cocarcinogenesis. Raven Press, New York.

Reeves, A. L. 1977. Beryllium in the environment. Pp. 37-48 in C. L. Winek and S. P. Shanor, eds. Toxicology Annual, Vol. 2. Marcel Dekker, New York.

Rosen, G., S. Suwansirikul, C. Kwon, C. Tan, S. J. Wu, E. J. Beattie, Jr., and M. L. Murphy. 1974. High-dose methotrexate with citrovorum factor rescue and adriamycin in childhood osteogenic sarcoma. Cancer 33:1151-1163.

Rubin, P., and G. W. Casarett. 1968. Clinical Radiation Pathology. Saunders, Philadelphia. 517 pp.

Shimkin, M. B., and G. D. Stoner. 1975. Lung tumors in mice: application to carcinogenesis bioassay. Adv. Cancer Res. 21:1-58.

Smith, P., D. Heath, and J. M. Kay. 1974. The pathogenesis and structure of paraquat-induced pulmonary fibrosis in rats. J. Pathol. 114:57-67.

Sostman, H. D., R. A. Matthay, and C. E. Putnam. 1977. Cytotoxic drug-induced lung disease. Am. J. Med. 62:608-615.

Tepper, L. B., H. L. Hardy, and R. I. Chamberlin. 1961. Toxicity of Beryllium Compounds. Elsevier, New York. 190 pp.

Terao, K., and H. Otsu. 1973. Special susceptibility of Clara cells of the murine terminal bronchiole to 4-nitroquinoline-1-oxide. Electron microscopic observation. Gann 64:179-181.

Ulland, B. M., J. H. Weisburger, R. S. Yamamoto, and E. K. Weisburger. 1973. Antioxidants and carcinogenesis: butylated hydroxytoluene, but not diphenyl-p-phenylenediamine, inhibits cancer induction by N-2-fluorenylacetamide and by N-hydroxy-N-2-fluorenylacetamide in rats. Food Cosmet. Toxicol. 11:199-207.

U.S. Department of Health, Education, and Welfare. 1977. Criteria for a Recommended Standard....Occupational Exposure to Vanadium. Department of Health, Education, and Welfare (NIOSH), Washington, D.C. 142 pp. Publication No. 77-222.

Wara, W. M., T. L. Phillips, L. W. Margolis, and V. Smith. 1976. Radiation pneumonitis: a new approach to the derivation of time-dose factors. Cancer 32:547-552.

Wattenberg, L. W. 1978. Inhibition of chemical carcinogenesis. J. Nat. Cancer Inst. 60:11-18.

Weibel, E. R., P. Gehr, D. Haies, J. Gil, and M. Bachofen. 1976. The cell population of the normal lung. Pp. 3-16 in A. Bouhuys, ed. Proceedings of a Brook Lodge Conference on Lung Cells in Disease. North-Holland, New York.

Witschi, H. 1976. Proliferation of type II alveolar cells: a review of common responses in toxic lung injury. Toxicology 5:267-277.

Witschi, H. P., and M. G. Côté. 1977a. Inhibition of butylated hydroxytoluene-induced mouse lung cell division by oxygen: time effect and dose-effect relationships. Chem. Biol. Interact. 19:279-289.

Witschi, H., and M. G. Côté. 1977b. Primary pulmonary responses to toxic agents. CRC Crit. Rev. Toxicol. 5:23-66.

Witschi, H., and S. Lock. 1979. Enhancement of adenoma formation in mouse lung by butylated hydroxytoluene. Toxicol. Appl. Pharmacol. 50:391-400.

Witschi, H. P., D. Williamson, and S. Lock. 1977. Enhancement of urethan tumorigenesis in mouse lung by butylated hydroxytoluene. J. Natl. Cancer Inst. 58:301-305.

Witschi, H., W. M. Haschek, K. R. Meyer, R. L. Ullrich, and W. E. Dalbey. 1980. A pathogenetic mechanism for lung fibrosis. Chest 78:395-399.

CHAPTER 8

CONDITIONS ALTERING TOXICOLOGICAL INTERACTIONS

Conditions often subtly or dramatically alter the "rules" that scientists like to provide in order to make the scientific universe move in an orderly fashion. This becomes obvious when dealing with chemicals and their biological effects and is even more apparent when several chemicals are superimposed on a biological system simultaneously. The "rules" may then become "guidelines" since the systems within organisms react in any number of ways to cope with the additional challenge of several new chemical intruders.

There may not be a high degree of predictability to the biological alterations that result from chemical interactions. Nonetheless, there are some general "guidelines" pertaining to the type of effects that result from multiple chemical exposures, the variations to be expected under certain environmental and stress conditions, the influences of dietary and nutritional factors, and alterations produced by the presence of preexisting disease states in the host system.

EFFECTS OF ENVIRONMENTAL AND STRESS CONDITIONS

Extreme environmental conditions, such as temperature and low oxygen or water levels, profoundly affect toxicity and the disposition of foreign chemicals in the various organs. The additional influence of physical factors, such as vibration or noise, produces comparable effects. These exogenous variables may also evoke typical stress responses. Microsomal oxidative stimulation, mediated via the

hypophyseal-adrenal axis, is usually an important mechanism for this effect (Driever and Bousquet, 1965; Fuhrman and Fuhrman, 1961; Fuller et al., 1972).

Cold Environmental Temperatures

Drastic lowering of the environmental temperature establishes a condition of stress that markedly alters the metabolism of drugs and foreign agents. Principally, it activates the drug-metabolizing capacity of the mammalian liver. Typical examples are the enhancement of acetanilide microsomal hydroxylation and ring hydroxylation of 2-naphthylamine (Inscoe and Axelrod, 1960). Exposure to cold also stimulates the metabolism of aniline, but it depresses the hydroxylation of hexobarbital (Dewhurst, 1963). Continuous exposure to low ambient temperatures accelerates $^{14}CO_2$ formation in rats that have been given several doses of ethanol (deBruin, 1976; Platonow et al., 1963)

High Environmental Temperatures

Investigators have observed that elevated environmental temperatures may exert a definite adverse influence upon the response to toxic chemicals and drugs. The potentiating effect of thermal stress (30°C-40°C) upon sublethal toxicity is demonstrable for drugs (Fuhrman, 1946), ozone (Stokinger, 1957), lead (Baetjer et al., 1960), mercury (Trakhtenberg et al., 1965), thiol poisons (Savitskii, 1967), antimony (Baetjer, 1969), and various pesticides, including anticholinesterase compounds (Grigorowa and Binnewies, 1973), DDT, warfarin (Keplinger et al., 1959), and 2,4-dinitro-o-cresol (Tesic et al., 1972).

Increased respiratory and dermal exposure may be responsible for the increased excretion rates of p-nitrophenol in subjects exposed to parathion in hot environments (Funckes et al., 1963).

Dehydration

The stress condition resulting from dehydration is similar to that caused by an elevation of temperature. Severe water deprivation is associated with lowered resistance to the acute toxic effects of lead (Baetjer et al., 1960), antimony (Baetjer, 1969), and methacholine chloride (Baetjer, 1973). Conversely, dehydrated animals are no more responsive to lipid-soluble solvents than they are under normal circumstances (Baetjer, 1973). Isolated hepatic microsomes derived from rats on a water deprivation regimen have a reduced capacity to metabolize hexobarbital (Baetjer, 1970).

Hypoxia

As expected, animals with hypoxia respond abnormally to chemical exposures. Alcohol metabolizes at a decreased rate, and experimentally induced lung tumorigenesis is increased (Hueper and Conway, 1964; Zapata-Ortiz et al., 1970).

Vibration and Noise

A mutual interaction between a vibrational stress and toxic factors has been established in animals subjected to the combined application of toxic metals and a vertical vibration load (50 Hz). Histopathological examination revealed that vibration accentuates the toxicity of manganese. It also intensifies the degenerative

action of mercuric salts upon nerve elements (Levakovskaya and Neizvestnova, 1972; Mavrinsaya and Tartakovskaya, 1972). Morphological changes in the internal organs were slight on exposure to repeated doses of the organophosphate trichlorfon, but were pronounced when the doses were combined with a prolonged noise stress (Tsapko and Rappoport, 1972).

EFFECTS OF NUTRITION AND DIETARY FACTORS

The activities of the hepatic enzyme systems that metabolize foreign compounds vary with nutritional status. Starvation results in decreased rates of hydroxylation of acetanilide, demethylation of meperidine, and metabolism of hexobarbital and other compounds. The activities of all the drug-metabolizing microsomal enzymes in the livers of female rats are enhanced by starvation. Fasting animals produce smaller amounts of glucuronide conjugates than normal (Miettinen and Leskinen, 1963). When maintained on a protein- or calcium-deficient diet, rats incur a diminished rate of drug metabolism due to decreased activity of the microsomal enzymes (Dingell et al., 1966). The toxicity of acetylsalicylic acid is increased by a protein-deficient diet and is further increased by a deficiency of magnesium (West, 1964). In guinea pigs that have been deprived of ascorbic acid, there is a reduction in the rates of metabolism of acetanilide and a variety of drugs.

Malnutrition and Starvation

Dietary deficiency arising from prolonged caloric restriction does not indiscriminately result in sensitization to toxic agents

(McLean and McLean, 1969). Nutrition is a highly structured entity. Consequently, dietary inadequacy may interact with toxic factors in a subtle and complex fashion, depending on the type of poisonous agent and the state of deficiency.

Reduced intake of calories usually inhibits, rather than promotes, the tendency of animals to develop spontaneous or chemically induced neoplasms (Tannenbaum, 1959; Tannenbaum and Silverstone, 1958). Malnutrition modifies the usual responses of organisms to noxious substances by interacting with their mechanisms of absorption, storage, and biotransformation. Some toxic agents have distinct antinutritive properties, and their action is confined to interference with a specific vitamin, coenzyme, or amino acid (Gontzea and Sutzescu, 1968).

A large intake of alcohol creates a general state of avitaminosis, thereby increasing the demand for vitamins. Simultaneous dietary deficiencies of the vitamins (especially the B-complex) predispose the organism to the adverse effects of alcohol. The effects of alcohol on the liver are minimized by intake of an adequate diet and are generally accentuated by dietary imbalance (Porta et al., 1970; Tomasulo et al., 1968).

The most prominent effects of nutritional deficiency occur at the level of microsomal biotransformation. Acting as stress stimuli, adverse nutritional factors may produce stimulatory effects by increasing the amounts of microsomal enzymes. Examples include the metabolic activation of DDT (Dale et al., 1962; Deichmann et al., 1972) and aniline (Kato and Gillette, 1965) due to severe fasting. Various types of unbalanced diets and nutritional variables

can modify chemically induced stimulation of hepatic microsomal drug-metabolizing enzymes. Fasting appears to be associated with a decline in the rate at which ethanol is metabolized (Smith and Newman, 1959; Vitale et al., 1953).

Protein Deficiency

Protein depletion materially alters the toxicity of numerous xenobiotic compounds, thereby exerting either favorable or adverse influences (McLean and McLean, 1969). Generally, protein-deficient diets greatly reduce the activity of hepatic microsomal enzymes and the level of cytochrome P_{450}, resulting in diminished ability of the organism to metabolize foreign compounds. Protein deficiency is associated with increased resistance to such hepatotoxic agents as carbon tetrachloride (McLean and McLean, 1966) and dimethylnitrosamine (McLean and Magee, 1970), which are toxic by virtue of their conversion into biologically active metabolites. Protein-free diets also suppress the microsomal hydroxylation of aflatoxin B_1 (Madhavan and Gopalan, 1965). Diets containing excess protein afford some degree of protection against the hepatocarcinogenicity of aflatoxin (Polrovsky, 1969).

Increased susceptibility to chloroform is apparent in protein-depleted rats, that have been treated with microsomal enzyme inducers (McLean and McLean, 1969). Both excessive ingestion of ethanol and protein-depleted diets tend to result in accumulation of hepatic triglycerides, and their simultaneous presence exerts additive effects. A low protein diet decreases hepatic alcohol dehydrogenase activity in conjunction with depressed clearance of ethanol from the blood

(Goebell and Bode, 1971). The amount of dietary protein given to animals relates linearly to the quantities of conjugated phenols and hippuric acid that are excreted following ingestion of benzene and toluene, respectively (Gontzea et al., 1970).

The susceptibility of animals to a wide range of pesticides is influenced markedly by the dietary level and quality of protein. In protein-deficient rats susceptibility is enhanced severalfold by dieldrin (Lee et al., 1964), DDT (Lasota et al., 1973), lindane (Shtenberg, 1972), chlordane (Boyd and Stefec, 1969), endrin (Boyd and Stefec, 1969), toxaphene (Boyd and Taylor, 1971), parathion (Webb et al., 1973), malaoxon (Webb et al., 1973), fenitrothion (Lasota et al., 1973), and carbaryl (Boyd and Boulanger, 1968). Protein inadequacy results in lower levels of the microsomal detoxifying enzymes. Diets of high quality protein promote the excretion of lindane and its metabolites, thereby reducing the degree of its storage in tissues (Chadwick et al., 1973).

Dietary Factors

Animals exposed to such hepatotoxins as carbon tetrachloride benefit from a diet that is high in carbohydrate and low in fat. The continuous feeding of hypocaloric diets intensifies the hepatogenic action of carbon tetrachloride (Shakman, 1974). In most instances, the consumption of high fat diets appears to enhance chemically induced carcinogenesis (Boutwell et al., 1957). The hepatoprotective effects of diets supplemented with certain sulfur-containing amino acids (such as methionine, cysteine, and homocysteine)

in animals challenged with liver-damaging doses of carbon tetrachloride, bromobenzene, or dichloroethane are well known (Binkley, 1949; Highman et al., 1951). Certain adverse responses to carbon tetrachloride in animals may be prevented or reversed by prior or simultaneous supplementation of aspartic acid, folic acid, cysteine, thioctic acid, or thiolactone plus cysteine (Fodor et al., 1971; Oeriu et al., 1966). Dietary methionine modifies the toxicity of halogenated hydrocarbon insecticides (Waliszewski, 1972). It also accelerates the detoxication of selenium, which combines with the methyl group of methionine. This process is additionally enhanced by the presence of vitamin E or antioxidants (Levander and Morris, 1970).

Deficiency of vitamin E (α-tocopherol) is associated with lowered resistance to the action of ozone. An excess in the diet reverses some of the usual biochemical responses to the inhalant. Vitamins have proved their antidotal value in various types of intoxication. Ascorbic acid probably plays a role in the activation of microsomal enzymes, as suggested by the diminished rate of metabolism of acetanilide and lindane in scorbutic animals (Chadwick et al., 1973). Other examples of interaction between nutrition and toxicity relate to altered susceptibility resulting from changes in dietary levels of trace elements. The dietary levels of calcium and iron strongly influence the toxicity of lead (deBruin, 1976).

EFFECTS OF PERSONAL HABITS

Individual lifestyle habits and activities, such as periodic or daily alcohol consumption, variable caffein intake through coffee or

tea drinking, and smoking of tobacco or mood-altering plant products, will alter physiological and biochemical functions. These effects, since they are commonplace and often not thought to be significant by the average person, may not be considered when potential interactions with other foreign chemicals are discussed.

Nevertheless, these personal, and often unique, chemical intakes as a result of lifestyle do present potentials for chemical interactions with other, more obvious foreign compounds from occupational and other environmental sources. Savolainen et al. (1979) reported that neurochemical effects were demonstrated by increased superoxide dismutase activity in the brains of 2-month old male rats exposed to 300 ppm concentrations of xylene vapors for 5 to 18 weeks. Concomitant ingestion of ethanol enhanced the xylene effect of proteolysis but did not affect superoxide dismutase activity. This has been further emphasized by Elovaara et al. (1980) who showed that inhalation exposure to xylene, a common solvent, when coupled with ingestion of ethanol produced severe liver damage while independent exposure to xylene or ethanol failed to do so.

Physicians, supervisors, and health officials should carefully explore the personal habits of workers or patients to determine if one or more of these "disguised" chemical intakes are occurring and to incorporate this information in any evaluation of adverse effects potentially or actually occurring through interaction with other compounds.

EFFECTS OF PREEXISTING DISEASE STATES

Human patients with liver damage have an increased sensitivity to a wide variety of drugs, a phenomenon attributed to impairment of the detoxicating function of the liver. Patients with obstructive jaundice, hepatitis, cirrhosis, and other liver diseases exhibit impaired formation of glucuronide and sulfate conjugates (Muting, 1963). This reduced ability of the diseased liver to metabolize foreign compounds has been confirmed by experiments with animals. Hepatic microsomes from rabbits with obstructive jaundice show impaired metabolism of acetanilide and a number of drugs. Infection of mice with murine hepatitis virus reduces the drug-metabolizing activity of the hepatic microsomes (Kato et al., 1963b). In rats with abdominal carcinosarcomas, microsomal metabolism of foreign compounds is impaired and the activity of the microsomal enzymes of various hepatic tumors is either impaired or absent (Kato et al., 1963a). Liver regeneration is accompanied by a decrease in glycogen content and microsomal enzyme activity, both of which are restored when regeneration is complete (Dixon, 1963-1964).

Similarly, diseases of the kidney and other avenues of excretion result in reduced elimination of drugs and environmental chemicals (deBruin, 1976; Parke, 1968). Higher than expected biological levels of these compounds result, and if threshold concentrations are reached, physiological alterations and toxicity can occur. The numerous chemicals encountered daily by humans during normal activities can readily induce toxic effects if disease interferes with or reduces the effectiveness of one or more steps in biological detoxication.

REFERENCES

Baetjer, A. M. 1969. Effects of dehydration and environmental temperature on antimony toxicity. Arch. Environ. Health 19:784-792.

Baetjer, A. M. 1970. Water deprivation and liver microsomal enzyme activity. Fed. Proc. Fed. Am. Soc. Exp. Biol. 29:350, abst. no. 595.

Baetjer, A. M. 1973. Dehydration and susceptibility to toxic chemicals. Arch. Environ. Health 26:61-63.

Baetjer, A. M., N. D. Joardar, and W. A. McQuary. 1960. Effect of environmental temperature and humidity on lead poisoning in animals. Arch. Environ. Health 1:463-477.

Binkley, F. 1949. The source of sulfate in the formation of ethereal sulfates. J. Biol. Chem. 178:821-826.

Boutwell, R. K., D. Bosch, and H. P. Rusch. 1957. On the role of croton oil in tumor formation. Cancer Res. 17:71-75.

Boyd, E. M., and M. A. Boulanger. 1968. Insecticide toxicology. Augmented susceptibility to carbaryl toxicity in albino rats fed purified casein diets. J. Agric. Food Chem. 16:834-838.

Boyd, E. M., and J. Stefec. 1969. Dietary protein and pesticide toxicity: With particular reference to endrin. Can. Med. Assoc. J. 101:335-339.

Boyd, E. M., and F. I. Taylor. 1971. Toxaphene toxicity in protein-deficient rats. Toxicol. Appl. Pharmacol. 18:158-167.

Chadwick, R., A. Peoples, and M. Cranmer. 1973. The effect of protein quality and ascorbic acid deficiency on stimulation of hepatic microsomal enzymes in guinea pigs. Toxicol. Appl. Pharmacol. 24:603-611.

Dale, W. E., T. B. Gaines, and W. J. Hayes, Jr. 1962. Storage and excretion of DDT in starved rats. Toxicol. Appl. Pharmacol. 4:89-106.

deBruin, A. 1976. Biochemical Toxicology of Environmental Agents. Elsevier/North-Holland, New York. 1544 pp.

Deichmann, W. B., W. E. MacDonald, D. A. Cubit, and A. G. Beasley. 1972. Effects of starvation in rats with elevated DDT and dieldrin tissue levels. Int. Arch. Arbeitsmed. 29:233-252.

Dewhurst, F. 1963. The effect of stress upon the metabolism of 2-naphthylamine in mice. Experientia 19:646-647.

Dingell, J. V., P. D. Joiner, and L. Hurwitz. 1966. Impairment of hepatic drug metabolism in calcium deficiency. Biochem. Pharmacol. 15:971-976.

Dixon, R. L. 1963-1964. A structure-function-relationship study of various factors altering microsomal drug metabolism. Diss. Abstr. 24:2513-2514.

Driever, C. W., and W. F. Bousquet. 1965. Stress-drug interactions: Evidence for rapid enzyme induction. Life Sci. 4:1449-1454.

Elovaara, E., Y. Collan, P. Pfaffli, and H. Vainio. 1980. The combined toxicity of technical grade xylene and ethanol in the rat. Xenobiotica 10:435-445.

Fodor, O., I. Szantay, St. Tamas, P. Szabo, and S. Cotul. 1971. The study of $C^{14}O_2$ elimination in experimental intoxication with carbon tetrachloride following d,l-aspartic acid-(4)-C^{14} administration under aspartate protection. Rev. Roum. Biochim. 8(2):113-116.

Fuhrman, F. A. 1946. The effect of body temperature on drug action. Physiol. Rev. 26:247-274.

Fuhrman, G. J., and F. A. Fuhrman. 1961. Effects of temperature on the action of drugs. Annu. Rev. Pharmacol. 1:65-78.

Fuller, G. C., W. F. Bousquet, and T. S. Miya. 1972. Effect of cold exposure on drug action and hepatic drug metabolism in the rat. Toxicol. Appl. Pharmacol. 23:10-19.

Funckes, A. J., G. R. Hayes, Jr., and W. V. Hartwell. 1963. Urinary excretion of paranitrophenol by volunteers following dermal expousre to parathion at different ambient temperatures. J. Agric. Food Chem. 11:455-457.

Goebell, H., and Ch. Bode. 1971. Influence of ethanol and protein deficiency on the activity of alcohol dehydrogenase in the rat liver. Pp. 23-30 in G. A. Martini and Ch. Bode, eds. Metabolic Changes Induced by Alcohol. Springer-Verlag, New York.

Gontzea, I., and P. Sutzescu. 1968. Natural Antinutritive Substances in Foodstuffs and Forages. S. Karger, Basel. 184 pp.

Gontzea, I., P. Sutzesco, S. Dumitrache, and E. Bistriceanu. 1970. Recherches sur le role de l'apport proteique sur les moyens de defense de l'organisme envers quelques toxiques chimiques. Arch. Mal. Prof. Med. Trav. Secur. Soc. 31:471-480.

Grigorowa, R., and S. Binnewies. 1973. Uber die kombinierte Wirkung von phosphororganischen Pestiziden und erhohter Umgebungstemperatur in inhalatorischen Kurzversuchen an Ratten. I. Toxikologische Aspekte. Combined action of organophosphorus pesticides and increased environmental temperature in short-term inhalation studies on rats. I. Toxicological aspects. Int. Arch. Arbeitsmed. 31:295-307.

Highman, B., L. A. Heppel, and R. J. Lamprey. 1951. The toxicology of 1,2-dichloroethane (ethylene dichloride). V. Effects of protective agents on visceral fatty changes in exposed rats. Arch. Pathol. 51:346-350.

Hueper, W. C., and W. D. Conway. 1964. P. 426 in Chapter XII, General considerations on bioassays. Chemical Carcinogenesis and Cancers. Charles C Thomas, Publisher, Springfield, Illinois.

Inscoe, J. K., and J. Axelrod. 1960. Some factors affecting glucuronide formation in vitro. J. Pharmacol. Exp. Ther. 129:128-131.

Kato, R., and J. R. Gillette. 1965. Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharmacol. Exp. Ther. 150:279-284.

Kato, R., G. Frontino, and P. Vassanelli. 1963a. Decreased activities of liver microsomal drug-metabolizing enzymes in the rats bearing Walker carcinosarcoma. Experientia 19:31-32.

Kato, R., Y. Nakamura, and E. Chiesara. 1963b. Enhanced phenobarbital induction of liver microsomal drug-metabolizing enzymes in mice infected with murine hepatitus virus. Biochem. Pharmacol. 12:365-370.

Keplinger, M. L., G. E. Lanier, and W. B. Deichmann. 1959. Effects of environmental temperature on the acute toxicity of a number of compounds in rats. Toxicol. Appl. Pharmacol. 1:156-161.

Lasota, W., H. Mlodecki, and W. Kwasniewska. 1973. Effect of phenitrothion on ezymes of rats in subacute intoxication. II. Serum glutamic-oxalactic [sic] transaminase and glutamic-pyruvate transaminase levels in rats fed a protein deficient diet with a different fat content. Bromatol. Chem. Toksykol. 6(4):435-444. [Chem. Abs. 82:93896c, 1975.]

Lee, M., K. Harris, and H. Trowbridge. 1964. Effect of the level of dietary protein on the toxicity of dieldrin for the laboratory rat. J. Nutr. 84:136-144.

Levakovskaya, A. I., and E. M. Neizvestnova. 1972. Morphological and histochemical characteristics of the adrenal glands under the influence of manganese and nonspecific vibration. Pp. 84-90 in B. T. Velichkovskii, ed. Komb. Deistvie Khim. Fiz. Faktorov Proizvod. Sredy. Sverdlovsk, USSR: Sverdlovsk. Nauch.-Issled Inst. Gig. Tr. Profzabol. [Chem. Abs. 79:88042e, 1973.]

Levander, O. A., and V. C. Morris. 1970. Interactions of methionine, vitamin E, and antioxidants in selenium toxicity in the rat. J. Nutr. 100:1111-1117.

Madhavan, T. V., and C. Gopalan. 1965. Effect of dietary protein on aflatoxin liver injury in weanling rats. Arch. Pathol. 80:123-126.

Mavrinskaya, L. F., and L. Ya. Tartakovskaya. 1972. Structural changes in the nerve elements of the spine and skeletal musculature during the separate and combined action of vibration and mercury. Pp. 67-76 in B. T. Velichkovskii, ed. Komb. Deistvie Khim. Fiz. Faktorov Proizvod, Sredy. Sverdlovsk, USSR: Sverdlovsk. Nauch.-Issled Inst. Gig. Tr. Profzabol. [Chem. Abs. 79:88039j, 1973].

McLean, A. E. M., and P. N. Magee. 1970. Increased renal carcinogenesis by dimethyl nitrosamine in protein deficient rats. Br. J. Exp. Pathol. 51:587-590.

McLean, A. E. M., and E. K. McLean. 1966. The effect of diet and 1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane (DDT) on microsomal hydroxylating enzymes and on sensitivity of rats to carbon tetrachloride poisoning. Biochem. J. 100:564-571.

McLean, A. E. M., and E. K. McLean. 1969. Diet and toxicity. Br. Med. Bull. 25:278-281.

Miettinen, T. A., and E. Leskinen. 1963. Enzyme levels of glucuronic acid metabolism in the liver, kidney and intestine of normal and fasted rats. Biochem. Pharmacol. 12:565-575.

Müting, D. 1963. Detoxication capacity of the diseased liver. Ger. Med. Mon. 8:198-202.

Oeriu, S., D. Winter, S. Sauvard, J. Winter, I. Tanase, N. Carp, V. Cojocaru, and E. Vochitu. 1966. Disturbances in protein metabolism during carbon tetrachloride intoxication. The protective action exerted by folcysteine. Rev. Roum. Physiol. 3(2):109-116. [Chem. Abs. 67:9855y, 1967.]

Parke, D. V. 1968. The Biochemistry of Foreign Compounds. Pergamon Press, New York.

Platonow, N., B. B. Coldwell, and L. P. Dugal. 1963. Rate of metabolism of radioactive ethanol in cold environment. Q. J. Stud. Alcohol 24:385-397.

Pokrovsky, A. A. 1969. Biochemical principles of dietotherapy. Vopr. Pitan. 28:3-10.

Porta, E. A., O. R. Koch, and W. S. Hartroft. 1970. Recent advances in molecular pathology: A review of the effects of alcohol on the liver. Exp. Mol. Pathol. 12:104-132.

Savitskii, I. V. 1967. Effect of high ambient air temperature on the course of thiol poisoning. Gig. Tr. Prof. Zabol. 11(10):30-35. [Chem. Abs. 68:58250b, 1968.]

Savolainen, H., P. Pfiaffli, M. Helojoki, M. Tengien. 1979. Neurochemical and behavioural effects of long-term intermittent inhalation of xylene vapour and simultaneous ethanol intake. Acta Pharmacol. Toxicol. 44:200-207.

Shakman, R. A. 1974. Nutritional influences on the toxicity of environmental pollutants. A review. Arch. Environ. Health 28:105-113.

Shtenberg, A. I. 1972. Diet background and the body sensitivity to toxic substances. Gig. Sanit. 37(6):73-76. [Chem. Abs. 77:97485n, 1972.]

Smith, M. E., and H. W. Newman. 1959. The rate of ethanol metabolism in fed and fasting animals. J. Biol. Chem. 234:1544-1549.

Stokinger, H. E. 1957. Evaluation of the hazards of ozone and oxides of nitrogen; Factors modifying toxicity. A.M.A. Arch. Ind. Health 15:181-190.

Tannenbaum, A. 1959. Nutrition and cancer. Pp. 517-562 in F. Homburger, ed. The Physiopathology of Cancer, 2nd ed. Hoeber-Harper, New York.

Tannenbaum, A., and H. Silverstone. 1958. Urethan (ethyl carbamate) as a multipotential carcinogen. Cancer Res. 18:1225-1231.

Tesic, D., L. J. Terzic, B. Dimitrijevic, D. Zivanov, and M. Slavic. 1972. Effect of environmental temperature and some medicaments on the toxic effect of dinitroortho-cresol. Acta Vet. (Belgrade) 22(2):45-52. [Chem. Abs. 83:54205c, 1975.]

Tomasulo, P. A., R. M. H. Kater, and F. L. Iber. 1968. Impairment of thiamine absorption in alcoholism. Am. J. Clin. Nutr. 21:1341-1344.

Trakhtenberg, I. M., I. V. Savitskii, and R. Ya. Shterengarts. 1965. Effect of low Hg concentrations on the organism (problem of combined action of toxic and thermal factors). Gig. Tr. Prof. Zabol. 9(12):7-12. [Chem. Abs. 64:20505h, 1966.]

Tsapko, V. G., and M. B. Rappoport. 1972. Structural changes in animal organisms during the combined action of intense noise and the organophosphorus pesticide chlorophos (Dipterex). Gig. Tr. No. 8:128-131. [Chem. Abs. 79:112203f, 1973].

Vitale, J. J., J. DiGiorgio, H. McGrath, J. Nay, and D. M. Hegsted. 1953. Alcohol oxidation in relation to alcohol dosage and the effect of fasting. J. Biol. Chem. 204:257-264.

Waliszewski, K. 1972. Effect of different dietary levels of casein and methionine supplements on pesticide toxicity. Bromatol. Chem. Toksykol. 5:317-321. [Chem. Abs. 78:24786x, 1973.]

Webb, R. E., C. C. Bloomer, and C. L. Miranda. 1973. Effect of casein diets on the toxicity of malathion and parathion and their oxygen analogues. Bull. Environ. Contam. Toxicol. 9:102-107.

West, G. B. 1964. The influence of diet on the toxicity of acetylsalicylic acid. J. Pharm. Pharmacol. 16:788-793.

Zapata-Ortiz, V., L. Batalla, and I. Gonzalez. 1970. Metabolism of alcohol at high altitudes. Pp. 38-41 in R. E. Popham, ed. Alcohol and Alcoholism. University of Toronto, Toronto, Canada.

CHAPTER 9

MATHEMATICAL MODELS FOR CHEMICAL INTERACTIONS

Mathematical models can be used to describe a variety of biological phenomena that cannot be characterized adequately in other ways. These models describe relationships among variables and can thereby provide better insight into basic biological phenomena and their implications.

In the biological sciences, interests often extend into dose ranges or other areas where experimental measurement is not practical. In these situations we can use models that fit the data in the observable range to extrapolate into the nonobservable range. Although more than one model may fit the observable data, the extrapolations that they suggest may differ substantially. Because of these possible discrepancies, it is highly desirable that a model have a biological basis. The models can, of course, be refined or extended as new information becomes available.

EXPLANATION OF THE ONE-HIT MODEL

The "one-hit" model has been used to describe the dose-response relationships of agents that produce cancer in humans. For cancer, a "hit" can be conceptualized as a permanent "change in cellular genetics resulting from interaction of one molecule of carcinogen with a critical receptor in one cell" (Maugh, 1978).

In general, if, at dose D, λD is the expected number of "hits", then the probability of exactly x hits is described by the Poisson Probability Law:

$$P[X = x] = \frac{e^{-\lambda D}(\lambda D)^x}{x!}$$

where e is exposure and λ is an unknown constant. The one-hit model assumes that only one hit is required to produce the disease response. Therefore, the probability (P) of response at dose D is:

$$P(D) = P[x \geq 1] = 1 - e^{-\lambda D}$$

P(D) can also be described as the proportion of individuals who would respond to dose D. Note that the one-hit model is essentially equivalent to the assumption of a linear dose-response relationship at low dosages, that is, $P(D) = \lambda D$ for low doses.

The one-hit model sometimes fits actual data reasonably well in the lower doses of the observable range. Various authors have argued that it is consistent with reasonable biological assumptions. However, others maintain that the one-hit model, or linear model at low doses, may not be adequate to explain all forms of carcinogenesis.

Several authors have proposed more complex models for these phenomena. In 1976, the Environmental Protection Agency (EPA) specified this model in its interim guidelines for risk assessment for suspected carcinogens.

INDEPENDENT ACTION MODEL vs. INTERACTION

The independent action model describes chemicals in a mixture whose joint effect is additive, i.e., the effect is neither more nor less than the sum of the independent effects of each chemical. This can be illustrated by the one-hit model. Assume that an individual is exposed to chemical A alone at dose D_1 and that this chemical follows the one-hit model in which $\lambda_1 D_1$ is the expected number of hits. Also assume that another individual is exposed to chemical B alone at dose D_2 and that this chemical follows the one-hit model in which $\lambda_2 D_2$ is the expected number of hits. If a person is exposed to both chemicals A and B at doses D_1 and D_2, respectively, and this mixture follows the one-hit model so that the expected number of hits is $\lambda_1 D_1 + \lambda_2 D_2$, then chemicals A and B are said to have independent action. That is, assuming a one-hit model for substance A and B and assuming independent action of A and B,

$$P(D_1,D_2) = 1 - e^{-\lambda_1 D_1 - \lambda_2 D_2} \quad \text{or}$$

$$P(D_1,D_2) = \lambda_1 D_1 + \lambda_2 D_2$$

for low doses.

The idea of independent action can also be generalized to other models. For example, if $P(D_1,D_2)$ is the probability of response for individuals with exposure to both chemicals A and B at doses D_1 and D_2, respectively, then an independent action model is characterized as:

$$P(D_1,D_2) = P(D_1) + P(D_2) - P(D_1)P(D_2) \quad (1)$$

In contrast to independent action, interaction, i.e., synergism or an antagonism, is said to occur when the combined effect of chemicals is greater or less than the sum of the independent action of each chemical. Three indices that measure the degree of interaction are related to equation (1) (Finney, 1971; Hogan et al., 1978; Walter, 1976);

$$I_H = R(D_1,D_2) - R(D_1) - R(D_2) + 2, \quad (2)$$

$$I_F = \frac{R(D_1,D_2) - 1}{R(D_1) + R(D_2) - 2}, \text{ and} \quad (3)$$

$$I_W = \frac{R(D_1, D_2)}{R(0_1,D_2)\ R(D_1 0)} \quad (4)$$

where I is the extent of interaction; H, F, and W refer to the Hogan, Finney, and Walter references, respectively; 0 refers to unexposed populations; and R(D) is the relative risk of individuals exposed to dose D compared to unexposed individuals, i.e.:

$$R(D) = \frac{P(D)}{P(0)}$$

Essentially, equations (2), (3), and (4) measure the degree to which equation (1), the criterion for independent action, is not true. Independence is indicated by values equal to 1, synergism by values greater than 1, and antagonism by values less than 1.

For low doses, the independent one-hit model results in values of approximately 1 for each index. Although these indices can be used to measure the degree of synergism or antagonism,

they may vary widely and yield conflicting results (Blot and Day, 1979; Hamilton and Hoel, 1978; Rothman, 1978a,b; Walter and Holford, 1978).

PARALLELISM

The property of parallelism can be defined formally as follows. If the probability of response to chemicals A and B at dose D are $P_A(D)$ and $P_B(D)$, respectively, then parallelism implies that:

$$P_A(D) = P_B(fD)$$

for all doses, where f is the relative potency of chemical A to chemical B.

In this model of parallelism, two chemicals acting alone respond as if one were a dilution of the other, dilution being defined as a mixture of two chemicals when one chemical is completely inert to the response of interest. This does not mean that one is in fact a dilution of the other but simply that the dose-response curves for these chemicals look like one could be a dilution of the other.

If two chemicals have the parallelism property and the chemicals act independently, then the transformed dose-response curve for the combined effect of these chemicals will be parallel to the curves for the individual chemicals. This is not necessarily the case for a model for independent action.

DOSEWISE ADDITIVITY

In a model of dosewise additivity, one chemical acts as if it were a dilution of the other but in a different way than discussed above (American Conference of Governmental Industrial Hygienists, 1977). For example, if 100 g of chemical A is equivalent in potency to 10 g of chemical B, this model indicates that a mixture of 50 g of A and 5 g of B would have the same potency as 100 g of A alone or 10 g of B alone. Furthermore, the mixtures--25 g and 7.5 g, 75 g and 2.5 g, 10 g and 9 g, and, in general, 100π and $10(1-\pi)$, where $0< \pi < 1$, would also have this same potency. Although chemical B is assumed to be 10 times as potent as A at a particular dosage, the dosewise additivity model does not guarantee that this particular relationship, the property of parallelism, will apply to other doses. On the other hand, the possession of this property by two chemicals does not in itself indicate what the efficacy of the mixtures might be.

Both parallelism and dosewise additivity have been described in terms of "apparent dilutions." However, if the dilutions were true, then both the parallelism and dosewise additivity properties should apply.

The independent action one-hit model has the property of dosewise additivity, but dependent versions of this model generally do not.

ACGIH THRESHOLD LIMIT CRITERION FOR EXPOSURE TO MORE THAN ONE CHEMICAL

The American Conference of Governmental Industrial Hygienists (ACGIH, 1977) has developed a threshold limit criterion for chemical mixtures or multiple exposures. When $C_i \equiv$ observed concentration for chemical i and $T_i \equiv$ the threshold limit value for chemical i for a mixture of n chemicals that affect the same body organs, if $\sum_{i=i}^{n} Ci/Ti>1$, the threshold limit of the mixture is considered to be exceeded. Gart, whose paper is attached to this report as Appendix B, made three important points with respect to this criterion.

1. For low doses, this criterion can be justified if one assumes that the chemicals follow the independent one-hit model.
2. Under the conditions stated in 1, the ACGIH criterion can be extended to include mixtures of chemicals that affect different organ systems.
3. Under certain circumstances this criterion can be misleading when applied to low doses if the chemicals follow a one-hit model that does not have independent action.

Models other than the one-hit or linear model may be useful in actual practice. One may be engaged in linear extrapolations when the underlying reality is not linear. The conclusions drawn may either underestimate or overestimate the risk, depending on the circumstances.

REFERENCES

American Conference of Governmental Industrial Hygienists. 1977. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1977. American Conference of Governmental Industrial Hygienists, Cincinnati. 94 pp.

Blot, W. J., and N. E. Day. 1979. Letter to the editor: Synergism and interaction: Are they equivalent? Am. J. Epidemiol. 110:99-100.

Finney, D. J. 1971. Probit Analysis, 3rd edition. Cambridge University Press, London.

Gaddum, J. H. 1953. Pharmacology, 4th edition. Oxford University Press, New York.

Hamilton, M. A. and D. G. Hoel. 1978. Detection of Synergistic Effects in Carcinogenesis. Paper presented at the Joint Statistical Meeting in San Diego, Calif., August 1978.

Hogan, M. D., L. L. Kupper, B. M. Most, and J. K. Haseman. 1978. Alternatives to Rothman's approach for assessing synergism (or antagonism) in cohort studies. Am. J. Epidemiol. 108:60-67.

Maugh, T. H. II. 1978. Chemical carcinogens: How dangerous are low doses? Science 202:37-41.

Rothman, K. J. 1978a. Estimation versus detection in the assessment of synergy. Am. J. Epidemiol. 108:9-11.

Rothman, K. J. 1978b. Occam's razor pares the choice among statistical models. Am. J. Epidemiol. 108:347-349.

Walter, S. D. 1976. The estimation and interpretation of attributable risk in health research. Biometrics 32:829-849.

Walter, S. D., and T. R. Holford. 1978. Additive, multiplicative, and other models for disease risks. Am. J. Epidemiol. 108:341-346.

U.S. Environmental Protection Agency. 1976. Interim procedures and guidelines for health risk and economic impact assessments of suspected carcinogens. Fed. Regist. 41:21402.

CHAPTER 10

LITERATURE SEARCH AND BACKGROUND ON INTERACTIONS

Preliminary attempts to locate articles dealing with toxicological interactions among 22 exemplary compounds listed by the Coast Guard (Table 11-1) involved searches in MEDLINE and TOXLINE under such headings as drug synergism, drug antagonism, and interactions. Although volumes have been written about potential drug-drug interactions (e.g., Newberne et al., 1978; Veldstra, 1956), there is a paucity of reports on interaction of common or widely used drugs and exposure to other chemicals of concern in marine occupations. Further search of the literature and communications with other scientists led to only a few additional reports.

Finally, all the references in another government data base were searched for the 11 compounds underlined in Table 11-1. All of these references have been incorporated into a single data base and constitute the presently available information on which the comments in this section are based. Certain well-known examples of interaction due to enzyme induction are not included because, for the most part, they did not relate to the compounds in Table 11-1 and have been adequately discussed in other sections of this report. Many reports that consider toxicological interactions are not retrievable by means of the usual key words intended to relate to interactions.

Published reports on the toxicity of various solvent-solvent or solvent-drug combinations can be divided into three groups. The first group contains those for which no synergism (only addition or summation) of effects was observed. The second group generally

Table 11-1

Candidate Materials of Interest to the U.S. Coast Guard as Possible Agents for Interaction Modeling

Compound	Molecular Weight	Boiling Point, °C	Melting Point, °C	Specific Gravity (°C)	Vapor Pressure, mm Hg @ 20 °C	Vapor Pressure, mm Hg @ 37 °C	Solubility: Water	Solubility: Ethanol	Solubility: Benzene	Threshold Limit Value, ppm	Short-Term Exposure Limit, ppm	Acute Inhalation Toxicity: Animal	Acute Inhalation Toxicity: Time of Exposure	Acute Inhalation Toxicity: LC50, ppm	References
Acetic Acid	60.05	117.9	16.7	1.050 (20)	0.95	0.60	Miscible	Miscible	Miscible	10	15	Guinea Pig	1 h	5,000	Ghiringhelli and Di Fabio, 1957
Acetone	58.08	56.1	-94.7	0.791 (20)	6.0	7.5	Miscible	Miscible	Miscible	1,000	1,250	Mouse	1 h	5,620	American Industrial Hygiene Association, 1972
Acrylonitrile	53.06	77.4	-83.6	0.807 (20)	1.8	3.5	Soluble	Soluble	Soluble			Mouse	3 h	130,000	Nekleosova and Kudrina, 1969
Benzene	78.10	80.1	5.5	0.879 (20)	1.6	3.2	Slightly soluble	Miscible	-	20(60)	(60)	Rat	4 h	500	Carpenter et al., 1949
										10	(25)	Guinea Pig	4 h	576	Hudley and Neal, 1942
												Rat	4 h	16,000	Carpenter et al., 1949
n-Butyraldehyde	72.11	74.8	-96.6	0.801 (20)	2.8	6.0	Soluble	Miscible	Soluble	-	-	Rat	7 h	10,000	Gerarde, 1960
												Mouse	7 h	9,980	Svirbely et al., 1963
Carbon tetrachloride	153.83	76.5	-23.0	1.590 (20)	1.9	4.0	Slightly soluble	Soluble	Miscible	10	20	Rat	30 min	60,000	Fassett, 1963
												Mouse	6 h	7,329	Gradiski et al., 1978
Cresols (isomers)	108.13	177.0	-	1.030 (20)	0.13	0.35	Soluble	Miscible	Miscible			Rat	30 min	23,900	Spector, 1956
										5	10	Mouse	7 h	9,528	Svirbely et al., 1963
Cumene	120.19	152.4	-96.1	0.866 (15)	0.28	0.56	Slightly soluble	Soluble	Soluble			Rat	1 h	>161 [a]	Industrial Bio-Test Laboratories, Inc., 1969a
										50	75	Rat	1 h	>277 [b]	Industrial Bio-Test Laboratories, Inc., 1969a
Ethanol	46.07	78.3	-114.0	0.790 (20)	0.90	0.22	Miscible	-	Miscible			Rat	1 h	>4,510	Industrial Bio-Test Laboratories, Inc., 1969b
										1,000	1,250	Rat	4 h	8,000	Smyth et al., 1951
Ethanolamine (mono)	61.08	170.8	10.3	1.010 (25)	-	-	Miscible	Soluble	Soluble			Rat	4 h	10,000-12,000	Treon, 1963
										3	6	-	-	-	
Ethylene diamine	60.10	117.0	11.0	0.909 (20)	0.20	0.56	Miscible	Soluble	Slightly soluble	10	20				
Ethylene dibromide	187.86	131.0	9.8	2.180 (20)	0.20	0.50	Slightly soluble	Miscible	Soluble			Rat	8 h	4,000	Smyth et al., 1951
										20	30				
Ethylene dichloride	98.96	83.5	-35.7	1.253 (20)	1.4	2.2	Slightly soluble	Slightly soluble	Soluble			Rat	2 h	400 [c]	Rowe et al., 1952
										50	75	Rabbit	1 h	8,425 [c]	Irish et al., 1940
Ethylene glycol (vapor)	62.07	197.6	-13.0	1.115 (20)	0.014	0.050	Miscible	Soluble	Soluble			Guinea Pig	3 h	400 [c]	Rowe et al., 1952
										100	125	Mouse	6 h	262	Gradiski et al., 1978
Gasoline	-	60-199	-	0.71-0.74 (20)	-	-	Slightly soluble	Miscible	Miscible			-	-	-	
										-	-	Mouse	2 h	121mg/l [d]	Spector, 1956
Methanol	32.04	64.5	-97.8	0.792 (20)	1.9	4.4	Miscible	Miscible	Miscible	200	250				
												Cat	6 h	33,600	Treon, 1963
Isopropylamine	59.11	32.4	-95.2	0.689 (20)	-	-	Miscible	Miscible	Soluble	-	-	Mouse	3-4.5 h	262,000-363,000	Spector, 1956
Styrene	104.16	145.5	-30.6	0.909 (20)	-	-	Soluble	Soluble	Soluble	100	125	Rat	3-4.5 h	174,000	Spector, 1956
												Mouse	60 min	7,000 [d]	Shell Oil Co., 1961
Toluene	92.-	110.6	-95.0	0.867 (20)	0.65	1.0	Slightly soluble	Miscible	Miscible	100	150	Rat	4 h	8,000 [d]	Spector, 1956
												Mouse	2 h	5,000	Shugaev, 1969
Toluene diisocyanate	174.16	250.0	20-22	1.220 (25)	-	-	Slightly soluble	Miscible	Miscible			Rat	4 h	2,809	Shugaev, 1969
										0.02	(0.02)	Mouse	7 h	5,300	Svirbely et al., 1963
Trichloroethylene	131.39	87.0	-86.4	1.460 (20)	1.2	2.5	Slightly soluble	Miscible	Miscible			Mouse	2 h	7,980-9,310	Spector, 1956
										100	150	Rat	4 h	14	Duncan et al., 1962
Vinyl acetate	86.09	72.9	-92.8	0.934 (20)	1.9	3.0	Soluble	Soluble	Soluble			Mouse	4 h	10	Duncan et al., 1962
										10	20	Rabbit	4 h	8	Duncan et al., 1962
												Guinea Pig	4 h	13	Duncan et al., 1962
												Rat	1 h	28,000	Carpenter et al., 1949
												Rat	4 h	4,000	Svirbely et al., 1963

[a] o-, p-

[b] o-

[c] LCLo, lowest published lethal concentration.

[d] LC, lethal concentration.

includes reports of studies on combinations, some of which produce synergistic effects while others evidence no potentiation. A few reports specify a potentiating interaction between two agents. Although antagonistic interactions may reveal details of mechanisms, emphasis in this literature search was placed upon synergistic interactions in view of the concern of potentially increased (rather than decreased) hazard to marine personnel exposed to multiple vapors.

Drew and Fouts (1974) pretreated rats with phenobarbital and chlorpromazine and then administered benzene either by inhalation or by intraperitoneal injection (in mineral oil). They observed no potentiation of benzene toxicity. In a second study, Drew et al. (1978) examined the changes in serum enzyme levels in rats that had been given organic solvents either singly or in combination. They found that neither tetrachloroethylene in combination with dioxane, butyl ether, or acetonitrile nor a combination of trichloropropane and dichloropropane resulted in greater than an additive effect on the levels of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), or ornithine carbamyl transferase (OCT). In many instances the biological effects of solvent combinations were significantly less than additive. Adams et al. (1952) and McCollister et al. (1956) studied the toxicology of fumigant mixtures, principally carbon tetrachloride, ethylene dibromide, and ethylene dichloride, and observed either simple summation of the separate toxicities or only slight potentiation.

In two classic studies, Smyth et al. (1969, 1970) reported a 3-year study in which they explored the joint toxic action (based on

LD_{50}'s of 27 industrial chemicals. They first examined the joint action of 1:1 v/v mixtures of the solvents. Altogether, 350 combinations of solvents were studied. In the second paper they compared the results obtained using mixtures of equal toxicity versus equal volumes for 53 solvent pairs. It makes a considerable difference whether one administers a solvent on the basis of equal toxicity or equal volume. When data from equal volume experiments were expressed as expected/observed LD_{50} ratios, the nine pairs showing the greatest antagonistic effect had ratios of 0.23 to 0.40 (ratios of 1.0 indicate additive effects), whereas the nine pairs with the greatest apparent potentiating effects had ratios that varied from 2.7 to 5.09.

Shugaev (1969) studied combinations of such hydrocarbons as butane, butadiene, isoprene, and styrene. Three of the 12 combinations exhibited synergism (when the results are expressed as LC_{50}). In nine tests, only summation of the individual chemical effects was observed.

Deguchi (1972) examined the effects of single and combinations of chlorinated hydrocarbons on the levels of serum transaminases in rats. Greater than additive increases in SGOT and SGPT were observed following administration of carbon tetrachloride with trichloroethylene. Other combinations were less synergistic. Elevation in enzyme levels was dose related.

Ohtsuji and Ikeda (1971) found increased metabolism and toxicity in rats exposed to combinations of styrene and phenobarbital, and Saida et al. (1976) reported potentiation of peripheral nerve changes when a combination of methyl n-butyl ketone and methyl ethyl ketone was administered to rats and cats. The combination of carbon tetrachloride

and acetonitrile (Radimer et al., 1974) was more toxic to the skin than either component applied separately, and systemic effects including septic shock, upper gastrointestinal bleeding, hypotension, and anuria were also observed following exposure to the combination. Moon (1950) reported that another combination, carbon tetrachloride and ethyl alcohol, was more hepatotoxic and nephrotoxic in humans than was either organic solvent alone. This toxicological interaction has also been observed repeatedly in studies with animals (Cornish and Adefuin, 1966, 1967; Cornish et al., 1973; Klaassen and Plaa, 1967; Kotub and Plaa, 1962). Van Doorn et al. (1978) has recently shown that phorone (diisopropylideneacetone) exhibits synergistic hepatotoxic effects with bromobenzene or paracetamol in mice. Also, Larionov and Broitman (1975) found synergistic effects when 2,6-dimethylphenol and methanol were given simultaneously by inhalation.

Two studies have reported striking carcinogenic responses to chemical combinations that maritime personnel might encounter. When combinations of ethylene dibromide (EDB) and disulfiram (antabuse) were administered chronically to male and female Sprague-Dawley rats, Plotnick (1978) observed hemangiosarcomas of the liver, spleen, omentum, and kidney as well as adenocarcinoma of the mammary gland in females and a high incidence of testicular atrophy. Neither morbidity nor mortality was observed in groups receiving the same doses of EDB or disulfiram alone.

Radike et al. (1977) found that a low oral dose of alcohol administered chronically to rats receiving vinyl chloride by inhalation causes a more rapid and greater incidence of tumors.

Data from these studies indicate that many toxic effects produced by combinations of organic solvents or of solvents and drugs are additive. However, other combinations obviously produce synergistic effects. Consequently, it is important to develop methods or principles whereby the potentially hazardous effects of chemical combinations can be predicted for humans.

REFERENCES

Adams, E. M., R. L. Hollingsworth, H. C. Spencer, and D. D. McCollister. 1952. Toxicity study of a spot fumigant. Mod. Sanitat. 4(7):39-41, 70.

American Industrial Hygiene Association. 1972. Hygienic Guide Series: Acetic Acid. Am. Ind. Hyg. Assoc. J. 33:624-627.

Carpenter, C. P., H. F. Smyth, Jr., and U. C. Pozzani. 1949. The assay of acute vapor toxicity, and the grading and interpretation of results on 96 chemical compounds. J. Ind. Hyg. Toxicol. 31:343-346.

Cornish, H., and J. Adefuin. 1966. Ethanol potentiation of halogenated aliphatic solvent toxicity. Amer. Ind. Hyg. Assoc. J. 27:57-61.

Cornish, H., and J. Adefuin. 1967. Potentiation of carbon tetrachloride toxicity by aliphatic alcohols. Arch. Environ. Health 14:447-449.

Cornish, H., B. Ling, and M. Barth. 1973. Phenobarbital and organic solvent toxicity. Amer. Ind. Hyg. Assoc. J. 34:487-492.

Deguchi, T. 1972. Threshold limit values for solvent mixtures in the air. Effects of single and mixed chlorinated hydrocarbons upon the level of serum transaminases in rats. Osaka Shiritsu Daigaku Igaku Zasshi 21(4-6):187-209. [Chem. Abs. 79:34688b, 1973].

Drew, R. T., and J. R. Fouts. 1974. The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol. Appl. Pharmacol. 27:183-193.

Drew, R. T., J. M. Patel, and F.-N. Lin. 1978. Changes in serum enzymes in rats after inhalation of organic solvents singly and in combination. Toxicol. Appl. Pharmacol. 45:809-819.

Dudley, H. C., and P. A. Neal. 1942. Toxicology of acrylonitrile (vinyl cyanide): I. A study of the acute toxicity. J. Ind. Hyg. Toxicol. 24:27-36.

Duncan, B., L. D. Scheel, E. J. Fairchild, R. Killens, and S. Graham. 1962. Toluene diisocyanate inhalation toxicity: pathology and mortality. Am. Ind. Hyg. Assoc. J. 23:447-456.

Fassett, D. W. 1963. Aldehydes and acetals. Pp. 1959-1989 in F. A. Patty, ed. Industrial Hygiene and Toxicology. Vol. 2, 2nd rev. ed. Interscience Publishers, New York.

Gerarde, H. W. 1960. Toxicology and Biochemistry of Aromatic Hydrocarbons. Elsevier, New York. 329 pp.

Ghiringhelli, L., and A. Di Fabio. 1957. Patologia de acido acetico: osservazioni negli animali da esperimento e nell'uomo. Med. Lav. 48:559-565. [Bull. Hyg. 1958. 33:247, English abstract].

Gradiski, D., P. Bonnet, G. Raoult, and J. L. Magadur. 1978. Toxicité aiguë comparée par inhalation des principaux solvants aliphatiques chlorés. Arch. Mal. Prof. Med. Trav. Secur. Soc. 39:249-257. [English summary].

Industrial Bio-Test Laboratories, Inc. 1969a. Bio-Fax Detailed Data on the Toxicological Properties of Chemicals: Cresols. Northbrook, Illinois. Bio-Fax Data Sheets No. 3,4,5.

Industrial Bio-Test Laboratories, Inc. 1969b. Bio-Fax Detailed Data on the Toxicological Properties of Chemicals: Cumene. Northbrook, Illinois. Bio-Fax Data Sheet No. 6.

Irish, D. D., E. M. Adams, H. C. Spencer, and V. K. Rowe. 1940. The response attending exposure of laboratory animals to vapors of methyl bromide. J. Ind. Hyg. Toxicol. 22:218-230.

Klaassen, C.,and G. Plaa. 1967. Relative effects of various chlorinated hydrocarbons on liver and kidney function in dogs. Toxicol. Appl. Pharmacol. 10:119-131.

Kotub, S., and G. Plaa. 1962. The effect of acute ethanol intoxication on chloroform-induced liver damage. J. Pharmacol. Exptl. Therap. 135:245-251.

Larionov, A. G., and A. Ya. Broitman. 1975. Combined action of 2,6-dimethylphenol and methanol. Gig. Tr. Prof. Zabol., No. 11:27-30.

McCollister, D. D., R. L. Hollingsworth, F. Oyen, and V. K. Rowe. 1956. Comparative inhalation toxicity of fumigant mixtures. AMA Arch. Ind. Health 13:1-7.

Moon, H. D. 1950. The pathology of fatal carbon tetrachloride poisoning with special reference to the histogenesis of the hepatic and renal lesions. Am. J. Pathol. 26:1041-1057.

Neklesova, I. D., and M. A. Kudrina. 1969. Comparative toxicological characteristics of certain chloronitroparaffins. (Sravnitel'naya toksikologicheskaya kharakteristika nekotorykh khlornitroparafinov). Hyg. Sanit. 34:429-432.

Newberne, P. M., R. L. Gross, and D. A. Roe. 1978. Drug, toxin, and nutrient interactions. World Rev. Nutr. Diet. 29:130-169.

Ohtsuji, H., and M. Ikeda. 1971. The metabolism of styrene in the rat and the stimulatory effect of phenobarbital. Toxicol. Appl. Pharmacol. 18:321-328.

Plotnick, H. B. 1978. Carcinogenesis in rats of combined ethylene dibromide and disulfiram. J. Am. Med. Assoc. 239:1609.

Radike, M. J., K. L. Stemmer, P. G. Brown, E. Larson, and E. Bingham. 1977. Effect of ethanol and vinyl chloride on the induction of liver tumors: Preliminary report. Environ. Health Perspect. 21:153-155.

Radimer, G. F., J. H. Davis, and A. B. Ackerman. 1974. Fumigant-induced toxic epidermal necrolysis. Arch. Dermatol. 110:103-104.

Rowe, V. K. H. C. Spencer, D. D. McCollister, R. L. Hollingsworth, and E. M. Adams. 1952. Toxicity of ethylene dibromide determined on experimental animals. Arch. Ind. Hyg. Occup. Med. 6:158-173.

Saida, K., J. R. Mendell, and H. S. Weiss. 1976. Peripheral nerve changes induced by methyl n-butyl ketone and potentiation by methyl ethyl ketone. J. Neuropathol. Exp. Neurol. 35:207-225.

Shell Chemical Company. 1961. Acute Toxicity Data. [10] pp.

Shugaev, B. B. 1969. Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health 18:878-882.

Smyth, H. F., Jr., C. P. Carpenter, and C. S. Weil. 1951. Range-finding toxicity data: List IV. Arch. Ind. Hyg. Occup. Med. 4:119-122.

Smyth, H. F., Jr., C. S. Weil, J. S. West, and C. P. Carpenter. 1969. An exploration of joint toxic action: Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol. Appl. Pharmacol. 14:340-347.

Smyth, H. F., Jr., C. S. Weil, J. S. West, and C. P. Carpenter. 1970. An exploration of joint toxic action. II. Equitoxic versus equivolume mixtures. Toxicol. Appl. Pharmacol. 17:498-503.

Spector, W. S., ed. 1956. Handbook of Toxicology, Vol. I. W. B. Saunders Company, Philadelphia. 408 pp.

Svirbely, J. L., R. C. Dunn, and W. F. von Oettingen. 1943. The acute toxicity of vapors of certain solvents containing appreciable amounts of benzene and toluene. J. Ind. Hyg. Toxicol. 25:366-373.

Treon, J. F. 1963. Alcohols. Pp. 1409-1496 in F. A. Patty, ed. Industrial Hygiene and Toxicology. Vol. 2, 2nd rev. ed. Interscience Publishers, New York.

Van Doorn, R., Ch.-M. Leijdekkers, and P. Th. Henderson. 1978. Synergistic effects of phorone on the hepatotoxicity of bromobenzene and paracetamol in mice. Toxicology 11:225-233.

Veldstra, H. 1956. Synergism and potentiation with special reference to the combination of structural analogues. Pharmacol. Rev. 8:339-387.

CHAPTER 11

CONCLUSIONS AND RECOMMENDATIONS

In the course of its deliberations, the panel discussed at length the paucity of information regarding the health effects, exposure histories, and analytical data relevant to chemical exposures experienced by marine personnel. There was considerable discussion of action required to acquire such information. The recommendations listed below reflect these discussions and the panel's considerations of the need to develop basic principles for predicting the toxicological interactions that constitute the principle subject of this report. These recommendations for action are listed in order of highest priority, as judged by the panel. Until more specific information becomes available, it seems that the most productive course to follow to determine limits for multiple exposures is to assume additivity and follow the guidelines for mixtures as recommended by the American Conference of Governmental Industrial Hygienists (1977).

1. A better characterization of the causative agents and the potential added risk to health from multiple chemical exposures is essential. The panel recommends that the health evaluation studies of Coast Guard marine inspectors, which have already been initiated on the advice of the panel, be extended to include the following:

   - The specific chemicals and the frequency with which they are encountered during inspection should be identified. The concentrations, temperatures, and durations of exposure should be logged in a daily

record maintained by each inspector. Data on personal use of chemicals should also be recorded.

- Urinary analyses should be performed to establish the usefulness of excretion profiles of agents as indices of exposure.
- If indicated by the data resulting from the performance of the first two recommendations, biomedical tests, e.g., SMA 1260, myoneural conduction, pulmonary function, or central nervous system function tests, should be conducted on marine inspectors and their coworkers to detect evidence of injury to organ systems.

In conjunction with these studies, health records should be augmented by information concerning any unusual dietary habits and the use and abuse of drugs, alcohol, and tobacco.

2. Current analytical procedures used by marine chemists appear to be limited in scope and are generally semiquantitative. Therefore, the panel recommends that a program be devoted to the improvement of analytical capabilities of both marine inspectors and marine chemists. This program should include both educational programs and the provision of improved analytical instrumentation.

3. The panel recommends that a list of priority chemicals be identified. The selection of chemicals for this list should be based on the nature, extent, and frequency of occupational exposure of marine personnel. These compounds should then be subjected to:

- A comprehensive literature review to determine existing knowledge concerning biological effects and potential

toxicological interactions, including a search for data on possible exposure of humans to the same chemical in other industries and occupations.

- Experimental studies, where necessary, to extend the toxicological data base, including: research to obtain quantitative information regarding reaction kinetics at potential sites of interaction; determination of effects of exposures of laboratory animals to pairs of chemicals over a dosage range that includes the threshold limit values with assessments of effects selected on the basis of the known toxicological end points of the individual chemicals; and, where there is reason for special concern, more extensive toxicological tests, such as in-vitro assays for reactive intermediates and tests for myoneural conduction, altered behavior, two-stage carcinogenesis, mutagenesis, teratogenesis, effects on reproduction, and other tests as appropriate.

4. The Coast Guard should collaborate with organizations such as the National Library of Medicine, the Oak Ridge Toxicology Information Response Center, and other groups to improve collection, collation, and retrieval of toxicological data.

5. When unusually high risk of serious toxicological interaction is expected, the Coast Guard should develop criteria for classifying chemicals to protect against toxicological incompatibility.

6. The Coast Guard should expand its research studies and educational programs regarding chemicals and health to include a

broader segment of the marine industry by cooperating with unions, shipping companies, and other naval personnel.

7. When sufficient data have been collected, as recommended above, the Coast Guard should proceed to an assessment of potential interactions from combinations of specific materials to which marine personnel are frequently exposed.

REFERENCES

American Conference of Governmental Industrial Hygienists. 1977. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1977. American Conference of Governmental Industrial Hygienists, Cincinnati. 94 pp.

APPENDIX A

PANEL SITE VISIT -- HOUSTON SHIP CHANNEL

On January 11, 1979, the panel visited and inspected the M/T Stolt Sheaf which was loading cargo in the Houston Ship Channel in Texas. Built at a cost of approximately $40 million in 1972, this vessel is designed specifically for the transportation of bulk liquid cargoes. It is divided into five main cargo sections by transverse cofferdams, has double bottoms throughout, and a double hull for 65% of the cargo area. Transverse and longitudinal bulkheads are used to divide the cargo space into 40 separate tanks with liquid capacities ranging from 239 to 1,573 m^3. There are 9 stainless steel, 9 zinc-lined, and 22 phenolic-coated tanks, all of which could contain different chemical cargoes. Each tank is equipped with an individual cargo line, hatch access, deepwell pump, steam heating lines, and ventilation pipes. Although it appears cluttered, the deck space is devoted to a complex, well-marked, interconnecting piping system of noncorrosive pipes lined with stainless steel. The entire system is closed, and ventilation pipes are exhausted well above the heads of crewmen. On-board pumps are operated from a central control room which also houses remote gauges to monitor and maintain cargo temperatures up to 82°C.

Cargo can be taken on board or discharged at either the port or starboard sides at a rate of 250 tonnes per hour per pump. Easily accessible, gasket-sealed standard manifolds facilitate attachment to flexible pipes from shore establishments and minimize leakage. This modern vessel is well equipped with up-to-date navigational aids.

After discussing with the ship's officers the problems of loading, storing, and discharging cargoes, the panel climbed down into a 737-m^3 stainless steel tank. It then asked questions concerning the operations involved in cleaning such a structure to remove traces of the previous cargo.

Prior to entry by the ship's personnel, air samples are taken the top, middle, and bottom of the tank with Draeger tubes, which react chemically and produce a color reaction. After entry, air samples are drawn from the corners. Two landings connected by stairs from the access hatch are located on one side of the tank, providing platforms from which the walls can be flushed down by warm or hot water or steam under pressure. The operators wear protective clothing and a gas mask or a self-contained breathing apparatus. The residue is pumped out to a holding tank and/or is transferred to a special disposal barge when in port. While workers are inside the tank, there is always someone on deck who periodically checks to ensure that they have not been affected by any volatile residues.

Subsequently, the panel examined the safety equipment of the officers and crew, including protective clothing and self-contained respirator packs for short- (20-minute) and long-term use.

The panel belives that this vessel was far superior to many of the vessels and barges that carry volatile and potentially hazardous chemical cargoes. This 6-year-old ship met a high level of safety standards, having been built specifically for this purpose unlike many older ships which were neither originally built nor converted to meet present day safety standards. The senior officers of the

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vessel were well aware of the dangerous and toxic nature of the cargoes carried. They had obtained such knowledge through courses held periodically by the company for their personnel. The panel was impressed by the responsibility with which the Stolt-Nielsen companies trained their officers, especially the executive officer who controls deck operations. It was obvious that few operations on this vessel would be conducted in a slipshod manner, a point confirmed by Lt. Cmdr. Robert Storch of the Houston Coast Guard inspectorate section. Unfortunately, time and opportunity did not permit the panel to visit other ships and barges to allow a comparison of safety features as well as loading and discharging features.

Stolt-Nielsen, Inc. requires an annual medical examination of its personnel. Reports of the results of these examinations are filed in the company's head office in Greenwich, Connecticut. The declaration of health forms pertain only to the physical examination and a chest X-ray. It does not include a blood chemistry or hematological assessment. The panel believes this to be a deficiency since such analyses might indicate alterations in organ function.

The impressive responsibility shown by Stolt-Nielsen, Inc. for its employees may not be the rule for many shipping firms; however, the panel has no comparative information with which to verify this.

APPENDIX B

MATHEMATICAL MODELS FOR CHEMICAL INTERACTIONS

A discussion of possible models for chemical interactions requires the review of some of the basic statistical concepts of bioassay.

PARALLEL LINE ASSAY AND RELATIVE POTENCY

Let the proportion response to chemical 1 at dose D_1 be denoted by $P_1(D_1)$ and to chemical 2 at dose D_2 be denoted by $P_2(D_2)$. Parallel line assay implies that $P_1(D) = P_2(fD)$ for all doses, D, where f is the relative potency of chemical 1 to chemical 2.

Example: Consider the "one hit" model, which is often appropriate to use in experiments with low doses of carcinogens and cell transformation:

$$P_1(D) = 1 - \exp(-\lambda_1 D),$$

$$P_2(D) = 1 - \exp(-\lambda_2 D),$$

where λ_1 and λ_2 are parameters (called "transformicities" by Gart et al., 1979) that are measures of the potency of the chemicals. For small doses, $P_1(D) \sim \lambda_1 D, P_2(D) \sim \lambda_2 D$, i.e., response is proportional to dose. When presented as a log-log plot, parallel straight lines are obtained:

$$\ln\{-\ln[1-P_1(D)]\} = \ln \lambda_1 + \ln D$$

$$\ln\{-\ln[1-P_2(D)]\} = \ln \lambda_2 + \ln D$$

The lines are parallel with slope 1. The relative potency is $f = \lambda_1/\lambda_2$, since

$$P_1(D) = 1-\exp[-(\lambda_1/\lambda_2)\lambda_2 D] = P_2(fD)$$

for all doses.

INDEPENDENT ACTION

Independent action may be defined by using the ordinary rules of probability in relation to the joint application of two doses, D_1 and D_2. If the probability of such a response is $P(D_1,D_2)$, then the independent action model implies:

$$P(D_1,D_2) = P(D_1,0) + P(0,D_2) - P(D_1,0)\ P(0,D_2).$$

Example: Using the "one hit" model again, we have:

$$P(D_1,D_2) = 1-\exp(-\lambda_1 D_1)+1-\exp(-\lambda_2 D_2)-[1-\exp(-\lambda_1 D_1)][1-\exp(-\lambda_2 D_2)] \text{ and}$$

$$P(D_1,D_2) = 1-\exp(-\lambda_1 D_1-\lambda_2 D_2). \quad (1)$$

Note that one possible dependent model is:

$$P(D_1,D_2) = 1-\exp(-\lambda_1 D_1 - \lambda_2 D_2 e^{\theta D_1}), \quad (2)$$

where, θ is a parameter which, when $\theta = 0$, measures the lack of independence of the model. Note that

$$P(D_1,0) = 1-\exp(-\lambda_1 D_1) \text{ and}$$

$$P(0,D_2) = 1-\exp(-\lambda_2 D_2).$$

That is, there is a parallel line assay, but independent action is not present.

DOSEWISE ADDITIVITY

If D_1' and D_2' individually yield the same response (e.g., 50%) then they are "dosewise" additive if the mixture consisting of $\pi D_1'$ and $(1-\pi)D_2'$ (for all $0<\pi<1$) leads to the same response (e.g., 50%) (Gaddum, 1953).

Example 1. Using the one hit curves and the independent model (Equation 1, above), the LD50's are $D_1' = (\ln 2)/\lambda_1$ and $D_2' = (\ln 2)/\lambda_2$. For all $0<\pi<1$, we find that $P[\pi D_1', (1-\pi)D_2'] = 1-\exp[-\lambda_1\pi(\ln 2)/\lambda_1 - \lambda_2(1-\pi)(\ln 2)/\lambda_2] = 1-\exp[-\ln 2] = 1/2$. This model is dosewise additive. Equation 2 will not generally enjoy this property. It is not necessary that all independent models be dosewise additive, but this one hit model (Equation 1) enjoys all three properties of parallelism, independence, and additivity.

Example 2. Gullino et al. (1956) used the concept of dosewise additivity in investigating the toxicity of essential amino acids in animals. In this effort, Cornfield (1975) served as statistical advisor. In an initial series of experiments, they determined the individual response curves of 10 such amino acids. More than 1,000 experiments would be required to determine all combinations. The investigators considered this to be impractical. Moreover, they believed that if the acids were dosewise additive, further such study would be unrewarding. They also studied pairs of acids at doses that yielded approximately 1% to 3%. A mixture under the dosewise additivity model should have yielded approximately this magnitude of response. However, 100% lethality was observed in all the pairwise combinations tested. When all 10 were combined at doses that should have yielded 50% response under the model, none died. From tests of all 10 combinations of 9 acids each, the investigators found that the one lacking L-arginine was less toxic than the rest. They attributed the protective effect of L-arginine to its ability to speed the metabolism of ammonia.

The concept of dosewise additivity can be quite useful in designing

a series of laboratory experiments.

MEASURES OF SYNERGISM AND ANTAGONISM

Much of the discussion of deviation from independence concerns its epidemiological applications (see, for example, Hamilton and Hoel, 1978; Walter, 1976). Such applications are typically defined in terms of the relative risk (R) of exposed individuals [P(D)] to unexposed individuals [P(0)], i.e., $R = P(D)/P(0)$. Unexposed individuals are assumed to have a background risk (α).

The modification of the one hit curves is as follows:

$$P(D_1,D_2) = \alpha + (1-\alpha)[1-\exp(-\lambda_1 D_1-\lambda_2 D_2)],$$

$$P(D_1,0) = \alpha + (1-\alpha)[1-\exp(-\lambda_1 D_1)], \text{ and}$$

$$P(0,D_2) = \alpha + (1-\alpha)[1-\exp(-\lambda_2 D_2)].$$

The relative risks to zero doses are:

$$R(D_1,D_2) = 1 + \frac{(1-\alpha)}{\alpha}[1-\exp(-\lambda_1 D_1-\lambda_2 D_2)], \tag{3}$$

$$R(D_1,0) = 1 + \frac{(1-\alpha)}{\alpha}[1-\exp(-\lambda_1 D_1)], \text{ and} \tag{4}$$

$$R(0,D_2) = 1 + \frac{(1-\alpha)}{\alpha}[1-\exp(-\lambda_2 D_2)]. \tag{5}$$

Various measures of synergism or antagonism have been proposed (see, for example, Hamilton and Hoel, 1978). Specific proposals of Hogan et al. (1978), Finney (1971), and Walter (1976) are designated below as S_H, S_F, and S_W, respectively:

$$S_H = R(D_1,D_2) - R(D_1,0) - R(0,D_2) + 2$$

$$S_F = \frac{R(D_1,D_2) - 1}{R(D_1,0) + R(0,D_2) - 2}$$

$$S_W = \frac{R(D_1,D_2)}{R(D_1,0)R(0,D_2)}$$

In all three examples, independence is indicated by S = 1, synergism by S > 1, and antagonism by S < 1. Risks in the first two are essentially additive, whereas in Walter's proposal, they are multiplicative.

For the independent action one hit model and for small doses, (e.g., $R(D_1,D_2) \sim 1+[(1-\alpha)/\alpha][\lambda_1 D_1 + \lambda_2 D_2)]$, all three equations yield approximately 1:

$$S_H \sim 1+ \frac{(1-\alpha)}{\alpha}[\lambda_1 D_1+\lambda_2 D_2 - \lambda_1 D_1-\lambda_2 D_2] = 1,$$

$$S_F \sim \frac{[(1-\alpha)/\alpha][\lambda_1 D_1+\lambda_2 D_2]}{[(1-\alpha)/\alpha][\lambda_1 D_1+\lambda_2 D_2]} = 1, \text{ and}$$

$$S_W \sim \frac{1 + [(1-\alpha)/\alpha](\lambda_1 D_1+\lambda_2 D_2)}{\{1 + [(1-\alpha)/\alpha]\lambda_1 D_1\}\ \{1+[(1-\alpha)/\alpha]\lambda_2 D_2\}} \sim 1.$$

This means that for a small absolute probability of response all these measures will yield approximately 1, indicating independence. In many situations, e.g., when smoking is associated with lung cancer, a low probability is reasonable although the relative risk may be high. This does not mean that they shall yield similar values under other circumstances.

Example 1: Consider the effect of smoking and asbestos on lung cancer, which was investigated by Hammond and Selikoff (1973). Let D_1 refer to smoking and D_2 to asbestos:

$$R(D_1,0) = 11$$

$$R(0,D_2) = 2$$

$$R(D_1,D_2) = 90$$

$$S_H = 90 - 11 - 2 + 2 = 79$$

$$S_F = \frac{90 - 1}{11 + 2 - 2} = 8.1$$

$$S_W = \frac{90}{11 \times 2} = 4.1$$

Thus, although all three measures show synergism, their magnitude can vary widely.

Example 2: Consider the effect of cigarette smoking, D_1, and exposure to radon daughters, D_2 (Lundin et al., 1969):

$$R(D_1,0) = 11$$

$$R(0,D_2) = 4$$

$$R(D_1,D_2) = 41$$

$$S_H = 41 - 4 - 11 + 2 = 28$$

$$S_F = \frac{41 - 1}{11 + 4 - 2} = 3.1$$

$$S_W = \frac{41}{4 \times 11} = 0.9$$

Although S_H and S_F indicate synergism, S_W does not. Clearly "additive" but not "multiplicative" synergism is present.

There is no consensus concerning the measure of synergism that is appropriate (Rothman, 1978a,b; Walter and Holford, 1978). Still other measures may also be proposed.

MATHEMATICAL JUSTIFICATION OF THE "THRESHOLD LIMIT" CRITERION [$\Sigma (C_i/T_i)>1$] BY THE INDEPENDENT ONE-HIT MODEL

The "Threshold Limit Document" (American Conference of Governmental Hygienists, 1977, p. 45 ff.) considers a criterion based on the quantity $\Sigma (C_i/T_i)$, where C_i denotes the observed concentrations and T_i the corresponding threshold limits for a series of chemicals i = 1,2,...n. If $\Sigma (C_i/T_i)$ exceeds unity, then the threshold limit of the mixture is considered to be exceeded. This criterion may be justified under the assumption of a generalized independent one hit model:

$$P(D_1,...D_n) = 1-\exp(-\lambda_1 D_1 - ...\lambda_n D_n).$$

Let P_o be an "acceptable probability" of an adverse response. Considering each individual chemical exposure only, this P_o may be used to define the threshold value:

$$P_o = P_i(T_i) = P(0,...0, T_i,0,...0) = 1-\exp(-\lambda_i T_i).$$

For small P_o, which is of course reasonable:

$$P_o \sim \lambda_i T_i, \text{ and thus,}$$

$$\lambda_i \sim P_o/T_i.$$

Conversely, for each small dose below the threshold,

$$P_i(C_i) \sim \lambda_i C_i \leq P_o, \quad i = 1,2,...n.$$

Substitution for λ_i yields the approximate relation, $C_i/T_i \leq 1$, for i = 1,2,...n, which is the single chemical threshold.

Now require that the risk of exposure to n chemicals at doses $C_1, C_2, \ldots C_n$ be less than the acceptable probability, P_o. Under the independent one hit model for small doses,

$$P(C_1, C_2, \ldots C_n) \sim \Sigma (\lambda_i C_i) \leq P_o,$$

since $\lambda_i = P_o/T_i$. This may be written:

$$P_o \Sigma(C_i/T_i) \leq P_o, \text{ or}$$

$$\Sigma(C_i/T_i) \leq 1.$$

This is the same criterion that was cited by the American Conference of Governmental Industrial Hygienists (ACGIH) (1977), but it has a different meaning. The probability model assumes a positive, but small, probability of response below T_i. Thus, it is possible that the n chemicals could affect different organs, but their accumulative probability of an adverse response at one or more organs will exceed P_o whenever $\Sigma(C_i/T_i) > 1$. The ACGIH does not use the additive criterion when the "harmful substances are not in fact additive, but <u>independent</u> as when purely local effects of different organs of the body are produced by the various components of the mixture." The ACGIH criterion assumes a perfect threshold, i.e., absolutely no adverse response below T_i for each T_i. Thus, this definition of "independent" and "additive" is very different from the sense in which these terms are used in the probability arguments advanced here.

When synergism may exist between two chemicals and the following model for low doses is assumed (see Equation 2, above):

$$P(D_1,D_2) = \lambda_1 D_1 + \lambda_2 D_2 \exp(\theta D_1).$$

It is easily seen that

$$T_1 = P_o/\lambda_1 \text{ and } T_2 = P_o/\lambda_2).$$

From this we may assume:

$$P(C_1,C_2) = \left(\frac{C_1}{T_1} + \frac{C_2}{T_2} \exp(\theta C_1)\right) P_o$$

Clearly, even if $C_1/T_1 + C_2/T_2 \leq 1$, it is possible when θC_1 is large enough for $P(C_1,C_2)$ to exceed P_o. Thus, in the presence of synergism this criterion can fail to provide suitable protection.

REFERENCES

American Conference of Governmental Industrial Hygienists. 1977. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1977. American Conference of Governmental Industrial Hygienists, Cincinnati. 94 pp.

Cornfield, J. 1975. A statistician's apology. J. Am. Stat. Assoc. 70:7-14.

Finney, D. J. 1971. Probit Analysis, 3rd edition. Cambridge University Press, London.

Gaddum, J. H. 1953. Pharmacology, 4th edition. Oxford University Press, New York.

Gart, J.J., J.A. Di Paolo, and P.J. Donovan. 1979. Mathematical models and the statistical analyses of cell transformation experiments. Cancer Res. 39:5069-5075.

Gullino, P., M. Winitz, S. M. Birnbaum, J. Cornfield, M. C. Otey, and J. P. Greenstein. 1956. Studies on the metabolism of amino acids and related compounds *in vivo*. I. Toxicity of essential amino acids, individually and in mixtures, and the protective effect of L-arginine. Arch. Biochem. Biophys. 64:319-332.

Hamilton, M. A. and D. G. Hoel. 1978. Detection of Synergistic Effects in Carcinogenesis. Paper presented at the Joint Statistical Meeting in San Diego, Calif., August 1978.

Hammond, E. C., and I. J. Selikoff. 1973. Relation of cigarette smoking to risk of death of asbestos-associated disease among insulation workers in the United States. Pp. 312-317 in P. Bogovski, J. C. Gilson, V. Timbrell, and J. C. Wagner, eds. Biological Effects of Asbestos. IARC Scientific Publication No. 8. International Agency for Research on Cancer, Lyon.

Hogan, M. D., L. L. Kupper, B. M. Most, and J. K. Haseman. 1978. Alternatives to Rothman's approach for assessing synergism (or antagonism) in cohort studies. Am. J. Epidemiol. 108:60-67.

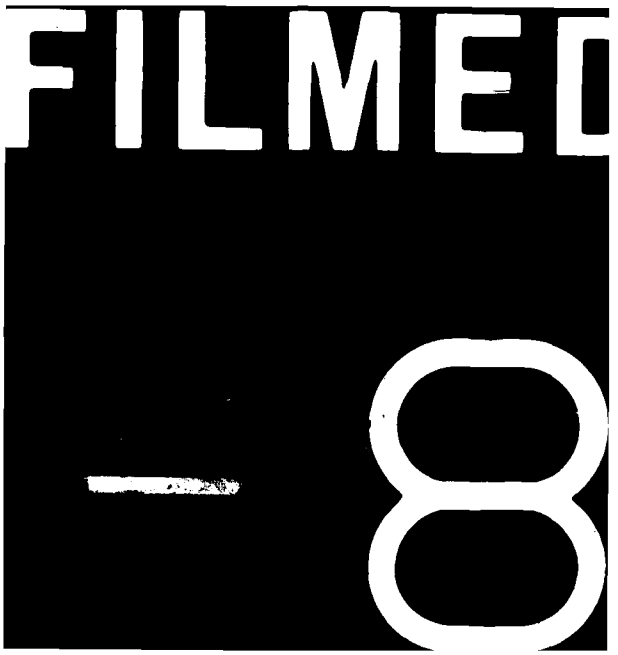
Lundin, F. E., Jr., J. W. Lloyd, E. M. Smith, V. E. Archer, and D. A. Holaday. 1969. Mortality of uranium miners in relation to radiation exposure, hard-rock mining and cigarette smoking--1950 through September 1967. Health Phys. 16:571-578.

Rothman, K. J. 1978a. Estimation versus detection in the assessment of synergy. Am. J. Epidemiol. 108:9-11.

Rothman, K. J. 1978b. Occam's razor pares the choice among statistical models. Am. J. Epidemiol. 108:347-349.

Walter, S. D. 1976. The estimation and interpretation of attributable risk in health research. Biometrics 32:829-849.

Walter, S. D., and T. R. Holford. 1978. Additive, multiplicative, and other models for disease risks. Am. J. Epidemiol. 108:341-346.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8