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PRINCIPLES OF TOXICOLOGICAL INTERACTIONS ASSOCIATED WITH MULTIPLE CHEMICAL EXPOSURES

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16. Abstract
This report is a first attempt to assess the added hazards to personnel such as Chemical Tankermen or Marine Inspectors, who are exposed to many hazardous vapors.

Because of the scarcity of data, the development of a set of general principles or guidelines for acquiring and evaluating data concerning Toxicological Interactions was proposed to facilitate future evaluation of hazardous vapor exposures.

Some specific factors considered include: Absorption, Elimination, Biotransformation Reactions, Storage Sites, Target Sites, Exposure Sequence and Mathematical Modeling.

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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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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 <u>ad-hoc</u> 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.

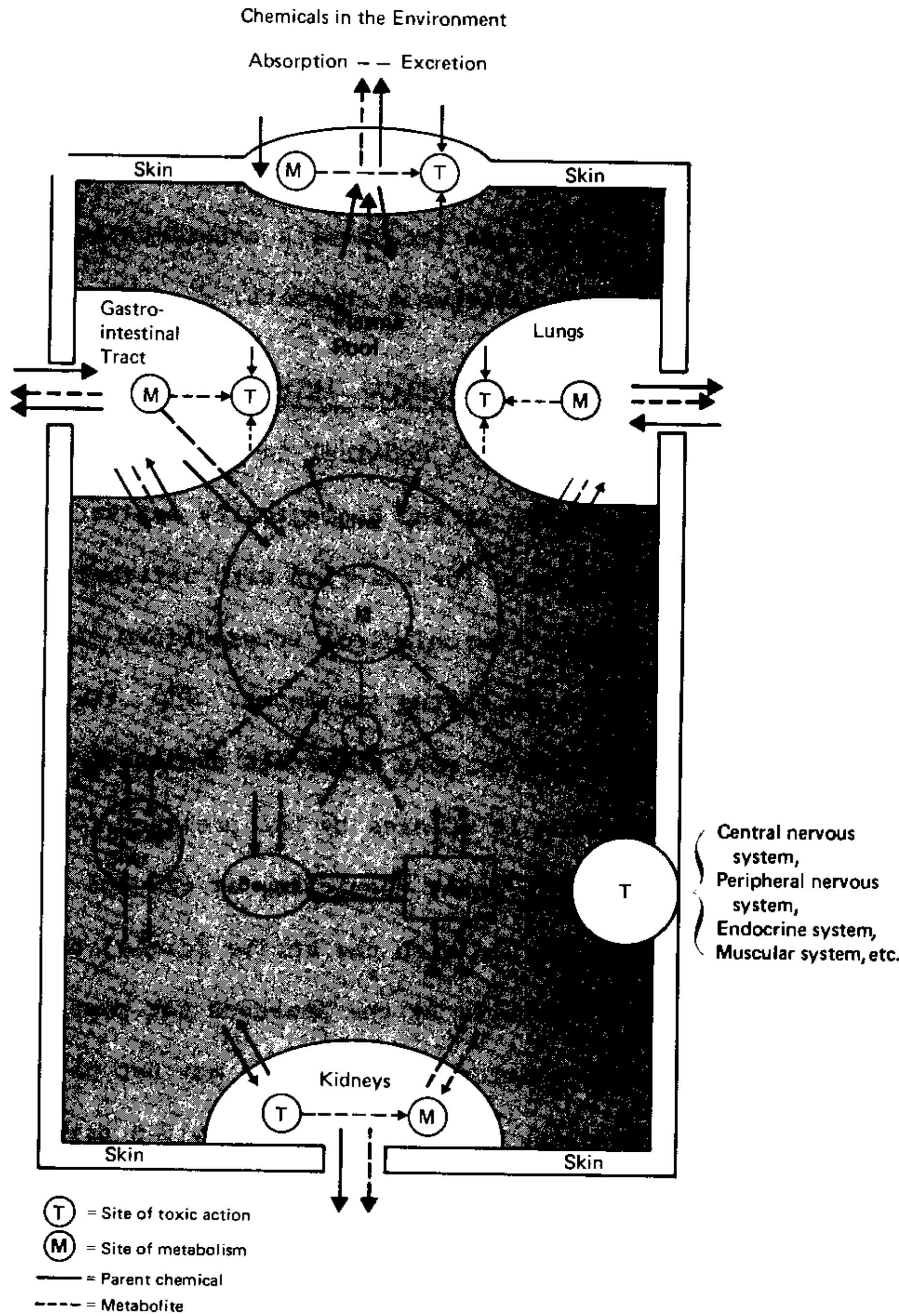

FIGURE 1-1. Sites for potential chemical interactions that may result in toxicological interactions. Each arrow represents a pathway or site for which chemicals may compete and thereby alter each other's biological disposition or intensity of action. Both parent compounds (solid arrows) and metabolites (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.