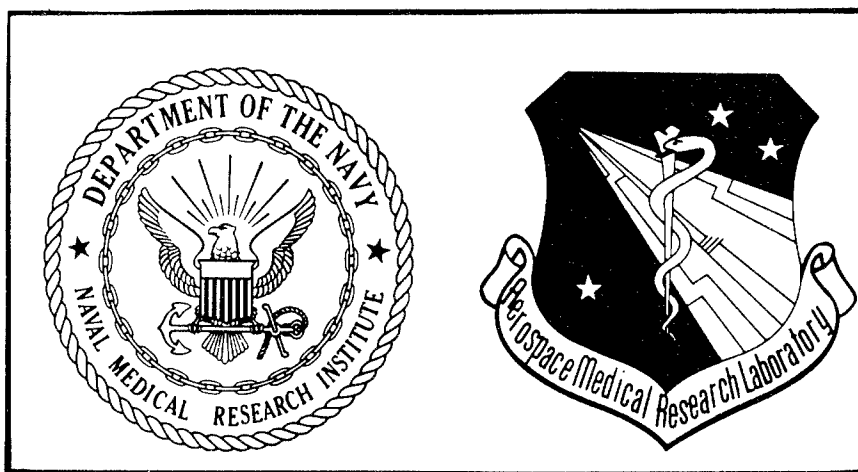AFAMRL-TR-79-121

PROCEEDINGS OF THE TENTH CONFERENCE ON ENVIRONMENTAL TOXICOLOGY 13, 14, AND 15 NOVEMBER 1979

UNIVERSITY OF CALIFORNIA, IRVINE
OVERLOOK BRANCH, P. O. BOX 3067
DAYTON, OHIO 45431

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AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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Major technical areas discussed included environmental hazards of fibrous dusts, toxicokinetics of inhaled gases and vapors, and the environmental hazards of toxicants in surface water systems. The oncogenic effects of hydrazine in animal models and immunotoxic reactions as manifested by skin and lung hypersensitivity was presented.

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PREFACE

The Tenth Conference on Environmental Toxicology was held in Dayton, Ohio on 13, 14, and 15 November 1979. Sponsor was the University of California, Irvine under the terms of Contract F33615-76-C-5005 with the Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio. Arrangements were made by the Toxic Hazards Research Unit of the University of California, Irvine. Anna M. Baetjer, D.Sc., Professor Emeritus, Environmental Medicine, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland served as Conference Chairwoman, and Mrs. Lois Doncaster, University of California, Irvine served as Conference Coordinator. Acknowledgement is made to Ms. Mildred Pinkerton and Ms. Sharon Seitz for their significant contributions and assistance in the preparation of this report.

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WELCOMING REMARKS

Colonel R. L. DeHart, USAF, MC

Air Force Aerospace Medical Research Laboratory
Wright-Patterson Air Force Base, Ohio

I have been requested to perform three tasks this morning. Or more precisely, I have been requested to first welcome you and then to make a few opening remarks. In point of fact, I requested the third task, that of introducing the Conference Chairwoman.

I will say good morning to you and get on to my first task of welcoming you, for we of the Aerospace Medical Division, the biotechnology arm of the Air Force Systems Command, are pleased to share with the University of California the sponsorship of this Tenth Conference on Environmental Toxicology. Our sponsorship and involvement in this type of conference was the subject of a recent commendation and resulted in a recommendation that other Air Force laboratories consider similar technical and scientific interchanges. I have been requested by Brigadier General McIver, Commander of the Aerospace Medical Division, to welcome each of you to this conference and to express his best wishes for a scientific and rewarding experience. I wish to add my own welcome and that of the staff of the Air Force Aerospace Medical Research Laboratory and to assure you that we stand ready to assist you in whatever way we can to optimize your visit to Dayton, Ohio.

In my comments to the participants of the Ninth Conference on Environmental Toxicology, I expressed a concern that the then popular press introduction of the cancer threat-of-the-week could ultimately result in a complacent, fatalistic, or skeptical public. Current polls suggest that such a situation may, in fact, be developing for the public at large is ignoring warnings that are coming out concerning potential carcinogenic risks. When the environment is soiled through ignorance, economic considerations, or a total disregard for the public health, the results may be sudden, swift, and catastrophic.

Permit me to expand this theme. Several papers to be presented at this conference will cite examples of soiling the environment. I use the word "soiling" to make a point. Among the higher order of animals, particularly those with established social interactions, the animals do not soil their den, their lair, or their burrows. This is not simply a behavioral trait or pattern for good housekeeping but, in fact, represents a practice for survival for it reduces disease, dilutes the scent, and serves as a territorial indicator.

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A recent congressional report indicates that hazardous waste dumps are a greater risk to the public health than was first believed. The report goes on to recommend stringent new laws and controls. Hazardous waste has been defined by the Environmental Protection Agency as those wastes that are flammable, corrosive, toxic to humans, or react violently with other chemicals. It is estimated that 10 to 15% of all industrial waste qualifies in this category. Hazardous wastes are produced by virtually every type of manufacturing process. It is estimated currently that in this country 36 million tons of hazardous waste are produced per year. Dangerous wastes at industrial dump sites can contaminate ground and surface waters, can pollute the air, cause fires and explosions, build up in the food chain, and have been reported to be causes of birth defects and cancer.

We will hear later in this conference of the Love Canal situation, of 235 families who live near that canal in Niagara Falls, New York, and hear that that area has been declared an imminent health hazard, consequently permitting Federal direct involvement.

About 75% of the waste products are disposed of on the properties of the companies that produce them. In other words, they soil their lair. We have no assurance that the same company will be occupying that site a year hence. Estimates by the EPA that much of the material is stored illegally have been made and that only 10% of waste products are stored in a manner consistent with proposed Federal guidelines. The EPA further estimates that there may be environmental health concerns at 34,000 industrial dumps throughout the United States. The Justice Department has taken an active role in trying to control and to bring to bear the powers of justice and the law against these corporations. But it is currently estimated that in order to clean up our environment which we have soiled, the economic impact represents 40 billion dollars.

Thus, it is incumbent on us, whether we are in academia, industry, or Federal agencies, to know the pharmacokinetics or toxicokinetics of those compounds we induce and introduce into the environment regardless of mode.

Are we prepared for the overreaction we recently witnessed in the nuclear industry? "Crisis!" screamed the headline of a Chicago Tribune series of articles on nuclear waste mismanagement. Washington's Governor Dixie Lee Ray, an individual not unfamiliar with nuclear waste, and Nevada's Governor List closed their low level waste disposal sites in their states. Nuclear medicine departments in hospitals all over this country were thrown into a panic for radiopharmaceuticals are used in the diagnosis and treatment of a wide variety of diseases. Only one low level site remains open. Some states are currently considering legislation banning trans-state shipment of any level of nuclear waste.

Only through a firm scientific base and technical expertise will you be able to answer the inevitable questions which are to come. I trust this scientific conference will contribute toward that goal.

My third task is a pleasant one. Not often are we given the opportunity to introduce our major professor on our own home grounds. In this case, a professor with the capacity to stimulate intellectual curiosity in her students, to teach that most difficult of challenges, taking the theoretical and applying it to the practical, and if she will forgive me, to put on a pair of tennis shoes and outrace her student charges on industrial site visits. Our Conference Chairwoman received her Doctorate of Science in Physiology at the Johns Hopkins University and proceeded to establish an enviable academic record at that institution. She has served on committees dealing with various aspects of the environment at all levels of government including both Federal and International. She has served with distinction in numerous professional societies, has written numerous professional articles in the technical literature, has contributed to the classic text of public health. Her contributions have been recognized by several universities which have granted her honorary degrees. During the commencement exercises in June at the Johns Hopkins University, her own faculty recognized her by granting an honorary doctorate in Humane Letters. It is my pleasure and to your good fortune that I introduce your Conference Chairwoman, Emeritus Professor of Environmental Medicine at the School of Hygiene and Public Health, Johns Hopkins University, Dr. Anna Baetjer.

INTRODUCTORY ADDRESS

PHYSIOLOGICAL VARIABILITY AS IT AFFECTS SUSCEPTIBILITY TO TOXIC CHEMICALS AND BIOLOGICAL AGENTS

A. M. Baetjer, D.Sc.
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Baltimore, Maryland

It is a great pleasure to serve as chairman of this Tenth Conference on Environmental Toxicology, and I thank Dr. Dwight Culver for giving me this opportunity. For this introductory speech, Dr. Culver suggested that I might talk about concepts I had learned in past experience and what I might predict as important research for the future. Since my basic background is physiology, I have chosen to discuss "Physiological Variability as it Affects Susceptibility to Toxic Chemicals and Biological Agents." In studies dealing with environmental exposures, we usually assume that all men are similar after adjusting for age, sex, and race. Yet men respond very differently to the same environment, and a man may differ greatly in his susceptibility to toxic and biological agents from day to day even in the same environment. What physiological changes in man are responsible for these variable reactions? This question was impressed on me recently by several very practical problems.

Last month, I was asked to interview a patient with aplastic anemia to determine whether her illness was related to her occupation. Her job for the past year had been to clean small metal parts with pieces of cotton dipped into an open container of clear fluid. With a bit of sleuthing, we found that the fluid was pure benzene, undoubtedly responsible for her illness. But the point in describing this case is that there were 10 young women doing the same job, all at the same bench, but as far as we could determine no other workers had become ill. Why didn't the other 9 workers also become ill? Obviously, there was something different in their physiological state that caused them to be less susceptible to benzene. Why was this one woman more susceptible to benzene than the other nine women?

Another problem involved employees who were heavy drinkers but were not considered alcoholics. An industry wanted to know whether these men would be more susceptible to chlorinated hydrocarbons than other employees. A third example was the workman who said, "I've worked with this chemical for years and never gotten sick from it. Why have I suddenly gotten sick now?"

There are many factors which can alter the physiological state of men and thereby affect their susceptibility to toxic and biologic agents in the environment. Table 1 shows the most important of these factors. Today, I will give examples of research in a few of these areas.

TABLE 1. FACTORS WHICH AFFECT THE PHYSIOLOGICAL STATE AND THUS ALTER SUSCEPTIBILITY TO TOXIC CHEMICALS

Genetic Background	Exercise, Physical Work
Nutritional State	Pregnancy
Smoking Habits	Age
Alcohol and Drug Intake	Emotions
Environmental Temperature	Preexisting Disease
Degree of Hydration	Previous Toxic Exposures
Barometric Pressure	

DEHYDRATION AND FOOD RESTRICTION

In my laboratory, we have been interested in the effects of water deprivation on metabolic processes. When rats are deprived of water, they reduce their food intake; hence, it is always necessary to study simultaneously rats allowed water but restricted in food intake to equal that consumed by the dehydrated animals (called pair-fed rats). The results of one experiment were as follows:

> "Water deprivation for 48 hours with its accompanying decrease in food intake significantly lowered the in vitro rate of hexobarbital metabolism by hepatic microsomes isolated from male rats. Pair-fed rats allowed water ad libitum had a significantly lower level of hexobarbital metabolism than those deprived of water. Rats starved for 24 hours with or without water also had levels of hexobarbital metabolism significantly lower than their controls; with those animals allowed water ad libitum, the level was significantly lower than for those deprived of water. The differences between hydrated and dehydrated animals were not attributable to reduction in concentration of microsomal protein or the water content of liver. It is concluded that water consumption accentuates the effect of food deprivation on hepatic microsomal metabolism." (Baetjer and Rubin, 1976).

Forty-eight hours of water deprivation is not a life threatening stress since rats fed a dry chow diet but deprived of water survive between 11 and 19 days.

These experiments emphasize that even 24 hours of food restriction markedly affects the state of rats. Yet it is common practice for experimenters to remove the food from animals the day before testing chemicals and drugs.

Other experiments in this or other laboratories have shown that water and/or food restriction markedly impaired the antibacterial defenses of the lungs in mice, decreased the number of alveolar macrophages recovered from the lungs of rats by lavage; altered the surface tension of lung extracts from rats, decreased the rate of cilia-mucus flow in the trachea of chicks and dogs, and increased the mortality of rats injected with lead, antimony, and some other chemicals. Thus, water and/or food restriction have a marked effect on susceptibility to environmental agents.

TEMPERATURE

Many studies have demonstrated that both high and low environmental temperature increase the reaction of most drugs. The same effect has been observed with environmental chemicals. My interest in temperature resulted from the observation that practically all serious cases and deaths from lead poisoning in young children, who had chewed lead paint and plaster, occurred during the summer months regardless of when they started chewing. Experiments with rats confirmed that exposure to high temperature markedly increased the mortality from lead. Studies in rats with other environmental chemicals also showed their toxic effects to be temperature dependent.

NUTRITION

Although scientific interest in nutrition is presently focused on specific foods as agents in the production of cancer, this discussion is concerned with experiments in which specific nutritional factors prevent cancer from known chemical carcinogens. The first example is the protective action of the antioxidants 2(3)-tert-butyl-4-hydroxyanisole (BHA) and 3,5-di-tert-butyl-4-hydroxytoluene (BHT) against the carcinogenic effects of benzo(a)pyrene. Mice injected with this carcinogen produce urine which is highly mutagenic by the Ames test. When these antioxidants are added to the diet for a few days preceding administration of the carcinogen, the mutagenicity of the metabolites of benzo(a)pyrene in the urine are greatly reduced. The levels of glutathione S - transferases and epoxide hydrases in the liver are elevated and glucuronide formation is enhanced.

Selenium is another dietary antioxidant which has been found to protect animals against the carcinogenic action of many of the polycyclic hydrocarbons. Vitamin A in the form of retinyl acetate is reported to protect animals from lung cancer induction by 3-methyl-cholanthrene.

Thus, the quality of the diet can greatly affect the biological reaction to carcinogenic chemicals.

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While I am talking about cancer, I would like to mention a somewhat different type of biological variability which may play a role in the development of cancer. Dr. Ernest Bueding at the Hopkins School of Hygiene developed a drug which was very potent for the treatment of schistosomiasis. This drug was not a mutagen when tested by all the usual methods but the urine from mice treated with this drug contained a strong mutagen. On the other hand, the urine of germ-free mice and mice treated with an antibiotic which changed their intestinal flora produced urine free of mutagenesis. Further experiments demonstrated that certain organisms in the intestinal tract of the untreated mice metabolized the nonmutagenic drug into a mutagenic product. Thus, the character of the intestinal flora may be an important variable.

PREVIOUS EXPOSURES AND TOLERANCE

Often a single or repeated exposure to a small amount of chemical alters the physiological state so that a person becomes more or less resistant to subsequent exposures of the same or different chemical. A very interesting example of this reaction concerns the development of tolerance to ozone. When animals are exposed to high concentrations of ozone, such as 10 ppm, they develop pulmonary edema followed by death. On the other hand, if the animals are first exposed to a low concentration of ozone (3 ppm) which causes no untoward effect and 4 hours later are subjected to 10 ppm, they fail to develop pulmonary edema. In addition, the animals also are resistant to the edemogenic effects of nitrogen dioxide and other oxidants. This tolerant effect lasts for weeks in both rats and mice.

Men also develop a form of tolerance to irritant gases at low levels. For example, men who work in areas where sulfur dioxide is present rapidly become unaware of the irritating effects of this gas.

HYPOXIA

Because this conference is associated with Wright-Patterson Air Force Base, it is suitable that my last example deals with a problem in hypoxia, specifically the effect of hypoxia on hepatic metabolism.

Rats exposed to 8% oxygen or to 500 ppm CO have approximately equal alterations in arterial oxyhemoglobin content (approximately 65% oxyhemoglobin). On the other hand, this degree of hypoxia had a much greater inhibitory potency on hepatic microsomal metabolism of drugs than did the carbon monoxide exposure. Further experiments demonstrated that the hypoxia (8% oxygen) caused a marked decrease in hepatic blood flow in the splanchnic area whereas 500 ppm carbon monoxide was without significant effect. It appeared that the difference in hepatic blood flow was responsible for the effects on hepatic metabolism. Thus, variability in hepatic blood flow could play a role in the susceptibility to toxic agents.

CONCLUSION

I hope these few examples will indicate the really great importance of the many factors which affect the physiological state of men and animals, and thus alter their reaction and susceptibility to toxic chemicals and other environmental agents.

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TECHNOLOGY AND ENVIRONMENTAL HAZARDS
OF FIBROUS DUSTS

Chairman

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THE PATHOLOGY OF ASBESTOS-RELATED DISEASES

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Asbestos causes four types of tissue changes: scarring of the lungs, cancer, localized plaques of scar tissue on the parietal pleura, and warts. The plaques occur on the lining of the thoracic cage and the upper surface of the diaphragm whereas the warts develop on the skin when slivers of asbestos become embedded in the epidermis. The warts do not become malignant.

With the exception of the contact of asbestos fibers with the skin, all other contacts with body tissues known to produce a health effect are traceable to their reception by the respiratory tract. The anatomy of the respiratory tract is such that only the smallest respirable particles gain entrance to the air spaces of the lungs. The larger inhaled particles are precipitated upon the sticky blanket of mucus that lines the air-conducting tubes. This film of mucus is in constant motion toward the throat with the result that the upper respiratory tract, represented by the air-conducting tubes, normally is cleansed of all deposited material within 24 hours. There is, in addition, a cleansing mechanism in the air spaces which operates less efficiently and more slowly but is, nevertheless, important. The existence of this deep-lung cleansing mechanism is demonstrated by the coal miner who, although retired on a farm for many years, still expectorates black sputum and will continue to do so for the rest of his life. The result of this combination of the two cleansing mechanisms is that about 98% of the inhaled particles are transported to the mouth where they are either swallowed or expectorated. It is in this manner that exposure of the respiratory tract to asbestos also results inevitably in exposure of the digestive tract to asbestos.

The burning question that is still unanswered is how many fibers are required to be inhaled by a person in order to result in a cancer 20 or more years later. On the basis that, theoretically, one molecule of a carcinogen can initiate a cancer, it has been proposed that perhaps one asbestos fiber contacting a suitable target cell can also initiate a cancer. If it is any reassurance to those who would take alarm at this possibility, it should be pointed out that asbestos is ubiquitous. The

urban air (of Philadelphia, for example) may contain as much as 70 ng/m^3 of asbestos. Since it has been estimated that each nanogram (one-billionth of a gram) of chrysotile is equivalent to about one million ultimate fibrils, the adults of some cities may inhale the equivalent of about 300 million fibrils each 24 hours. It should, therefore, be no surprise that postmortem examination of lungs of people, not occupationally exposed, in Pittsburgh, New York, London, and Paris has demonstrated the presence of asbestos fibers in all of them and that the presence of these fibers was not associated with recognizable disease attributable to the asbestos.

The most common result of an <u>excessive</u> inhalation of asbestos dust is the development of scar tissue in the lungs, known as asbestosis. This scar tissue causes thickening of the walls of the air spaces, often resulting in obliteration of the blood capillaries within these walls. The result of this scarring is a stiffening of walls so that greater effort must be expended in ventilating the lung, and because many fine blood vessels have been destroyed, the gaseous exchange is reduced. Because the scarring is nonuniform, some air spaces, although ventilated, are not perfused with blood; some that are perfused with blood are not ventilated; and some are neither perfused nor ventilated. Early in the disease a sufficiently large number of air spaces remain normal so that no symptoms develop. It is not until more than 50% of the substance of the lungs has become affected (usually requiring 20 or more years) that symptoms of exertional air hunger develop. It is generally agreed that asbestosis is a progressive disease, i.e., progression occurs even though exposure has ceased. This, of course, does not mean that everybody who has ever been occupationally exposed to asbestos dust will die of asbestos-related disease. The amount of disease and the related disability is dependent upon how much asbestos has been deposited in the lungs.

One of the distinguishing features of the inflammation caused by asbestos and that results in scarring is the presence of asbestos bodies. These are microscopic structures evolved from asbestos fibers by the action of scavenger cells that deposit an iron-containing protein derived from hemoglobin around the fibers. These structures may resemble straight or curved drumsticks that may be segmented. They are golden-yellow to brown and of various sizes and shapes. Because similar structures may be formed from inhaled glass fibers and other insoluble fibers and because they all have a coating of iron-containing protein, they have been given the generic name of ferruginous bodies.

Asbestotic scarring of the lungs, although diffuse, characteristically lacks uniformity. It is most severe in the lower portions of the lower lobes. There may also be severe thickening of the pleura which further increases the effort required to breathe. Scarring may also affect the pleural surface covering the ribs and the diaphragm, and resulting in plaque formation. This, a strictly benign, asymptomatic,

localized process, has been demonstrated by chest X-ray films in many people who worked in fields near asbestos mines and in fields where the soil contained asbestos fibers. Of course, plaques are also found in occupationally exposed workers.

The most talked about asbestos-related diseases are cancers. These are, in the order of their frequency: cancer of the lung, mesothelioma (cancer of the lining of the chest or abdominal cavities), cancer of the lower intestinal tract, and cancer of the larynx. It is of interest that when asbestos causes cancer in animals, this cancer is always found originating within scar tissue. Likewise, asbestotic lung cancers in humans occur in those workers who had been most heavily exposed, i.e., those whose lungs are most severely scarred. Also of interest is the fact that in a large epidemiologic study of asbestos insulation workers, it was found that nearly all lung cancers occurred in cigarette smokers. Nonsmoking asbestos workers had only a slightly greater chance of developing lung cancer than a nonsmoking non-asbestos worker. A smoking asbestos worker's chance of developing lung cancer was calculated to be about 90 times that of a nonsmoking nonasbestos worker. For this reason, a large asbestos manufacturing company has forbidden cigarette smoking on its premises and urges its workers to stop smoking at home.

Mesothelioma is a rare tumor. It has been estimated to have a background incidence of 7 to 10 per million population. Even in association with asbestos exposure, a world-wide total of only 4539 meso-thelioma cases has been recorded between 1960 and 1976. This compares with the 85,000 lung cancer death toll last year in the U.S. alone, all but a small fraction of which was caused by cigarette smoking. Meso-theliomas are clinically silent until the disease is far advanced. Death usually occurs within a year after the diagnosis is made. There is no certainty that all the reported mesotheliomas are caused by asbestos exposure. On an average, about 15% of these reported tumor cases give no history of any asbestos exposure. Furthermore, reports from Turkey indicate the occurrence of mesotheliomas in endemic proportions in small communities where no asbestos is present. The soil, however, does contain zeolite mineral fibers of vulcanic origin. Aside from the fact that some of the cases of mesothelioma that are assumed to be asbestos-related give no history of asbestos exposure and that similar cases occur in the absence of asbestos exposure but in the presence of exposure to vulcanic fibers, there are other puzzling aspects. For instance, there are cases where the history indicates only a scanty exposure, and this is corroborated by the post-mortem finding of no asbestotic involvement of the lungs. Furthermore, this combination of minimal exposure with the absence of asbestosis is more likely to be seen in those cases where the mesothelioma is in the abdomen. The addiction to cigarette smoking appears to play no role in the development of mesothelioma.

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A much smaller risk of developing cancer from occupational asbestos exposure has been demonstrated epidemiologically for the lower intestinal tract and the larynx. Presumably, the exposure of the intestinal wall to asbestos stems from the swallowing of the inhaled asbestos that is brought up from the respiratory tract by its cleansing mechanisms.

The ubiquity of asbestos fibers is also demonstrated by their presence in the water of many rivers. The sources of these fibers are outcroppings of serpentine rocks over which the rivers are flowing. These rocks contain chrysotile fibers. The asbestos fibers eroded from the rocks differ from those encountered in occupational settings insofar as the former are generally less than 2 μm long, whereas the latter consist of the entire spectrum of fiber lengths, from the ultrashort to the very long. Since the discovery of electron-microscope-sized asbestiform amphibole fibers in the waters of Lake Superior and in the drinking water of Duluth, fears have been generated by the press regarding the development of gastro-intestinal cancers from the ingestion of these fibers in the water. Epidemiologic studies of Duluth people who had been ingesting the fibers in drink and food for about twenty years failed to disclose an increase in the cancer rate. However, these negative results were considered inconclusive because insufficient time has elapsed. In an attempt to settle the problem of whether or not the ingestion of these short fibers in the drinking water pose a health problem, Congress has authorized the expenditure of millions of dollars for animal-feeding studies. These are now underway. Unfortunately, because of the difficulty in obtaining the required kilogram-amounts of short asbestos fibers without significant admixture by long fibers, the animals are being fed a spectrum of short and longer fibers. The results of these studies will not become available for several years.

MAN-MADE MINERAL FIBERS - THEIR HEALTH EFFECTS

J. L. Konzen, M.D.

Owens-Corning Fiberglas Corporation
Toledo, Ohio

DEFINITIONS AND APPLICATIONS

Several considerations are involved when examining the health aspects of man-made mineral fibers. These include a definition of man-made mineral fibers, their geometry, the products they form, the airborne concentrations of fibers during the manufacture of the fibers and subsequent products, the animal experimental data, and the human health experience of workers exposed during the manufacture and fabrication of man-made mineral fiber products.

Man-made mineral fibers, for purposes of this examination, include fibrous glass, rock and slag wool. These fibers are amorphous silicates which, after melting, are fiberized by a variety of methods. In general, these substances can be divided into wool products such as batt and board insulation, textile products which would go into a variety of decorative and reinforcement applications, and a special category best described as finer diameter fiber products (Pundsack, 1976; Smith, 1976).

FIBER RANGES

Wool fibrous glass products have been manufactured since the 1930s. They have been manufactured by a variety of processes including steam blown, flame attenuated, and rotary. They produce a variety of insulation type products varying from the familiar insulating batts for home and industry to granulated fibers for blown-in applications to various density board type products used in manufacturing air-transmission systems, ceilings, and sound treatment panels.

These products contain fibers ranging in nominal diameter from approximately 5 to 9 microns. A portion of the fibers in these products will be less than 3.5 microns in diameter, and a small fraction may be less than 1 micron in diameter. The lengths of the fibers are infinitely long in relation to the diameters. This spread of fiber diameters has been present in the products since they were first manufactured, which in the case of rock and slag wool fibers dates from the early days of this century, and for fibrous glass from the early 1930s.

It has been frequently stated that the nominal diameter of fibers in fibrous glass products generally is getting smaller. This observation is based on published statements that the nominal diameter of wools in the forties and fifties was in the range of 10 or more microns. These values result from using an air porosity resistance method to measure fibers for process control methods. The early fibrous glass contained a higher portion of very large fibers (greater than 15 microns) compared to today's fibers. Using the air porosity resistance method of measurement, these very large fibers unduly influenced the so-called nominal diameters of fibers. Present fiberizing techniques limit the number of very large fibers, hence dropping the nominal diameter measured by air porosity to the range of 6 to 8 microns. Thus, fiberizing techniques in use today produce a spread of fibers in the 3.5 micron and lower diameter range, very similar to those fibers produced in past decades. It is likely that fibers which become and stay airborne today would be little changed in size as compared to when man-made mineral fibers were first produced for wool type insulation products.

Recent studies by both light and electron microscopic analysis on fibrous glass materials produced in the forties, fifties, and seventies by different fiberizing processes confirmed this finding.

Textile fibrous glass has been produced since the late 1930s. This material is die drawn; hence, the spread of fiber diameters beyond the "nominal" values is quite limited. The finest diameter textile fiber which can be drawn in commercial quantities has an average diameter of approximately 3.5 microns. However, this diameter material has been produced only since the early 1960s. It is produced in relatively small quantities while the majority of textile die drawn continuous filament fibrous glass goes into the reinforcement of a variety of materials where the larger the diameter, the better the reinforcement properties. For this reason, the majority of textile continuous fiber glass will be found in the diameters of 9 microns and larger. In this reinforcement type of textile fibrous glass, the amount of fiber less than 3.5 microns will be minuscule.

Fibers referred to as specialized finer diameter fibers are produced by a flame-attenuated process which is a lower volume process than its rotary counterpart. This process can produce fibers which have a nominal diameter as low as 1 micron or less. The material that has a nominal diameter of 1 to 2 microns has been produced since the early 1940s on a commercial basis in rather limited quantities (Smith, 1976). Therefore, the number of workers exposed to the material is also limited. Today the amount of this fiber production in the United States is less than 1% by weight and dollar volume of all the fibers produced. This wool product is used in a variety of highly specialized applications including filtration, reinforcements for aerospace applications, and high efficiency insulation for aircraft.

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Extremely fine diameter fiber, with a nominal diameter of well under 1 micron, has been more recently manufactured for certain applications. This was first put into production in the 1950s. Its use is highly specialized, and it is produced in only one plant in the United States in very limited quantities.

ASBESTOS VERSUS MAN-MADE MINERAL FIBERS

It is important to contrast man-made mineral fiber from asbestos. The contrasts include:

1. Asbestos is a naturally occurring group of fibrous materials which contains large quantities of very fine fibers. Man-made mineral fibers are manufactured for specific purposes. The fiber diameters are controlled to achieve these specific purposes. Unless specifically manufactured, the fine fiber content (less than 1.5 microns in diameter) of products is quite low.

2. The airborne concentration of asbestos, especially the submicron fibers, is considerably in excess of similar man-made mineral fiber exposures.

3. Asbestos fractures along its long axis, producing additional long thin fibers. Man-made mineral fibers fracture transversely, producing shorter, less biologically active fibers.

4. Asbestos has definitely been proven to cause disease in laboratory animals by both physiological as well as artificial routes of administration. Man-made mineral fibers have been shown to produce malignant disease only by highly artificial surgical instillation methods. Fibrosis has been definitely demonstrated only by intratracheal instillation – an artificial method of administration.

INDUSTRIAL HYGIENE STUDIES

Several industrial hygiene studies have been reported by Dement (1975), Corn and Sansone (1974), Esmen et al. (1979), and Konzen (1976) in this country and Dodgson et al. (1979) in England. The results of these studies are remarkably consistent, considering the variation in measuring and analytical techniques involved in these sampling studies. In general, the airborne fiber concentration in ordinary wool fibrous glass manufacturing operations is 0.1 or less fibers per ml for airborne fibers less than 3.5 microns in diameter. It is unusual to find airborne concentrations approaching 1 fiber per ml. While the lengths were variable, the majority of the fibers noted

were longer than 10 microns. In the finer diameter fiber products (average diameter of 1 micron and less) the airborne concentration may exceed 1 fiber per ml in manufacturing and fabrication areas. Esmen et al. (1979) demonstrated that, generally speaking, as the nominal diameter of the products increased, the airborne concentration decreased. Similar findings were demonstrated in the fabrication, packing, and cutting areas of the plants. Relatively low airborne concentrations of fibrous glass compared with similar operations involving asbestos appear to be partly explained by the manner in which man-made mineral fibers fracture compared with asbestos. Fibrous glass fractures transversely when energy is applied, while asbestos fractures longitudinally, producing more and thinner fibers (Assuncao and Corn, 1975). As will be discussed later, based on current results of animal studies, the shorter the fiber, the less biologically active fibers have been shown to be.

Generally, the industrial hygiene findings have shown that for any wool type product, the average diameter of the airborne fiber resulting from manufacture or fabrication operations will be smaller than the average diameter of the parent product (Esmen et al., 1979). As an example, the average diameter of building insulation is approximately 7 microns, while the average diameter of the airborne fiber resulting from the manufacture or fabrication of this material will be in the range of approximately 2 microns. About 25% of the airborne material will be less than 1 micron in diameter. There is generally an overlap between SEM and optical microscopic sensitivity thus making comparison of light microscopic to electron microscopic analytical findings somewhat difficult, although it can be generally stated that the light microscopic counts will be approximately equal to the electron microscopic counts for ordinary type fibrous glass. This does not hold true for the special finer fiber products (average product diameter 1 micron or less) where the electron microscopic findings may be greater than the light microscopic analysis.

In general, when analyzing industrial hygiene samples, light microscopic methods are quite satisfactory for ordinary fibrous glass products.

Since the absolute production of fibrous glass has increased over the last decade, the question is frequently asked whether this has resulted in higher airborne concentrations. One company has been carrying out routine industrial hygiene sampling of fibrous glass manufacturing facilities for the past eight years. A review of the sampling results has failed to demonstrate any appreciable increase in airborne concentrations in the manufacturing facilities both for wool type and textile type products. Ventilation has remained essentially unchanged over this period.

ANIMAL STUDIES

Concern over possible adverse health effects of man-made mineral fibers was first raised soon after the first commercialization of fibrous glass in the mid and late 1930s. This is in contrast to asbestos where the health aspects of asbestos were studied only after chronic pulmonary disease was manifested in exposed workers. The concern over possible adverse health effects of man-made mineral fibers and in particular fibrous glass arose out of the concern as to whether this material, even though an amorphous silicate, could produce silicosis or possibly an "asbestosis." This concern was heightened because fibrous glass products were extremely irritating to the skin.

As a result, animal inhalation and intratracheal injection studies were undertaken beginning in the late 1930s (Annual Report of the Saranac Laboratory for the Study of Tuberculosis of the Edward L. Trudeau Foundation, 1940, 1941, 1942). These inhalation studies, utilizing initially textile type fibrous glass which had a nominal diameter in the range of 6 microns failed to produce either fibrosis or tumor formation in the rats and guinea pigs exposed. It was felt that the fiber was too large to be respired. Therefore, a flame-attenuated product which had a nominal diameter of approximately 1 micron was substituted for the textile fiber following animal exposure of twenty months. A succeeding twenty-month exposure in these same animals again failed to demonstrate either fibrosis or tumor development. It is interesting to note that the researchers did find fiber in the peribronchiolar region of the lungs in small quantities which were 10 to 12 microns long. It was felt that the material was cleared rapidly from the lungs.

These early studies, due to the untimely death of Gardener, the principal investigator, were not widely known until Schepers published in 1955 (Schepers, 1955; Schepers and Delahant, 1955). These studies and others (Schepers et al., 1958; Schepers, 1958) which included fibrous glass as well as fiber glass reinforced plastic dust failed to demonstrate either significant fibrosis or malignant tumor. The studies did, however, elicit a nonspecific, nonprogressive cellular dust response.

In the late 1960s, Gross (1970) began a series of inhalation studies exposing rats and hamsters to 100 mg/m^3 of fibrous glass which had a nominal diameter of approximately 1 micron and in the chamber air of 0.5 microns. Seventy percent of the airborne material was reported to be fibrous. The fiber lengths were between 5 and 20 microns. These studies again demonstrated no appreciable alteration of lung architecture, minimal pleural change, no significant fibrosis, and no tumor development in animals exposed for 24 months and domiciled for the remainder of their lifetimes. Other inhalation studies were carried out in the early seventies by Botham and Holt (1971), Trimbrell (1965), Harris and Fraser (1976), Harris (1976), Lippman et al. (1976), and Brain et al. (1976). These studies

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again demonstrated non-specific pulmonary responses with a relatively wide variation but no significant fibrosis and no tumor formation were reported. It is interesting to note that Botham and Holt confirmed Gardener's and Gross' observation that the long thin fiber can elicit a ferrugenous body response.

Another recent inhalation study (Lee et al., 1979) involving fibrous glass with a nominal diameter of approximately 1 micron but with few fibers over 10 microns long in high concentration failed to demonstrate either malignant or nonmalignant disease and produced little change in the lung architecture. It did demonstrate an alveolar proteinosis which would suggest that the exposure was extremely close to a maximum tolerated dose.

Very recently an inhalation study (Johnson and Wagner, 1979; Wagner et al., 1979) has been reported in which focal fibrosis was noted following inhalation of 10 mg/m^3 of dust for one year in rats. The material used included UICC chrysotile B, extremely fine glass fiber with a mean geometric diameter of 0.3 microns, glass wool and rock wool with mean geometric diameters of 0.8 microns. The rats were sacrificed at 3 and 12 months. The focal fibrosis noted at 3 months was quantitatively greater in the chrysotile exposed animals and, while chrysotile exposed animals showed marked progression at the twelfth month sacrifice, man-made mineral fiber exposed animals did not demonstrate progression at the twelfth month interval. This study is still underway and must await the final analysis of the remaining animals in order to identify the significance of this reported minimal focal fibrosis.

Another very recent inhalation study (Reinhardt, 1978) involved not fibrous glass but potassium titanate fibers in extremely high concentration which varied from 3,500 to 43,000 fibers per ml. This study demonstrated, in less than 2 years, the production of both pulmonary carcinomas and mesotheliomas. However, firm conclusions could not be drawn from the study because of the small number of animals in this study. These nonvitreous fibers would be expected to have different properties of durability compared to vitreous man-made fibers.

While inhalation studies have, in general, failed to demonstrate significant chronic irreversible health effects in animals, the less physiologic administration of man-made mineral fiber by intratracheal injection has been carried out by a number of investigators beginning with Gardener (Annual Report of the Saranac Laboratory for the Study of Tuberculosis of the Edward L. Trudeau Foundation, 1942) in the late 1930s and followed by Wenzel et al. (1969), Gross et al. (1970), and Kuschner and Wright (1976).

Again, these studies demonstrated a wide variety of cellular response with no significant chronic pulmonary effects with the exception of the Kuschner-Wright study. In that study, the investigators demonstrated a peribronchiolar fibrosis in guinea pigs intratracheally injected with long, thin fibrous glass. This response was qualitatively similar to a response to chrysotile but quantitatively much less. The authors commented that this information, because the method of administration by-passed respiratory defense mechanisms, could not be directly extrapolated to man. However, the study demonstrated that massive doses, placed at the target site of action in the body, could cause fibrosis if the fibers were long and thin (less than 3.5 microns in diameter and greater than 10 microns long).

The authors felt that additional inhalation studies using long thin fibers were indicated. Tumors have not been produced by intratracheal injection of fibers known to produce them by intrapleural implantation.

Intracavitary implantation of man-made mineral fibers, as well as a variety of other fibrous material, has been carried out by a number of investigators (Stanton and Wrench, 1972; Stanton et al., 1977; Pott, 1978; Wagner et al., 1973; Wagner et al., 1976), both in this country and abroad. Mesotheliomas have been produced using long thin fibers (fibers less than 1.5 microns in diameter and greater than 8 to 10 microns long), by surgical implantation and injection in the pleura and in the peritoneum. It is interesting to note that implantation of ordinary building insulation, which does contain some fibers less than 1.5 microns in diameter, failed to elicit mesothelioma production (Stanton et al., 1969). This suggests a dose response relationship.

However, massive doses of long thin fibers of diverse composition can produce mesothelioma. Stanton, who has performed the most critical study, notes that care must be used in extrapolating the implantation study findings to man since the dose was massive and the route of administration was unphysiologic.

A study involving intrapleural surgical implantation was reported by O'Neill et al. (1949). The purpose of the research was to develop an alternate method of collapse therapy for the treatment of tuberculosis. Fibrous glass, with a nominal diameter of 1 micron, in the form of bulk glass wool was placed in the intrapleural space of dogs. It was found that the massive fibrosis produced in the animals precluded its use in humans. However, no fibrosis was generated when this material was placed inside a sack manufactured from large diameter fibrous glass textile material. This type of collapse therapy was, in fact, used in a clinical trial in six patients as a result of the dog experiments. It is interesting to note that when the concentration of fine fiber was reduced, by use of the sack, no pathology was noted six months after implantation. Some of the fine fiber most surely would have spread through the interspaces of the glass sack and some in contact to the pleura. This further suggests a dose response relationship.

HUMAN STUDIES

The health aspects of fibrous glass in man have been studied for many years. The first study published by Seibert in 1942 was limited to fibrous glass manufacturing workers exposed in the United States. His study which included a review of chest x-rays, physical examinations, and skin biopsies concluded that workers exposed at that time demonstrated no respiratory pathology which could be attributed to fibrous glass but the study did identify the well known phenomenon of transitory mechanical skin irritation.

A number of cross sectional morbidity studies were carried out by Bjure et al. (1964), Wright (1968), deTreville et al. (1970), Nasr et al. (1971), Utidjian and deTreville (1970), and Hill et al. (1973). The Bjure study reported the cardiac and pulmonary function in 14 workers, 6 exposed to glass wool and rock wool and 8 exposed to asbestos. The workers were exposed from 7 to 30 years. No disease was demonstrated for glass and rock wool exposures, while the asbestos exposure group showed marked pulmonary dysfunction. Wright studied 1389 fibrous glass manufacturing workers who were exposed from 10 to 25 years. The study demonstrated no unusual pattern on roentgenograms which could be attributed to the glass exposure. Utidjian reported the results of evaluation of a randomly selected sample of 232 fibrous glass manufacturing workers and failed to demonstrate any major effect on lung function in long-term exposed fibrous glass workers. deTreville used a subsample of Utidjian's workers, including 30 long-term fibrous glass workers, 15 with minimal and 15 with heavy subjective exposure, who were carefully evaluated for cardiac and pulmonary status including blood gas studies. There was no significant difference noted between the two groups of exposed workers. Nasr, examining 2028 chest x-rays of fibrous glass workers from the same plant studied by Wright and Utidjian, observed no difference in the prevalence of abnormalities between exposed factory workers and nonexposed office workers. No radiographic effect resulting from fibrous glass exposure was noted. Hill, in evaluating 70 workers with occupational exposure to fibrous glass with an average exposure time of approximately 20 years, found that chest x-rays and pulmonary function studies of the fibrous glass exposed workers were not significantly different from an unexposed control group matched for age, height, and weight. He concluded that none of the exposed workers demonstrated evidence of respiratory effect from occupational exposure to fibrous glass.

Gross et al. (1971), in studying the lungs of 20 fibrous glass manufacturing workers with exposure ranging from 16 to 32 years, compared to 26 urban nonfibrous glass exposed controls, noted no significant difference in lung fiber content or fiber dimensions between the two groups. No pulmonary pathology was identified which could be attributed to fibrous glass.

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It has been reported that a naturally occurring zeolite whose fibers have the same general dimensions as asbestos has caused mesothelioma in individuals who are naturally exposed to the material. This finding needs further investigation and clarification of exposure levels, fiber dimensions, and other environmental factors before a definitive comment can be made on its relevance to disease causation (Baris et al., 1978).

EPIDEMIOLOGICAL STUDIES

Mortality studies have been carried out on man-made mineral fiber workers. Enterline and Henderson (1975), studying 416 retired fibrous glass workers with exposures of up to 30 years, noted that the mortality experience after age 65 of male fibrous glass workers and the reasons for disability retirement prior to age 65 did not reveal any unusual health hazards. He did note 3 deaths, compared to an expected 0.5 deaths, due to chronic bronchitis. The author also observed that the numbers involved were small and that the disease was too poorly defined to draw conclusions.

Bayliss et al. (1976), in studying 1448 workers occupationally exposed to fibrous glass, demonstrated no significant respiratory disease even 20 years after onset of exposure. He did demonstrate 19 nonmalignant respiratory disease deaths, excluding pneumonia and influenza, as compared to an expected of 10. However, more than half had previous exposure in dusty environments including 9 in a foundry, 1 in a silica batching operation, and an additional individual with exposure in coal mining.

Several of the previous studies, including Wright and Nasr, Utidjian and deTreville's cross sectional morbidity studies, were carried out on this same plant population. It would be unlikely that any significant nonmalignant respiratory disease would not have been suggested by these studies. The proposition that susceptibles would select themselves out of the working population is not a tenable explanation.

The case control study reported by Bayliss as a subpart of the previously mentioned epidemiological study was flawed since the case control study used to examine the relationship between respiratory disease and exposure to the finest fiber manufactured during the 1940s incorrectly classified some of the workers who had no contact with this fiber during their work environment as having been exposed to it (Konzen, 1976; Bayliss et al., 1976). Additionally, the case control study was inadequately designed and analyzed.

Very recently, Enterline reported on the first 8 plants in a study involving 17 man-made mineral fiber manufacturing facilities. The study group included 7049 workers: 6023 workers from 5 fibrous glass manufacturing plants and 1026 workers from 3 mineral wool plants. The total study will include almost 17,000 workers and will be completed by mid 1980. Compared to expected deaths in the U.S., there were 7% less deaths than expected for all causes of death. For respiratory cancer, there were 12% fewer deaths

than expected; for malignant disease of the digestive organs and peritoneum, there were 13% fewer deaths than expected; for nonmalignant respiratory disease, excluding pneumonia and influenza, there were 5% more deaths than expected; this was not statistically significant.

Employees with less than 20 years employment showed a slight deficit, while employees with 20 or more years of exposure showed a very slight excess for the standard mortality ratios for all causes of death, respiratory cancer, digestive system cancer, nonmalignant respiratory disease, and most other causes of death. There was no statistical significance to indicate increased mortality with longer exposure.

Enterline and Marsh (1979) observed that there could have been an initial selection of healthy workers and a disappearance of the healthy worker effect with time. Also, it would be misleading to think of any mortality ratio greater than 100 as "an excess" since it is rare to have a mortality ratio of exactly 100, and even in the absence of an increased risk, half of the observations will be slightly greater and the other half slightly less than 100.

Enterline broke the entire cohort into other subgroups. Although the numbers in each subgroup were small, making trend identification difficult, the mortality rates for respiratory cancer did not increase with increasing duration of employment. For one mineral wool plant, there were 3 respiratory cancer deaths, versus an expected of 0.9, for workers with more than 30 years of employment. However, there was no consistent progression showing increasing death rates with increased duration of exposure.

Likewise, when analyzing the entire cohort for nonmalignant respiratory disease, there was no pattern of increasing mortality rates with increasing duration of exposure.

It has been established that the working population usually smokes more than the general population. In this study, smoking histories could not be determined. This could be important when analyzing data or drawing conclusions about both malignant and nonmalignant respiratory disease.

There are fairly wide variations in mortality ratios from one plant to another based on rather small numbers for each individual plant. When the plants are grouped, however, the mortality ratios are generally much closer to the 100 which is expected.

Another recent study by Robinson et al. (1979), has reported on a cohort of 596 workers from just one of the mineral wool plants included in Enterline's study. The SMR for all causes of death was lower than the adjusted U.S. population. There was no statistically significant excess of malignant or nonmalignant respiratory disease or digestive cancer for the

entire cohort. The author suggested that lung cancer, nonmalignant respiratory disease, and digestive cancer showed a progressive rise related to time from first exposure and that this was particularly suggestive in workers exposed for more than 30 years. There was no statistical significance. The numbers are extremely small, and a careful examination of the data does not demonstrate any consistent pattern for malignant respiratory disease, nonmalignant respiratory disease, or digestive cancer. The data must also be interpreted keeping in mind the healthy worker effect and the fact that smoking histories were not considered. Enterline's study, encompassing a much larger group of employees, is more pertinent and indicates no significant excess for those causes of death.

There are individual case reports in the world literature suggesting a possible association between man-made mineral fiber and acute disease. Infection appeared to play a role in these cases. The first was a case reported by Kahlau (1947) in which an upholsterer, applying a textile wall covering, developed fatal pneumonia one month after the exposure ceased. At autopsy, fibrous glass was found in the lung. This may have been incidental to his pneumonia. In 1956, a worker repairing an incubator developed a lobar pneumonia which was resistant to treatment (Bezjak, 1956). Following a violent coughing episode, a bolus of what appeared to be a plug of fibrous glass was expelled and the patient responded quickly to antibiotics. In this case, this was a foreign body type reaction. The third case was reported in 1961 in which a worker who was dismantling water heaters with fibrous glass insulation was diagnosed as having segmental bronchiectasis (Murphy, 1961). A resection was followed by clearing of the clinical condition.

Upper respiratory irritation has been reported by a variety of authors (Champeix, 1944; Roche, 1946; Cirla, 1948; Mungo, 1960; Milby and Wolf, 1969). It is often found following unusually heavy dusty conditions, often in tear-out situations. After the initial irritation is passed, there is complete resolution of the condition.

The effect of man-made mineral fibers on the skin are well known. This has been reported by Siebert (1942), Sulzberger and Baer (1942), Heisel and Mitchell (1957), Heisel and Hunt (1968), and Possick et al. (1970). The authors are all in agreement that it is a mechanical transitory irritation which lessens in intensity after several days of continuous occupational exposure. The irritation is directly related to the fiber diameter. That is, as the fiber diameter of the product increases, so does the skin irritation. There is no evidence that the skin irritation is other than a transitory mechanical one. There is no allergic component.

In summary, the health aspects of fibrous glass have been studied for almost 40 years. To date, there is no satisfactory evidence that man-made mineral fibers cause chronic adverse health effects, either malignant or non-malignant, in workers occupationally exposed to the material. Fibrous glass, since it has been manufactured, has contained small amounts of fiber less than 1.5 microns in diameter with lengths varying from a few microns to hundreds of microns. This concentration, although very small, appears to have been consistent in products manufactured over the years, including present material. Airborne concentrations of fibrous glass in manufacturing operations appear consistent and very low -- for ordinary fibrous glass, the airborne concentrations are less than 0.1 fiber per ml. These low concentrations are explained in part by the observation that fibrous glass, unlike asbestos, fractures transversely rather than longitudinally.

Fibrous glass, by intercavitary implantation of long thin fibers, will cause mesotheliomas. This finding cannot be readily extrapolated to man since the dose and method of administration are remote from ordinary human exposure. Intratracheal instillation of fiber results in a mild peribronchiolar fibrosis. Again, the method of administration bypasses important body defense mechanisms and delivers an unusually high dose to the target organ. For this reason, the data cannot be directly extrapolated to man.

Inhalation studies using highly respirable fibers have not demonstrated pathology with the exception of a nonvitreous man-made mineral fiber (potassium titanate) and an inhalation study now in progress which requires compilation to determine if a mild nonprogressive focal fibrosis is of significance.

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USE OF RODENTS IN TESTING BIOLOGICAL EFFECTS OF FIBROUS MATERIALS

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Because of their convenient size and lifespan, rodents, notably rats, hamsters, guinea pigs, and mice, have been utilized in a majority of experiments on biologic effects of fibrous minerals. In the present paper, I will cite some of the more recent reports. These contain references to earlier work and give a picture of the present state of knowledge.

Materials have been introduced into rodents by inhalation, orally, or by intratracheal, intrapleural or intraperitoneal injection. After inhalation exposures, pulmonary fibrosis, pulmonary carcinomas and mesotheliomas have been found in rats exposed to the principal varieties of asbestos, e.g. chrysotile, crocidolite, amosite, and anthrophyllite (Wagner et al., 1974). The inhalation route thus provides a model for study of biologic effects of asbestos corresponding to the effects seen in man. Inhalation exposures of rats and hamsters to glass fibers failed to show biologic activity related to these fibers (Gross, 1976).

Because of the large amounts of materials required for inhalation exposures, many experiments with animals have been carried out by injection techniques that require relatively small amounts, e.g. intra-tracheal, intrapleural or intraperitoneal injection. These injection techniques do not, of course, parallel conditions of human exposure; however, they have yielded some interesting information.

Lung cancer has been reported in rats exposed to chrysotile by intratracheal injection. In hamsters, it was known that lung cancers could be induced in high percentages of animals by intratracheal injection of benzo(a)pyrene (BP), a compound known to be present in cigarette smoke. In experiments with hamsters exposed by repeated intratracheal injections in our laboratory, lesser amounts of BP were tested until a dose was found that seldom induced lung cancer. When hamsters exposed to that dose of BP were also given chrysotile, numerous lung cancers developed. No lung cancer occurred in hamsters exposed to the tested dose of chrysotile without BP (Smith et al., 1970).

We did, however, find a pulmonary adenocarcinoma and a mesothelioma in hamsters given amosite by intratracheal injection. Intratracheal injections of asbestos, a synthetic fluoroamphibole, or glass fibers into guinea pigs produced striking quantitative differences but some qualitative similarities in tissue responses depending upon physical characteristics of the fibers (Kuschner and Wright, 1976).

Many preparations of fibrous minerals have been tested for carcinogenicity by injection into the pleural space. Methods and results of such experiments have been discussed (Smith and Hubert, 1974). Numerous tumors have been found at injection sites in rats in several laboratories and in hamsters in our laboratory. In contrast to the predominantly epithelial-type tumors diagnosed as mesotheliomas in human beings, the majority of tumors found at intrapleural injection sites in rats and hamsters have been sarcomatous, only occasional cases showing epithelial-like histological characteristics of mesotheliomas in man. However, electron micrographs of histologically sarcomatous tumors in hamsters show that at least some of the tumor cells have ultrastructural characteristics of mesothelium (Sobel et al., 1978). Accordingly, we use the term "mesothelioma" in tabulating intrathoracic tumors which spread over pleural surfaces in animals given intrapleural injections.

After implantation into the pleural space of rats, Stanton and his associates have obtained tumors with a variety of preparations of asbestos, glass, silicon carbide, potassium octatitanate, and other minerals. From their results, they associate high tumor probabilities with fibers less than 0.25 μm in diameter and longer than 8 μm (Stanton and Layard, 1978). The question of biologic activity of fibers in shorter lengths has been discussed (Selikoff and Lee, 1978), but there is a question as to whether cited experiments involved samples free of larger fibers.

In intrapleural injection experiments with Golden Syrian hamsters, we found mesotheliomas with preparations of chrysotile and tremolite containing numerous fibers up to and longer than 20 μm, but not with preparations milled until relatively few fibers exceeded 5 μm in length (Smith et al., 1979). Also by intrapleural injection of hamsters, we tested 3 pairs of glass fiber samples matched for diameters, but varied in fiber lengths. Tumors were induced only by the longer fiber sample in each pair. In that experiment, samples that induced tumors showed 34 to 82% of fibers longer than 20 μm, whereas in samples that did not induce tumors only 0 to 2% of fibers were longer than 10 μm (Smith et al., 1979a). In contrast to frequent induction of tumors in rats or hamsters, intrapleural injection of crocidolite induced tumors in only 1 of 100 guinea pigs and 2 of 150 mice (Davis, 1974).

Intraperitoneal injection is another route that has been used to compare samples of mineral fibers for biologic activity. After injection of various preparations of fibrous minerals by this route, rats appear to be the most susceptible species for induction of mesotheliomas, tumors having been obtained in high percentages of animals within 2 years. In rabbits, mesotheliomas were induced only after 5 years (Pott et al., 1979). From experiments by intraperitoneal injection of rats, Pott has reported that fibrotic reactions are not a precondition for development of tumors and that tumors resulted from a preparation of chrysotile milled until 99% of fibers were shorter than 3 μm (Pott et al., 1976). However, this percentage was calculated by electron microscopy. On a recent visit by one of our group (W. E. Smith), Dr. Pott demonstrated that optical microscopy of this sample shows numerous fibers from 5 to 50 μm in length. This is a problem that often confronts investigators working with samples of fibrous minerals. Long fibers may be numerically fewer than short fibers, but, on a gravimetric basis, they can represent a substantial part of a sample. For example, with comparable diameter, a single fiber 10 μm long would weigh the same as 10 fibers 1 μm long. In comparing different samples for biologic activity, it has been customary to use doses that are comparable on the basis of weight of the total sample. For future work, attention should be given to weight of fibers in various size categories. For example, with fibers of comparable length, the number of fibers per mg would, of course, be far greater for very thin than for thick fibers.

As noted above, injection experiments are an artificial route of exposure. In contrast, oral exposures are a physiological route. Oral exposures to asbestos in food did not induce tumors in rats in several laboratories or in hamsters in our laboratory, as discussed by Gross (Gross et al., 1974). Oral exposures have come to be of interest because of reports of asbestos fibers in drinking water supplies. Recently, we carried out an experiment in which hamsters were maintained throughout life on drinking water to which we added the UICC Standard Reference Sample of the amosite variety of asbestos at three dose levels. In the animals on the middle dose, there were a few tumors, including one peritoneal mesothelioma, that might have been related to treatment. No tumors attributed to treatment were found in hamsters drinking water containing tailings from milling of taconite ore rich in cummingtonite/ grunerite mineralogically related to amosite. Electron micrographs revealed numerous fiber-shaped particles in both of the tested samples, but examination by optical microscopy (400X) readily demonstrated large numbers of fibers in the amosite sample but very few in the tailings (Smith et al., 1979b).

In order to determine size of fibers in mineral samples, our experience has been that counting should be done by direct measurement of length and diameter of individual fibers from montages of low and high power photographs. Publication of such photographs, showing micron scales, should be included with reports of tests for biologic activity of fibrous minerals, so that comparison between various studies can be made.

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PAPER NO. 4

THE MOLECULAR BIOLOGICAL ASPECTS OF ASBESTOS INTERACTIONS WITH CELLS IN CULTURE

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INTRODUCTION

The carcinogenicity of asbestos fibers is well known, and it has been recently stated based on epidemiological findings at Lyon, France (Shapiro, 1979) that their effects have no threshold and are linear with dosage. Therefore, it is important to begin to establish reasonable test models for asbestos. The models in vitro employing direct tests for mutagenicity (Chamberlain and Tarmy, 1977) or DNA damage (Huang, 1979) have only partly been successful. Although other systems in vitro have been employed (hemolysis of RBC, macrophage enzyme release) as measures of asbestos activity, Brown and Chamberlain in collaboration with many others (1980) have found that the most predictive model for tumorigenic potential of fibers is a measurement of extent of cytotoxicity of fibroblasts in culture. The present investigators describe studies of the processes which may contribute to either cell death or cell morbidity after asbestos uptake by fibroblast cells. Among these are changes in the ratio of metal ions on ingested asbestos which may increase aryl hydrocarbon hydrolase activity, a first step in the damage of DNA by these compounds, changes in plasma membrane surface-carbohydrate-containing components and in benzo(a)pyrene binding.

METHODS

Metal ion ratios on asbestos were studied with cultured human foreskin cells (Detroit 550, CCL 109, American Type Culture Collection, Rockville, Maryland) which were grown in Earles MEM with 10% fetal bovine serum

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(FBS) at 37°C in 5% CO_2 and serially passaged every 4-5 days at a 1:3 split ratio. The test mineral fibers were suspended in Hank's Balanced Salt Solution at 200 mg/ml. The suspensions were autoclaved at 121°C for 20 minutes and working solutions prepared at from 1.0 to 100 µg/ml concentrations in EMEM supplemented with 10% FBS. The cells were treated with varying concentrations of mineral fibers for 24 hours at 37°C. At the completion of the incubation period, the medium was decanted and the cells washed 3 times with incomplete EMEM medium, scraped, pelleted at 6000 x g for 15 minutes, suspended in 0.2 M cacodylate buffer, dehydrated in ethanol-propylene oxide, and resin-embedded for EM processing.

TEM-STEM x-ray microanalysis was performed on 500 to 1200 Å sections which were unstained and were mounted on grids for EDAX assay. Vicinal sections were poststained with uranyl acetate-lead hydroxide. Reference samples were carbon and colloidin sandwiches of precipitates of standard suspensions of asbestos mounted on grids. The TEM-STEM (x-ray) analysis was performed with a Hitachi HUIID and a modified JEM100C that had a STEM module and an EDAX-EDIT system interfaced with a T1707A computer.

The cells employed in the cell membrane studies were also a fibroblast culture representing the predominant cell type in Syrian hamster embryo primary culture at 3rd passage. These cells grown in EMEM with 10% FBS were incubated for up to 72 hours with 10 µg/ml chrysotile fibers. Thus, (Figure 1) after the inoculation and incubation, cultures were treated with 25 U/dish of galactose oxidase and 2mM phenyl methyl sulfonyl fluoride for 3 hours, scraped and the washed pelleted cells (600 x g 10 minutes) suspended with 1 mCi of NaB^3H_4 and shaken for 30 minutes. The pelleted cells were suspended in 200 µl PBS, and aliquots were taken for glycolipid and glycoprotein determinations. One portion was directly soaked in chloroform-methanol for determination of distribution of labeled glycolipids and the remainder treated with mercaptoethanol – SDS at 60°C to extract labeled glycoproteins.

CELL SURFACE LABELLING
OF GLYCOLIPIDS & GLYCOPROTEINS

CELL–GALACTOSE

↓ Galact–oxidase

CELL–GALACT–CHO

↓ NaB^3H_4

CELL–^3H–GALACTOSE

CHCl$_3$:MeOH
2:1

SDS–Gel
Electroph.

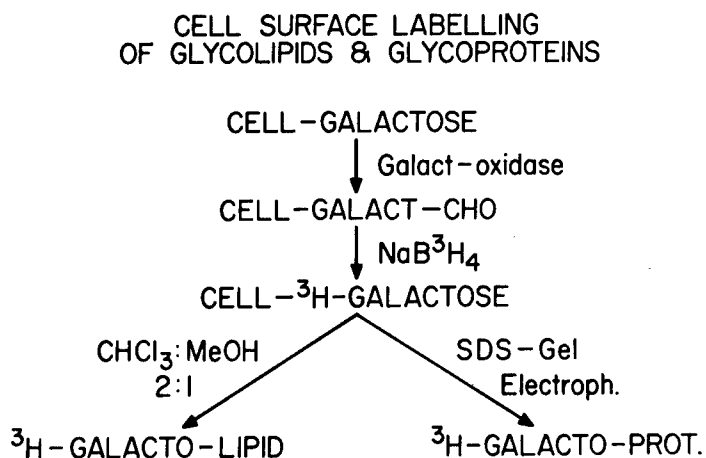^3H–GALACTO–LIPID

^3H–GALACTO–PROT.

Figure 1. Flow diagram of cell surface labeling procedure.

RESULTS

The crystal morphology of the standard samples of mineral fibers is shown in Figures 2-4. Chrysotile, a serpentine asbestos, has a soft silky fibrous structure consisting of parallel sheets of brucite and silica jointed in a characteristic tubular "Swiss roll" formation with brucite (Figure 2) forming the outer layer. Figures 3 and 4 show the structure of the amphoboles, crocidolite and amosite, respectively. Although both are chain-like structures, amosite is single-chained whereas crocidolite is a double set of linkages. Both in micro view are of a rougher texture than chrysotile, and both contain higher amounts of Fe^{+++} than the chrysotile.

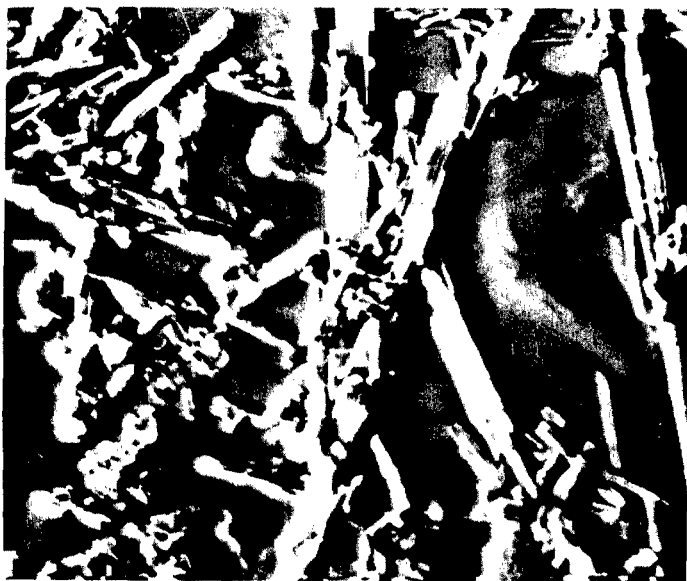Figure 2. Crystal morphology of chrysotile asbestos.
(X 15,000)

Figure 3. Crystal morphology of crocidolite asbestos.
(X 15,000)

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Figure 4. Crystal morphology of amosite asbestos.
(X 9,900)

Next, we studied the structure of control foreskin fibroblasts as revealed by TEM (Figure 5). These are characteristic fibroblasts in culture with numerous intracytoplasmic granules, mitochondria, and considerable interdigitation of the cytoplasmic membranes with nuclei exhibiting irregular membranes. Typically, asbestos-inoculated cells (Figure 6) contain both "intracytoplasmic and intranuclear" crystal inclusions as well as entrapped crystals within the elongated and interdigitated extensions of the cytoplasmic membrane.
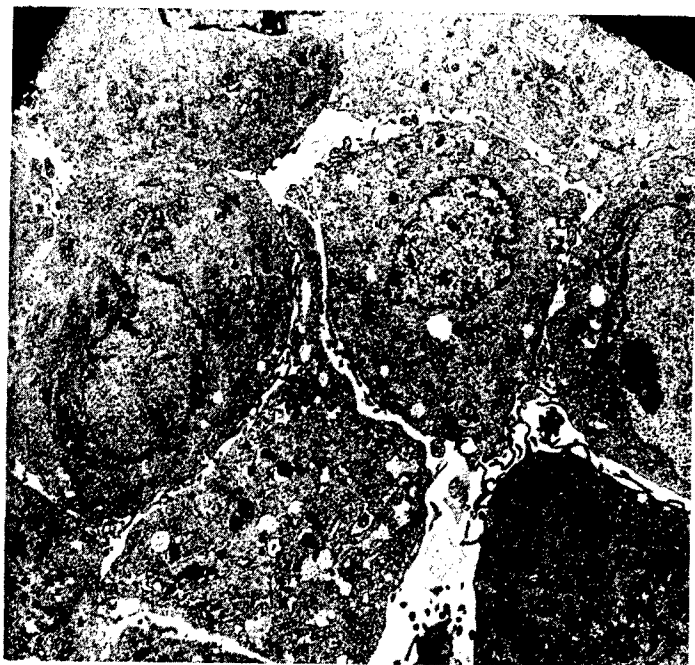

Figure 5. Control foreskin fibroblasts.
(X 4,245)

45

Figure 6. Asbestos
incubated foreskin
fibroblasts.
(X 5,000)

By x-ray intensity EDAS (Figure 7) NIEHS intermediate chrysotile
exhibited the least alteration of its elemental composition on being
engulfed. Intracellular chrysotile exhibited a 1:1 ratio of Mg/Si (Figure
8) which was comparable to a similar ratio in the extracellular fibers,
and this ratio was observed at a range of x-ray intensities based on 10
random readings. The EDAX of crocidolite (Figure 9) Mg/Si showed a
reduced amount of Mg^{++} in intracellular crocidolite and a reduction in
intracellular Mg/Si. More striking is the reduction in the intracellular
Fe^{+++} (Figure 10) with crocidolite inoculation. With amosite, the reduction
in Mg^{++} in the cell-engulfed particles (Figure 11) is compared to extra-
cellular fiber Mg^{++}, but Fe^{+++} (Figure 12) appears to be labile.
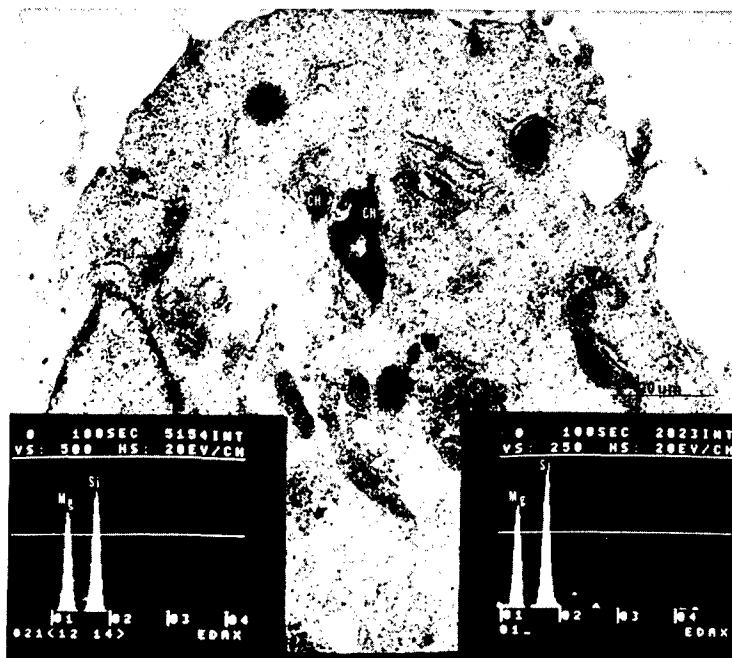
Figure 7. An intracyto-
plasmic crystal of chryso-
tile. Inset left panel
represents the x-ray
intensity ratios of Mg/Si
in the standard sample,
and inset right panel
demonstrates the energy
intensity ratios in the
included crystal.

X-RAY ENERGY RATIOS

Figure 8. Comparative
Mg/Si energy intensity
ratios of intracytoplas-
mic and standard
chrysotile.

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Figure 9. Comparative Mg/Si energy intensity ratios of intracytoplasmic and standard crocidolite.

Figure 10. Comparative Fe/Si energy intensity ratios of intracytoplasmic and standard crocidolite.

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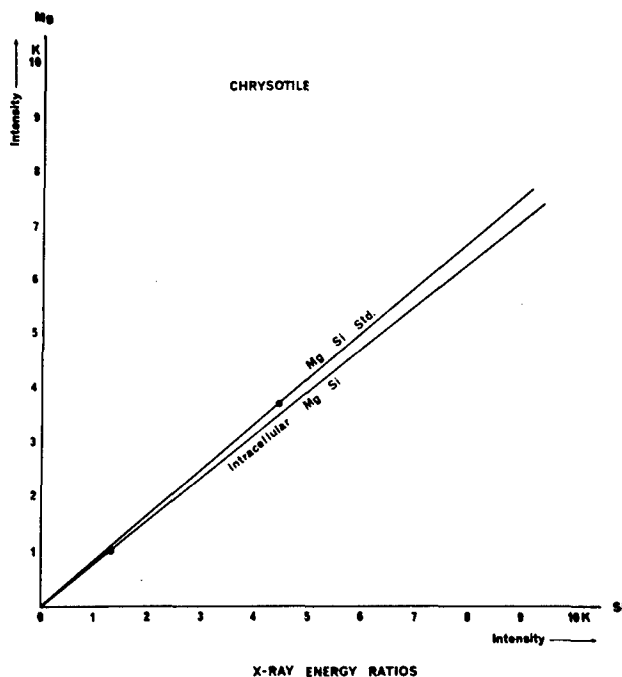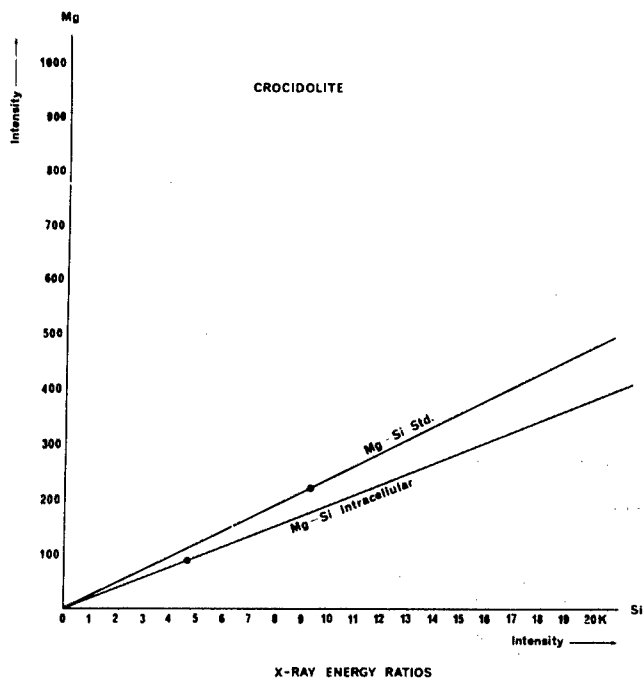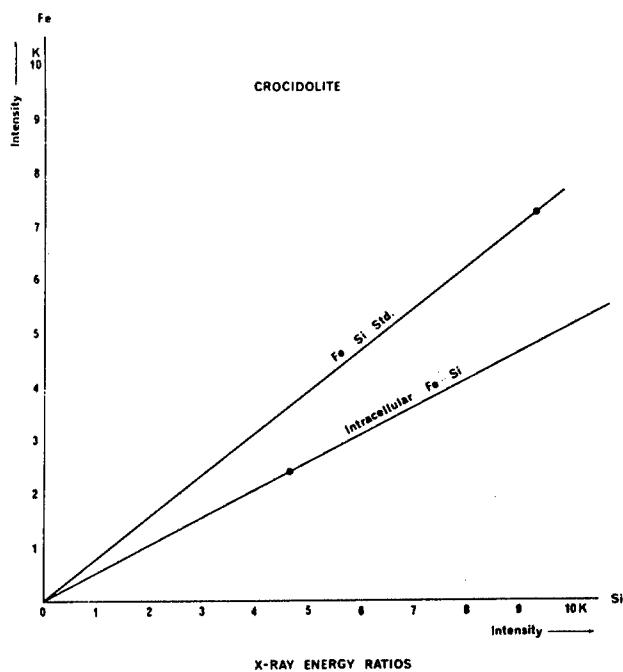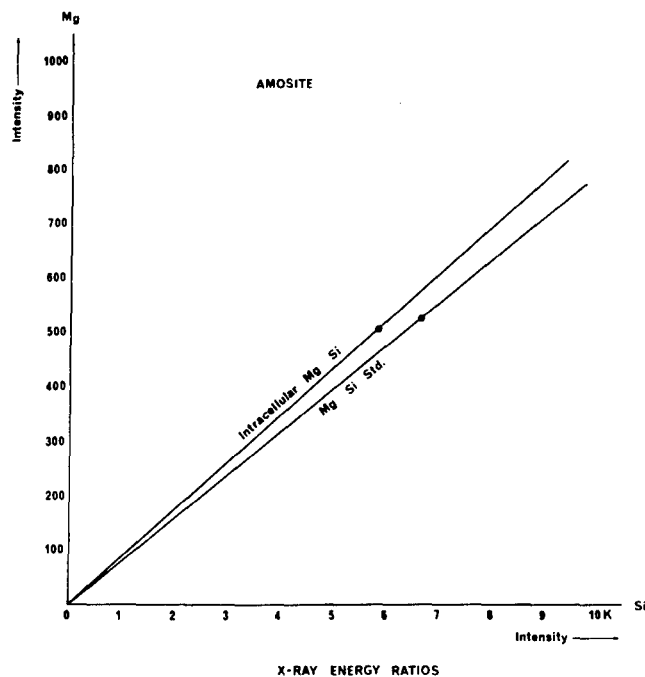Figure 11. Comparative Mg/Si energy intensity ratios of intracytoplasmic and standard amosite.
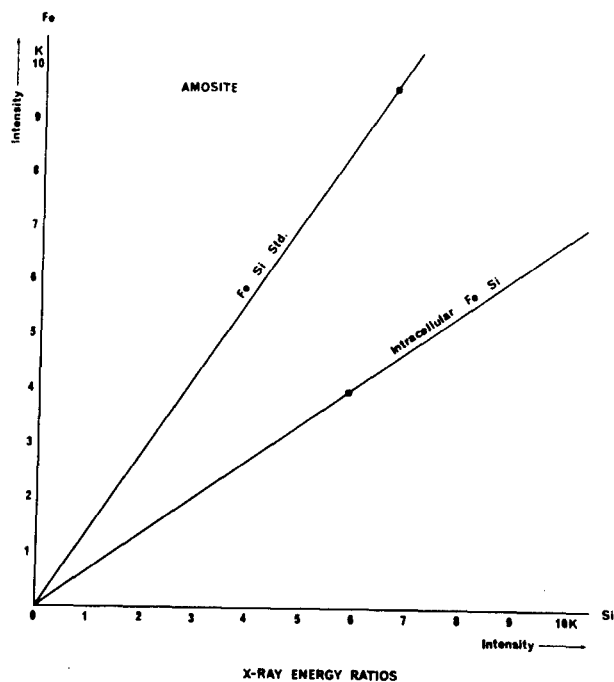

Figure 12. Comparative Fe/Si energy intensity ratios of intracytoplasmic and standard amosite.

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The entry into cells of asbestos fibers has modified the asbestos/
cation ratios in 24 hours, but has this entry caused an immediate modi-
fication of the cell membrane? By labeling the cell membrane glycolipids
and glycoproteins, we have made strides in the direction of answering
the above question. The action of mixed chrysotile asbestos on Syrian
hamster embryo fibroblast surface glycolipids is demonstrated in Table 1.
Asbestos caused no significant change in neutral or polar glycolipids in
2 hours, but in 24 hours considerable change had taken place in ^{3}H
surface distribution among both neutral and polar glycolipids. Reminis-
cent of transformed cells, there was a simplification of gangliosides with
reduced label in the more complex G_{D1A} and G_{T1} gangliosides with con-
comitant increases in G_{M2} (Table 1). This increase was greater in 48
and 72 hours. G_{M1} also increased at the expense of G_{D1A} and G_{T1}.
Globoside label correspondingly went up at 24 hours and declined subse-
quently. The sequence of changes in label distributions among surface
glycolipids was paralleled to some extent by labeling distributions in glyco-
proteins. Glycoproteins were separated by SDS-PAGE electrophoresis, the
gel sliced and ^{3}H determined in each gel slice. The distribution of label
in glycoproteins of control and 2-hour incubated cell cultures is shown in
Figure 13. Similar to the results seen in the glycolipids, there is no
change in labelling pattern in 2 hours. Incubation for 24 and 48 hours
(Figure 14) showed overall losses of label with greater losses in higher
molecular mass proteins. At 72 hours (Figure 15), there was a rebound
in the amount of glycoprotein labeled as compared to control, but there
was a loss of a 86,000 dalton protein.

TABLE 1. RELATIVE DISTRIBUTION OF SURFACE LABELED GLYCOLIPIDS OF SYRIAN HAMSTER EMBRYONIC CELLS

Compounds	Untreated Cells	Chrysotile Asbestos Treated Cells			
		2 Hr.	24 Hr.	48 Hr.	72 Hr.
Polar Glycolipids					
Monosialoganglioside (G_{M1})	24.9 ± 2.33	25.2 ± 2.05	26.0 ± 2.47	37.5 ± 1.62	48.1 ± 2.05
Monosialoganglioside (G_{M1})	30.3 ± 3.74	29.8 ± 1.76	48.1 ± 2.89	51.2 ± 0.91	43.1 ± 1.20
Disialoganglioside (G_{D1A})	20.7 ± 1.97	20.2 ± 2.96	6.9 ± 1.89	3.9 ± 1.12	4.4 ± 1.68
Trisialoganglioside (G_{T1})	24.0 ± 3.39	24.7 ± 3.25	18.8 ± 1.48	7.3 ± 1.86	4.2 ± 0.89
Neutral Glycolipids					
"Glucocerebroside"	32.8 ± 1.69	30.5 ± 1.20	21.2 ± 1.76	26.4 ± 0.56	29.6 ± 1.06
Cerebroside	31.1 ± 1.56	36.0 ± 1.20	35.4 ± 4.24	42.4 ± 1.69	56.5 ± 1.62
Globoside GL-4	35.9 ± 0.42	33.4 ± 2.40	43.3 ± 2.47	31.2 ± 2.26	13.8 ± 0.56

Percent Labeling*

* Based on TLC comparison with known glycolipid standards

Mean 1 standard deviation, n = 2

Figure 13. Comparisons of ^{3}H-labeling patterns of electrophoretically separated glycoproteins from mixed chrysotile - (2 hrs.) - (---) and buffer - (——) treated SHE cells.

Figure 14. Comparisons of ^{3}H-labeling patterns of electrophoretically separated glycoproteins from mixed chrysotile - (24, ······, 48 hrs., ------) and buffer - (———) treated SHE cells.
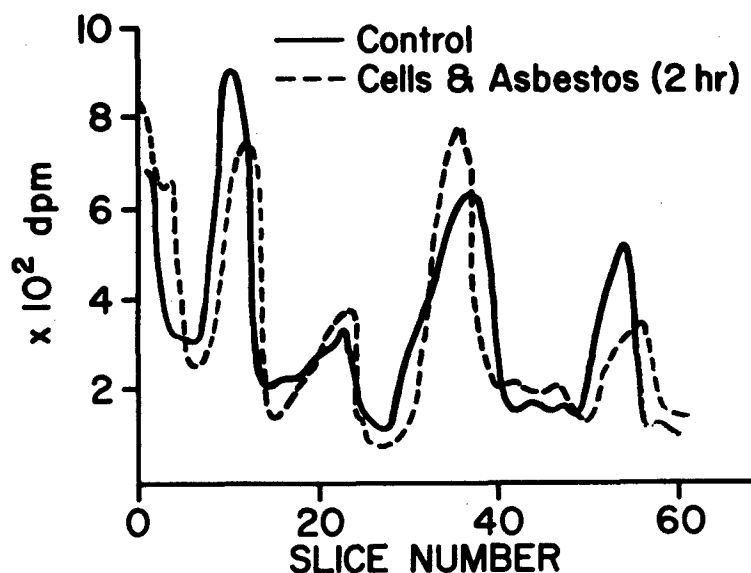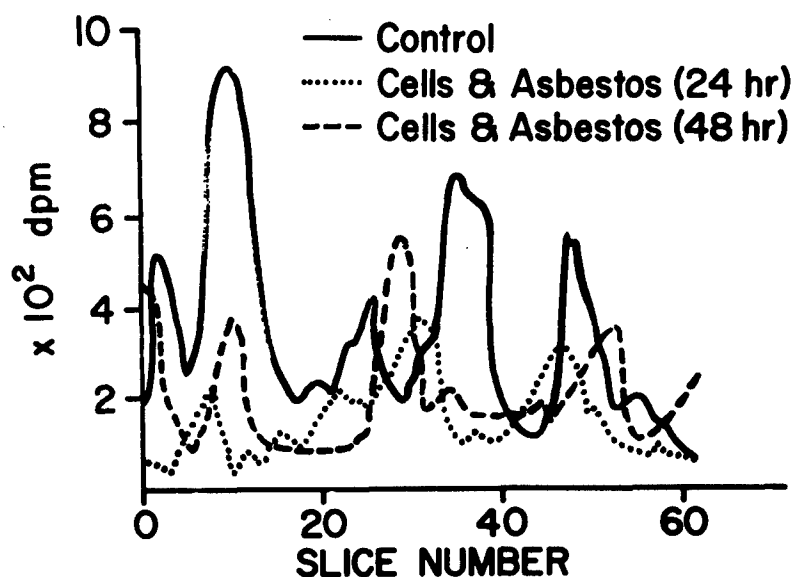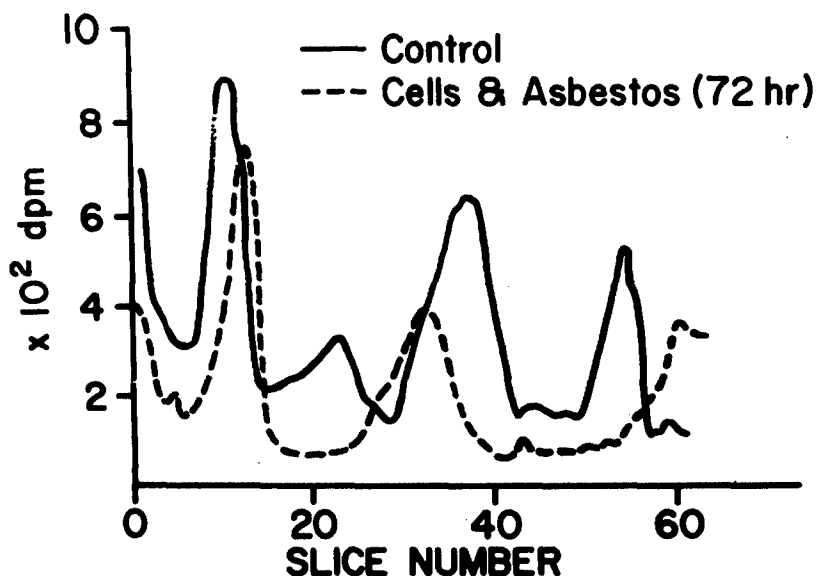
Figure 15. Comparisons of ^{3}H-labeling patterns of electrophoretically separated glycoproteins from mixed chrysotile - (72 hrs., - - -) and buffer - (————) treated SHE cells.

Thus, it appears that changes in the plasma membrane surface are related to a metabolic change rather than a specific removal of glycolipids and glycoproteins from the cell surface by interaction with asbestos. This alteration in the membrane surface in a 48-hour incubation with various asbestos fibers shows (Table 2) a maximum change in glycolipid label pattern with crocidolite and a minimum change with amosite. This same difference in cellular response was also shown with glycoproteins (Figures 16-18).

TABLE 2. RELATIVE % DISTRIBUTION OF SURFACE LABELED GLYCOLIPIDS OF SYRIAN HAMSTER EMBRYONIC CELLS TREATED WITH DIFFERENT ASBESTOS FIBERS FOR 48 HOURS

| | Percent Labeling[a,b] | | | |
| | | Asbestos Fibers | | |
Compounds	Untreated Cells Control	Amosite	Chrysotile (Intermediate)	Crocidolite
1. Polar Glycolipids:				
Monosialoganglioside (G$_{M1}$)	27.2 ± 2.35	34.4 ± 6.50	38.9 ± 4.21[d]	51.8 ± 4.62[e]
Monosialoganglioside (G$_{M2}$)	36.0 ± 3.60	35.8 ± 3.60	48.5 ± 5.90[d]	40.2 ± 3.37[e]
Disialoganglioside (G$_{D1A}$)	19.4 ± 2.95	12.7 ± 1.55[c]	4.69 ± 1.01[d]	4.25 ± 1.26[e]
Trisialoganglioside (G$_{T1}$)	17.4 ± 3.33	17.1 ± 3.21	7.97 ± 1.30[d]	3.82 ± 0.83[e]
2. Neutral Glycolipids:				
"Glucocerebroside"	28.3 ± 2.41	25.9 ± 2.96	25.1 ± 1.41	21.9 ± 2.21[e]
Cerebroside	31.2 ± 1.66	46.3 ± 3.66[c]	48.5 ± 4.27[d]	58.6 ± 0.60[e]
Globoside GL-4	39.9 ± 2.40	27.8 ± 3.60[c]	26.4 ± 3.74[d]	19.5 ± 1.82[e]

[a]Based on TLC comparison with known glycolipid standards.

[b]Mean ± standard deviation, n = 3.

[c]Statistically significant p < 0.05 (Amosite vs. Control).

[d]Statistically significant p < 0.05 (Chrysotile Intermediate vs. Control).

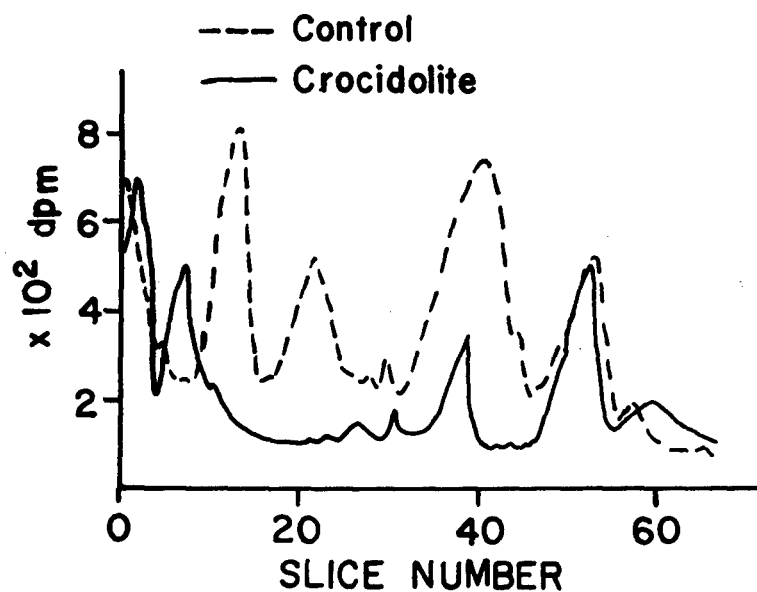[e]Statistically significant p < 0.05 (Crocidolite vs. Control).

Figure 16. Comparisons of ^{3}H-labeling patterns of electrophoretically separated glycoproteins from crocidolite - (48 hrs., ---) and buffer - (———) treated SHE cells.
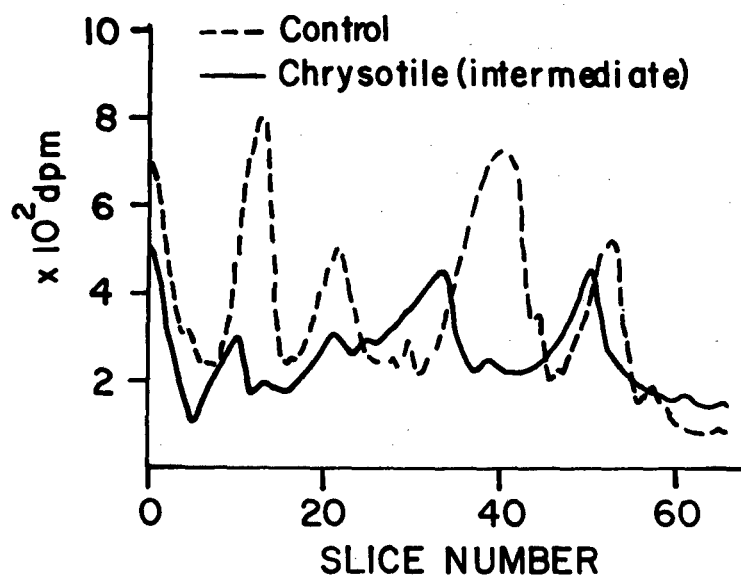
Figure 17. Comparisons of ^{3}H-labeling patterns of electrophoretically separated glycoproteins from intermediate chrysotile - (48 hrs., ---), and buffer - (———) treated SHE cells.
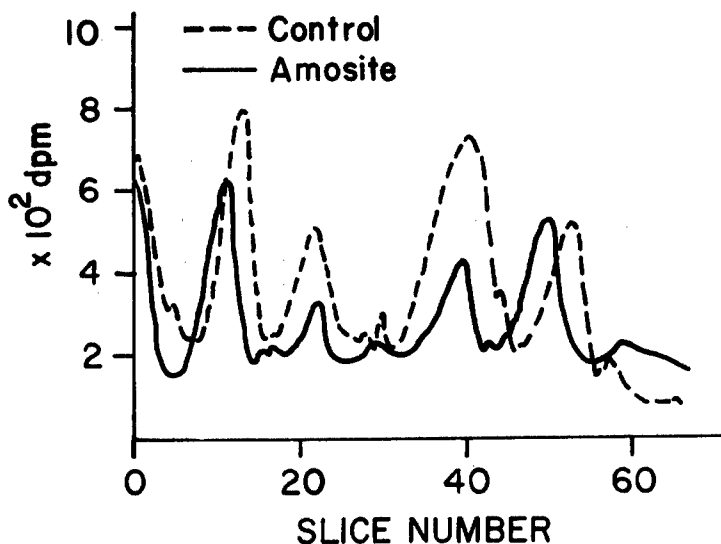
Figure 18. Comparisons of ^{3}H-labeling patterns of electrophoretically separated glycoproteins from amosite – (48 hrs., ---) and buffer – (———) treated SHE cells.

Thus, along with cytotoxicity, membrane changes appear to be another biological endpoint with some correspondence with fiber tumorigenicity.

The classical action of either physical or chemical carcinogens is on DNA as expressed by measurement of DNA damage or cell mutation. All tests of DNA modification by asbestos have shown no effect. The alternative possible action of asbestos might be on the promotion and/or activation of chemical carcinogens. First, a test of the enhancement of promotion through the microsomal activation step was tested by incubation of liver microsomes with up to 2 mg/ml of chrysotile asbestos fibers and 7,12 dimethylbenz(a)anthracene activation was unchanged (Table 3) (Figures 19-20).

TABLE 3. RAT LIVER MICROSOMAL METABOLISM
OF 7,12-DIMETHYLBENZ(A)ANTHRACENE

Concentration Chrysotile (mg/ml)	% Metabolized to Water Solubility ± S.D.
0	71 ± 1.8
0.5	68 ± 2.0
1	70 ± 1.5
2.5	69 ± 2.3

Reactions contained 100 nmoles of ^{3}H-DMBA, 2.0 mg microsomal protein, and required cofactors (9) in a 1 ml volume buffered with 0.1 M Tris pH 7.45 at 37°C.

DMBA METABOLITES – RAT LIVER
NO CHRYSOTILE
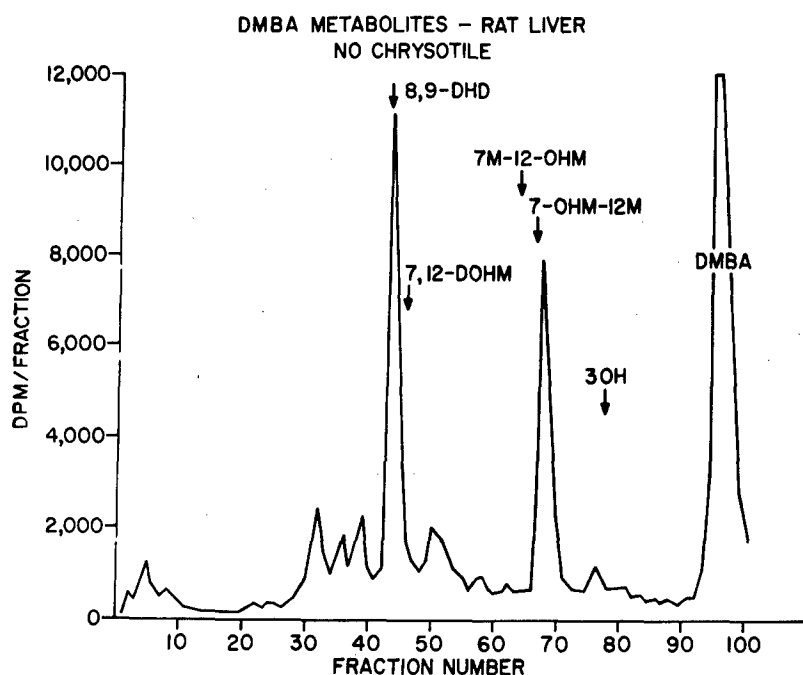
Figure 19. HPLC profile of ^{3}H-DMBA rat liver (3 MC induced) microsomal metabolites. Spherisorb 0.46 x 25 cm ODS column with 25% to 100% methanol in water convex gradient. Fractions of 0.5 ml collected and radioassayed by liquid scintillation procedures.

DMBA METABOLITES – RAT LIVER
2.5 mg/ml CHRYSOTILE
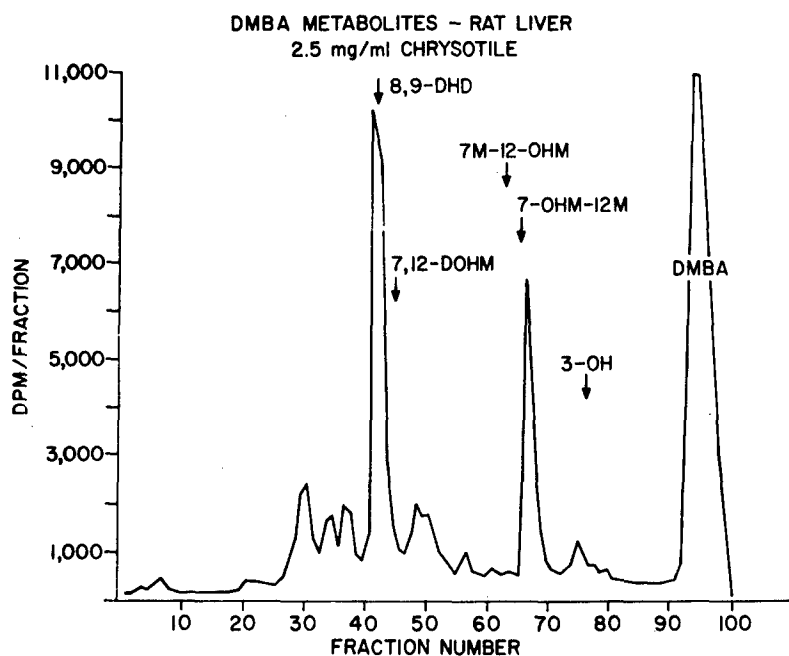
Figure 20. HPLC profile of ^{3}H-DMBA rat liver (3 ml) microsomal metabolites in presence of 2.5 mg/ml NIEHS chrysotile (conditions same as for Figure 19).

NIEHS chrysotile asbestos with and without ^{3}H-benzo(a)pyrene (BaP) was incubated with Detroit 550 skin fibroblast cells for 72 hours according to the schedule shown in Table 4. Group A received simultaneously the BaP and asbestos and Group C received asbestos at -24 hours and BaP at 0 hour. There is a definite enhancement of binding of BaP in the experiment in which there was a prior addition of asbestos. The enhanced binding of ^{3}H-BaP did not appear to be accompanied by a shift in the known products of BaP although there was an increase in unknown ^{3}H in uncharacterized polar constituents of the HPLC eluants in asbestos-pretreated cells (Figures 21-23), but no change in pattern was observed when asbestos and BaP were applied concurrently.

TABLE 4. BINDING OF BENZO(A)PYRENE TO HUMAN FIBROBLASTS IN VITRO

Group	Time of Addition of Chrysotile Relative to BP	Binding Level μmoles BP/mole Deoxynucleotide
A	None	0.45
B	Simultaneous	0.48
C	24 Hours Before	0.63

Cells treated with ^{3}H-BP at 2 μM at 60-70% of confluency. Concentration of NIEHS chrysotile - 1 μg/ml.
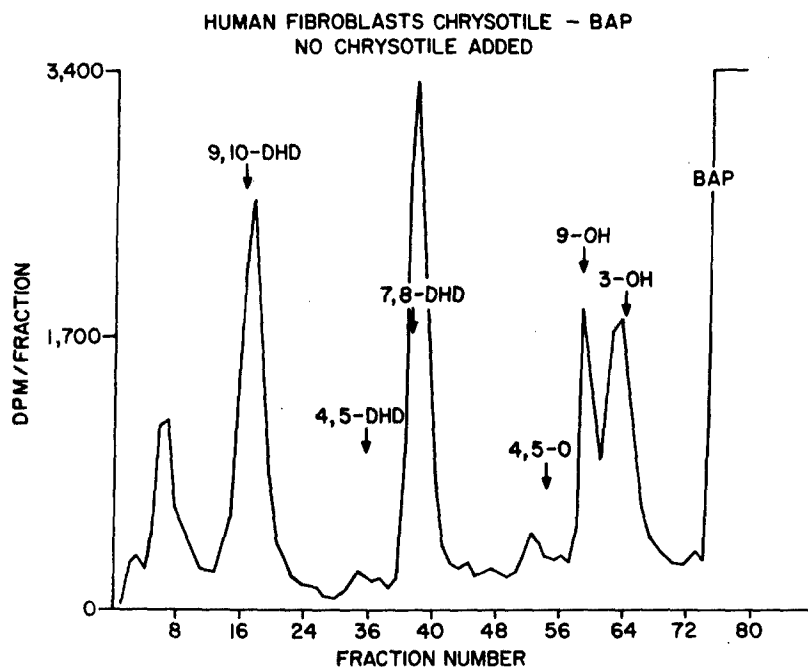
Figure 21. HPLC of ^{3}H-BaP metabolites produced by human fibroblasts in vitro. Spherisorb 0.46 x 15 cm ODS column with 60-100% linear gradient. Fractions of 0.4 ml are collected and radioassayed.
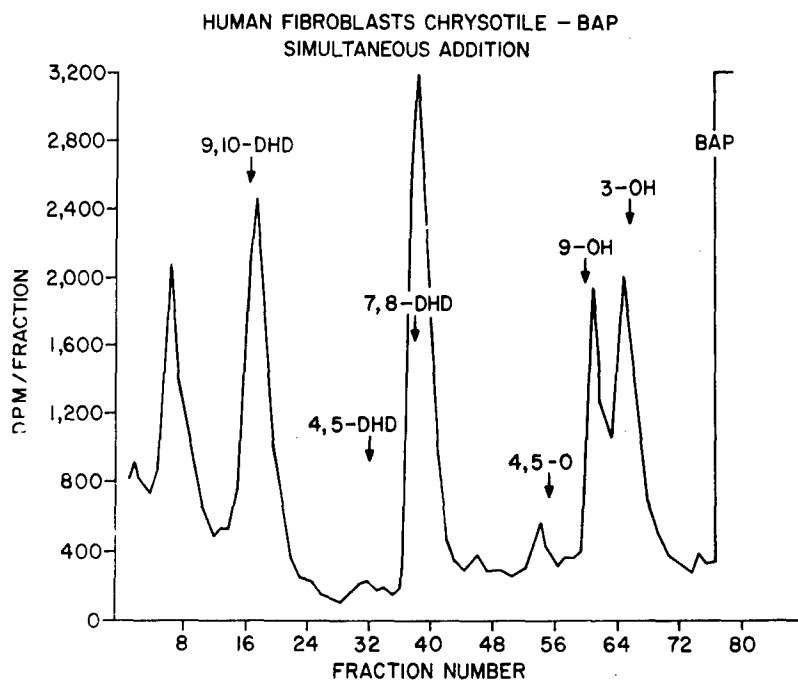
Figure 22. HPLC profile of ^{3}H-BaP metabolites produced by human fibroblasts in vitro with simultaneous addition of 1 mg/ml NIEHS chrysotile (conditions same as Figure 21).
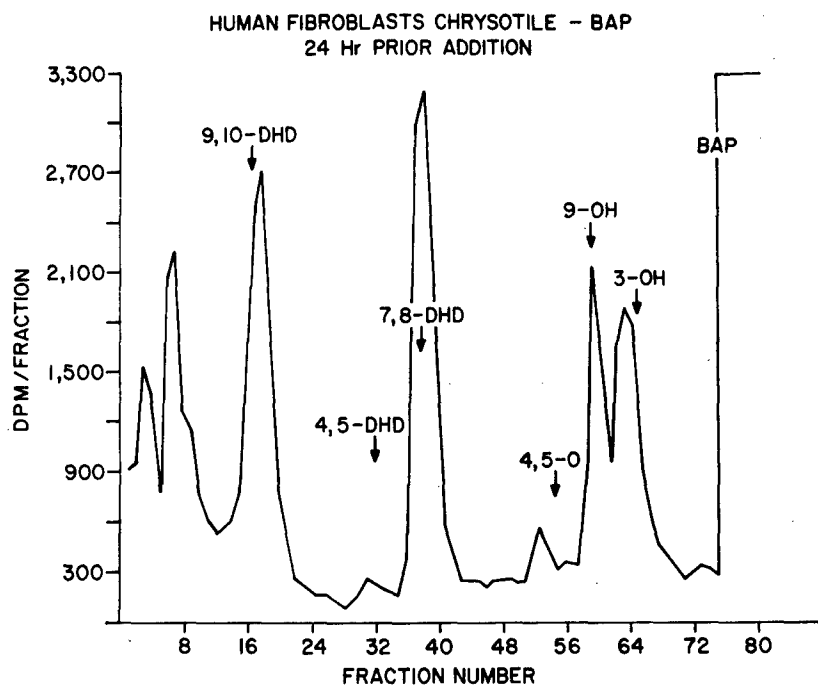
Figure 23. HPLC profile of ^{3}H-BP metabolites produced by human fibroblasts in vitro when 1 mg/ml NIEHS chrysotile is added 24 hours prior to ^{3}H-BaP (conditions same as Figure 21).

A most interesting aspect of this study vis-a-vis other cytotoxicity studies was the finding (Figure 24) that cytotoxicity was enhanced when asbestos was applied 24 hours prior to BaP addition of fibroblast cultures.
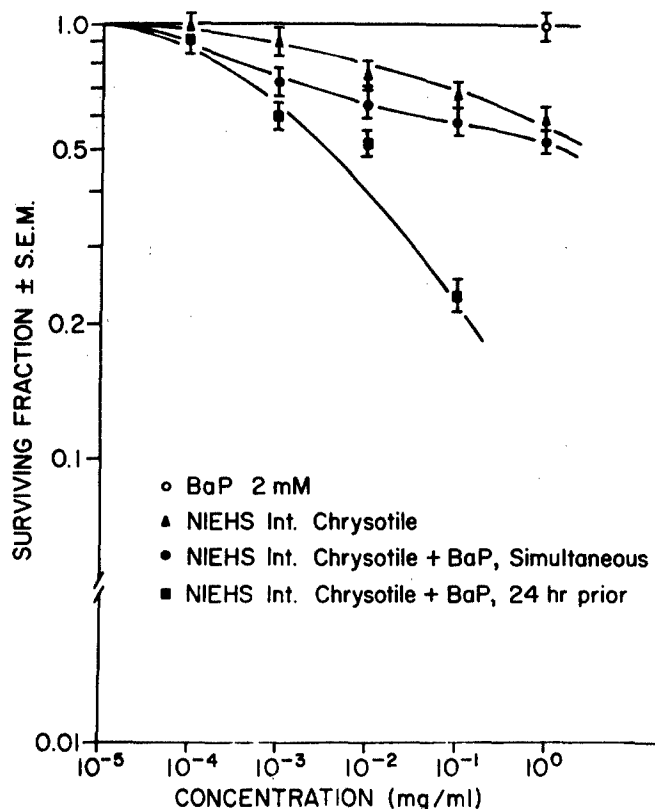
Figure 24. Dose-survival relationship of human fibroblast cells treated with NIEHS chrysotile and/or asbestos.

DISCUSSION

The simplification of the molecular structure of glycolipids and glycoproteins appears to be a manifestation of metabolic changes within cells brought about by interaction of asbestos with intracellular elements rather than through direct masking or removal of cell surface components since the removal or masking of surface components would be an immediately manifest reaction and would certainly be consumated in a 2-hour time span, a period of time in which no significant alteration in glycolipid or glycoprotein pattern could be discerned. That these changes are brought about by alterations in the metabolism of the Golgi and/or the lysosomes requires additional study. Evidence by Harington et al. (1976) with macrophages points to enhanced lysosomal enzyme release with the consequent modification of cell membranes through the production of lysophosphatides at the expense of diacyl phospholipids which are predominantly situated in cellular membranes. In addition, peritoneal macrophages prepared by intraperitoneal

injection of asbestos 4-5 days prior to cell harvesting caused marked increases in cell surface areas as determined by scanning electron microscopy and results in greatly increased cell receptors for IgG and the third component of complement (Miller and Kagan, 1976, 1976a). If the macrophage receptors are ganglioside in nature, surface labeling of these cells should show a modification of surface distribution due to asbestos treatment.

The differential effect of amphobolic double chained or serpentine (crocidolite, chrysotile) and single-chained amosite may not be fortuitous but instead may relate to the molecular configuration of these particles vis-a-vis their interaction with subcellular elements.

Our treatment of embryonic Syrian hamster cells with asbestos shifted the percent labeling from G_{D1A} to G_{M2} and increased the label in GL-4. Overall, however, the labeling of gangliosides, and therefore, possibly the amount of exposed ganglioside, was reduced with asbestos treatment. This effect may be related to either masking of the labeling sites or a reduction in the amount of ganglioside present on the cell surface. In contrast, when lectins bind to the cell surface (Hakomori, 1975) or when cells become transformed (Gahmberg and Hakomori, 1975), there is an increased labeling observed. The changed distribution of surface gangliosides after asbestos treatment is consistent with such distributions after cell tranformation, but since in the presence of asbestos the surface labeling is reduced, this finding is unlikely to be a reflection of early changes en route to transformation. It is more likely to be related to asbestos interactions with membrane co-enzymes such as retinol phosphate which may be responsible for surface glycosylation of gangliosides and ganglioside precursors (Yogeeswaran et al., 1974; Laine et al., 1974a), and for glycoproteins.

After asbestos treatment, a considerable shift in distribution of label toward lower molecular weight cell surface glycoproteins was recorded, but there was also an overall reduction of labeling. This shift in distribution is similar to that observed in transformed cells. A glycoprotein, galactoprotein "a" (possibly the same as LETS), with a molecular weight of 200,000 daltons was found to be deleted in highly tumorigenic NIL py cells (Gahmberg and Hakomori, 1973). Lectin treatment also suppressed labeling of surface galactoprotein "a" in normal cells (Hakomori, 1975), but had little effect on lower molecular weight surface galactoprotein. This could be a step in the direction of transformation by these cells or more likely a direct inhibition of glycoprotein glycosylation at the membrane surface through the removal of retinol phosphate (Yogeewaven et al., 1974).

Most mammalian carcinogenesis appears to occur through a multistep sequence. Two clearly identifiable steps, initiation and promotion, have been delineated (Boutwell, 1978).

Although the nature of promotion is unclear, a consensus has been established that the initiation process has DNA damage as a critical biochemical event (Brookes, 1975). Initially, at least, such damage is thought to require interaction of the chemical or its products with cellular DNA.

Due to the pioneering work of the Millers (1977), it appears that all chemical carcinogens must be either (1) electrophiles or (2) are converted to electrophiles by the catalysis of cellular enzymes; the critical event in chemical carcinogenesis is the reaction of these species with cellular nucleophiles, i.e., DNA (Hart et al., 1978). Both benzo(a)pyrene (BaP) and 7,12-dimethyl-benzo(a)anthracene (DMBA) are powerful carcinogens which have been thoroughly studied (Gelboin and T'so, 1978). BaP, in particular, has been investigated with respect to its tumorigenicity, mutagenicity, metabolism, and DNA binding (Gelboin and T'so, 1978).

The major steps in BaP metabolism in regard to carcinogenic activity are outlined in Figure 25. BaP is oxidized to 7,8-dihydro-7,8-dihydroxy-9,10-dihydro-9,10-oxy-benzo(a)pyrene (BaP-diol epoxide or BaP-DE), and it is this compound that reacts with cellular DNA (primarily at the guanine N^2 position) to produce the critical DNA damage (Osborne et al., 1978). After BaP exposure, this damage is detected in vitro (Osborne et al., 1976) and in vivo (Grover et al., 1976) by finding B(a)P-DNA adducts in isolated DNA.
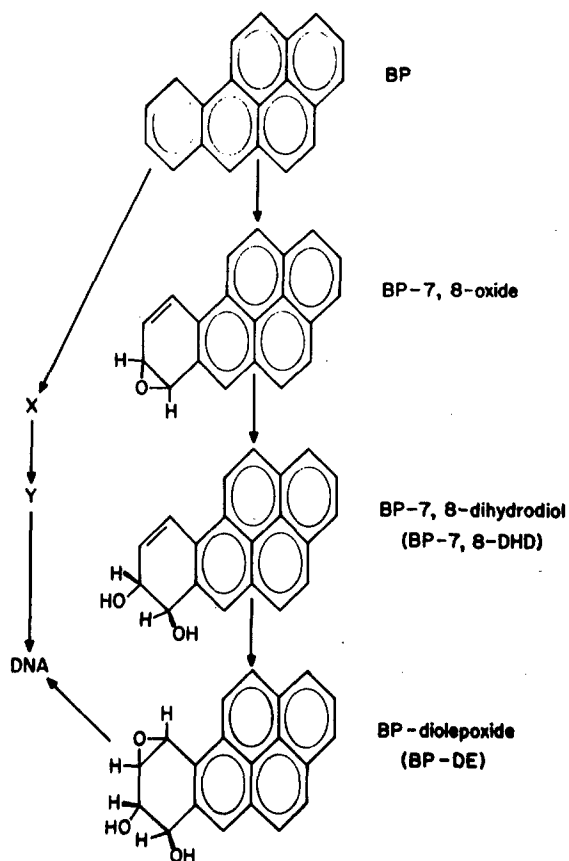
Figure 25. Outline of benzo(a)-pyrene (BaP) metabolism related to carcinogenesis.

The enzyme systems responsible for the generation of these reactive electrophiles are arylhydrocarbon hydroxylase (AHH), a cytochrome P450 containing mixed function oxidase found both in the rough endoplasmic reticulum, the nuclear membrane and membrane-bound epoxide hydrase (EH). Both enzymatic activities are induced by many xenobiotic stimuli (Levin et al., 1977).

Our studies have shown that pretreatment of human fibroblasts increases both DNA binding and cytotoxicity of BaP. A number of possible mechanisms could be proposed for these phenomena:

1. the asbestos increases the concentration of cellular AHH and/or EH which results in increased concentrations of activated BP_1,

2. the asbestos "redirects" the metabolic sequence along different pathways to metabolites with even higher DNA binding potential ("X" and "Y" in Figure 25),

3. the asbestos fibers could cause modification of the cell membrane so that there was a facilitated hydrocarbon transport across the plasma membrane causing an elevated concentration of substrate for the oxidizing and reducing enzymes,

4. various divalent cations leached from the fibers could perturb hydrocarbon metabolism, possibly leading to increased DNA binding,

5. interaction of the highly charged asbestos fibers with DNA could change the purine and pyrimidine bases leading to tautomeric forms with enhanced nucleophilicity for BaP and its metabolites.

6. other mechanisms may be operating.

Currently, it is unclear which system(s) is(are) most responsible for the enhanced cytotoxicity and DNA binding. The increased binding only occurs if the cells are preconditioned to asbestos 24 hours prior to exposure to the polycyclic aromatic hydrocarbon. This point in time is similar to that in which cell membrane glycolipid and glycoprotein changes take place.

Recently, Naseem et al. (1978) showed that AHH is more inducible in lymphocytes of both currently working and former asbestos workers than in matched controls. It has also been demonstrated that the combination of crocidolite and 3-methyl-cholanthrene is a more effective inducer of AHH in cells than the hydrocarbon alone (Mossman et al., 1980). Chrysotile, in this study, has no such enhancing effect.

The second hypothesis has merit since we have observed increased amounts of highly polar uncharacterized metabolites in the cell medium (Figures 21-23). If these are valid observations, then the DNA adducts from asbestos-pretreated cells should differ from those in the control plates, a study currently in progress.

The transfer of hydrocarbons, adsorbed to UICC chrysotile B, to cell membranes greatly exceeds the transfer from an aqueous medium (Lakowicz et al., 1980; Lakowicz et al., 1978). However, these findings which support the current studies, differ significantly from our procedure. In Lakowicz and Bevans' studies, the hydrocarbons were adsorbed to the asbestos particles prior to being added to the medium.

Previous investigators (Thompson et al., 1978) have demonstrated that Mn^{++}, Mg^{++}, and Fe^{++}, cations found on asbestos, change the ratios of BaP metabolites and amount of BaP-DNA binding in a microsomal system. We have been unable to make comparable observations since (1) the chrysotile had no detectable effect on either the relative or absolute amounts of various DMBA metabolites after incubation with liver microsomes (Figures 19 and 20), and (2) the ratio of identifiable metabolites of BaP (both in absolute and relative terms) did not change, and thus, although no direct experiments with cations have been undertaken, it appears that the cation leaching is not sufficient to induce metabolic changes.

The last possibility, charged particles causing a perturbation in the electronic configuration of nitrogen bases in DNA, has some merit, but support for this hypothesis would require the presence of asbestos fibers in the nucleus. Light and Wei (1980) have presented arguments for such a possibility.

In summary, we have found a synergistic effect of BaP and asbestos for both an enhancement of cytotoxicity and DNA binding in human fibroblast cells. This enhanced binding may be mediated by asbestos-induced changes in the plasma membrane structure, a "redirection" of BaP metabolism, a direct facilitated transport of BaP through asbestos penetration of the cell, or direct asbestos-DNA interactions. Notwithstanding the mechanism, the enhanced binding of DNA by BaP can be related to an enhanced carcinogenic effect of this PAH. How this relates to the observed increased lung carcinoma in smoking asbestos workers is uncertain at this time.

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OPEN FORUM

DR. NEWMAN (Ohio State University): I have two questions for Dr. Gross. At a recent NIEHS meeting in Washington, there was a discussion of a scientific meeting in Lyon. At that meeting, I believe that there was an epidemiological study reported by an epidemiologist from Johns-Mansville. I understood that they claimed there was a zero cancer threshold, but I wasn't sure which particular fibers were involved and which type of cancer. I would also like to know if the response was linear for exposure time and for dosage. Could that information be further elaborated here? The second question I have is that I understand that when asbestos workers are exposed to asbestos over a period of years there are changes in terms of their immune systems. I'd like some comment about that.

DR. GROSS (Medical University of South Carolina): I appreciate that it is generally held there is no threshold for asbestos. However, it seems to me that the fact that at autopsy the lungs of all urban dwellers examined contained asbestos fibers and inasmuch as the asbestos content in the lung was not usually associated with either scarring of the lung or cancer, these facts should be considered as evidence of a cancer threshold for asbestos. There does appear to be an asbestos dose at which there is no disease. Inasmuch as I am not an immunologist, I cannot expand on your second question regarding the immune response. However, we do know an occasional asbestos worker does develop large masses in the lungs the same as coal workers do due to rheumatoid factor. That is the only instance of which I am aware where an immune reaction results from asbestos exposure.

DR. CROCKER (University of California, Irvine): There has been a great deal of attention paid to particle size in both the fibrous glass studies and asbestos exposure studies. I still did not discern whether there is a distinction between man-made fibers and asbestos fibers in terms of composition which would account for any difference that is not accounted for in particle size alone. I'd like to ask either gentleman to comment on that.

DR. GROSS: We are dealing with a whole spectrum of particle sizes where asbestos related disease is concerned. The size limit of the disease producing fiber, whether glass or asbestos, is that size which is capable of reaching the pulmonary air spaces. The upper limit is determined by the diameter rather than length but length also plays a part. Generally speaking, the diameter of 3.5 micrometers has been given as the upper limit of diameter which can penetrate into the deep air spaces. That diameter, however, is much too large according to the work of Harris at the University of North Carolina and Dr. Trimbrough of Wales. The aerodynamic equivalent diameter of a 3.5 micron diameter fiber is in the neighborhood of 7 micrometers and a unit sphere of that diameter will not

ordinarily penetrate into the deep air spaces of the lung. Thus, diameter does play an important part insofar as asbestos is concerned in producing disease in lungs. I am personally convinced that inhaled glass fibers of any diameter will not produce fibrotic disease in the lungs and certainly no tumors.

DR. CULVER (University of California, Irvine): I believe Dr. Crocker also asked about the difference in chemical composition of fibers and whether this may be one of the reasons for the observed effect.

DR. KONZEN (Owens-Corning Fiberglas Corporation): A wide variety of fibrous materials have been studied. Stanton was able to demonstrate that the geometry of the fibers seemed to be the critical factor in causing mesotheliomas under laboratory conditions. Pott, in Germany, has implanted short fibers of a variety of materials into the peritoneal sac of mice and produced mesotheliomas. Many researchers are concerned that the results Pott observed may have been artificially produced by longer tramp fibers which may have been present in very small quantities in that material which he implanted. If so, these longer tramp fibers may have been the ones that produce the disease.

DR. CULVER: Dr. Konzen, you reported a study in which using potassium titanate fibers produced pulmonary cancer as well as mesothelioma. Does the potassium titanate fiber have a crystalline or an amorphous structure?

DR. KONZEN: I'm not sure. It's not a vitreous fiber, and I don't know what the exact composition of the fiber is.

DR. CULVER: That would be of interest because the obvious difference between the naturally occurring fibers and the man-made mineral fibers is the issue of crystalline versus amorphous structure.

COLONEL DE HART (Air Force Aerospace Medical Research Laboratory): Dr. Gross, as you very vividly pointed out, the synergistic action of smoking and asbestosis results in a 90-fold increase in cancer. Would you venture an opinion as to the mechanism of action for this high rate?

DR. GROSS: Smoking will interfere with the cleansing mechanism of the lung. The result of smoking consequently results in higher retained fiber dosage of the smoker exposed to asbestos than a nonsmoking asbestos worker. I think that is the main factor. Another important factor may be that asbestos fibers are capable of adsorbing polycyclic hydrocarbon carcinogens.

DR. NEWMAN: I might also suggest that the polycyclic hydrocarbons are the original damaging agent on DNA and that the asbestos may prove to be a tumor promoter of further metabolism of the cyclic hydrocarbon. There is an alteration in the kinds of cations that may

exist within the cell when asbestos enters the cell. So there are a number of things that could be happening. This event is occurring in a fibrous area where there has been a lot of proliferation of fibroblast-like cells. It's possible that the enhancement of cell proliferation has caused the DNA damage to be further expressed in a number of daughter cells. The final expression may be in tumors. This is just my hypothesis.

DR. NEWBALL (The Johns Hopkins School of Medicine): I think both of the speakers have referred to the work of Dr. Stanton from the National Cancer Institute, and I'd like to just quote very briefly from one of his editorials. He states, "Data from several laboratories, as well as my own, indicate that asbestos causes cancer, not because it is asbestos, but because of the unique physical structure it attains." He then continues to say, "The most convincing evidence of a structural relationship to carcinogenicity is that fine, long fibers of durable material, completely unrelated to asbestos, such as glass and aluminum oxide, are similarly carcinogenic in the pleura of the rat." We have been able to recover fibrous glass from both the lungs of man and of dogs in the range of diameter and length that have been reported by Dr. Stanton to be carcinogenic. Dr Gross suggested that, if I understood him correctly, he was not concerned about the carcinogenicity of the glass fibers. I was just wondering if he would present some of the data which he has to substantiate that suggestion.

DR. GROSS: First of all, the label of carcinogenicity on fibers other than asbestos is based on intracavitary placement of these materials into rodents rather than by inhalation. The results of these experiments cannot be and should not be extrapolated to man. This sort of carcinogenesis has been termed solid state carcinogenesis which has no relevance to man. I think that would be my main reply to your question. Another aspect of my answer would be that in my studies using rats and hamsters which had been exposed to fiberglass that had a diameter of six-tenths of a micrometer and 50% of the fibers were between 10 and 20 micrometers in length, none of the exposed animals held for their lifetime developed cancer. This is well within the diameter and length range that Stanton considers significant for carcinogenesis.

DR. CAVENDER (ToxiGenics, Inc.): I participated in an earlier study that was sponsored by NIH which was the companion study of Wagner and Johnson that was described by Dr. Konzen. Part of that study involved the tracking of inhaled asbestos fibers during early exposure to rats. We look at rat lungs immediately after exposure, fixed both by intratracheal perfusion and by vascular perfusion. In animals killed immediately after exposure, there were fibers all over the bronchial airways even down to the alveoli. But if we waited 24 hours to kill the animals, we saw only a few large fibers, and they were trapped around the bifurcations. If we looked at lungs 24 hours postexposure with transmission electron microscopy, the fibers were beginning to move into the interstitial spaces. I think that Nettesheim's group feels that the mechanism of cancer production by asbestos is related to its movement into the interstitial spaces. Can either of you

comment on whether similar studies have been done with man-made fibers and if they indeed move as freely at least as asbestos seems to within the lung?

DR. KONZEN: I think Dr. Gross has done some fiber translocation studies, and I'd like him to comment on that. We are involved at the present time in some studies using sized fibers, fibers that are very precisely sized and took a great deal of time to prepare. This work is being carried on at the present time here in this country by Dr. Kushner, Dr. Drew, and Dr. Bernstein. It's also being carried on in England by Dr. Morgan.

DR. GROSS: The mechanism by which asbestos produces disease of any kind is unknown at the present time.

DR. CROCKER: I'd like to go back to the issue about particle diameter which appears to be a point of some conflict. Stanton seems to make diameter and shape be the determining factor. Gross finds that does not apply. We do have two different structures, crystalline and amorphous. But we also have the capacity of each of those two structures to adsorb differently, a factor that may also participate in whatever biologic effect we get from either fiber, asbestos or man-made glass. It is possible that a difference other than size could account for the difference in biologic effect. The first issue is, is the biologic effect different? I gather that is in contention. Would either of you comment on your understanding as to the possible part played by the composition of material, amorphous versus crystalline, and adsorbed organic materials in comparing fibrous glass and asbestos?

DR. GROSS: First of all, all of my experiments with glass whether fibrous or not fibrous, have been negative. I have been unable to demonstrate any effect in the lungs other than a macrophage reaction. With inhaled asbestos, I have shown cancer of the lungs and severe fibrosis. An inflammatory reaction is demonstrable within a very short time after asbestos has been either injected or inhaled by the animal. I think that there is a clear cut difference in the effect of glass on the one hand and asbestos on the other. I have just come from a conference in Spain on silica. Dr. Eyler, who is a physical chemist, reported quite convincingly that whether a material is crystalline or amorphous played an important role in adsorption of organic molecules on the surface and that this adsorption is dependent upon regularity in the molecular structure of the material. Since crystalline material does have a regularity in the molecular and atomic arrangement, it will combine with and hold the organic molecules and change its character. That ability of a crystalline material to hang on and transform an organic molecule is considered to be the basic reason why quartz on one hand, or asbestos on the other hand, is capable of producing its biologic effect. Amorphous material has a surface which has no regular molecular arrangement, and its ability to similarly transform an organic molecule is much reduced.

DR. CULVER: Dr. Gross, the pleural plaques seen in asbestosis are located on the parietal pleura, and mesotheliomas are located on the visceral pleura. Is that true?

DR. GROSS: Yes, that is correct.

DR. CULVER: The production of pleural plaques has been postulated to be partially due to the abrasive action of the asbestos fiber on the surface of the lung. Is that correct?

DR. GROSS: The actual explanation is not known.

DR. CULVER: One would suspect that there must be a difference in the mechanism of production of pleural plaques and mesothelioma.

DR. GROSS: Yes, that difference is exemplified by the situation in Finland where pleural plaques are found in people who live near the anthophyllite mines and in Bulgaria where anthophyllite is found in soil. Many of the people in these areas have pleural plaques but no cases of mesothelioma have been reported in Finland. There is not a single case of mesothelioma in Finland even though there are many people who have pleural plaques.

DR. CULVER: I think those differences are important in the field of occupational medicine.

DR. BLAKESLEE (Ohio State University): Dr. Newman, with the membrane changes that you find in hamster embryo cells, is it possible that the concentrations of asbestos being used are causing a cytotoxic effect?

DR. NEWMAN (Ohio State University): In the first 24 to 48 hours, there is no significant reduction in the number of cells. Now, this was not in the conventional cytotoxic study in which the asbestos was introduced with the cells and then washed away and cell colonies counted. We saw no real change at 24 hours and 48 hours just counting cells in cultures that were carried in parallel with our other studies. At 72 hours, there was some reduction in the number of cells. In the case of fiberglass, it is possible that our population was somewhat heterogenous, and some of the effects could have been attributed to that. We are in the process of looking at a Detroit 550 culture, which is a homogenous preparation, in order to further validate our original observations.

DR. HENDERSON (Olin Corporation): Before it is forgotten, I'd like to reinforce Dr. Baetjer's comments on nutrition and the need to look at the nutritional status of employees in relation to occupational health. I've just been involved in doing a pilot survey of the nutritional status in 26 people in one of our plants. Their caloric intake ranged from 1000 to 4000, which ranges from approximately one-half to well above the recommended dietary allowance for women. Over 50% of the people surveyed were getting less than the recommended dietary allowance of Vitamin A. One person was getting 50% of the calories from

fat. The majority of the people were getting more than 35% of their calories from fat which is contrary to the American Heart Association's recommendation in diet. I could go on and on with the nutritional deficiencies shown up in just a pilot survey. If you are really going to pay attention to the total health of the employee, you should take a look at their nutritional habits.

DR. NEWTON (University of California, Irvine): While looking at the slides of fibrous dust particles this morning, I was reminded of a similar slide, which I saw recently, from which they were making fiber length measurements. However, when the same sample was looked at stereoptically, the fibers were found to be standing on end, apparently due to a static charge, and consequently, the length measurements were totally inaccurate. I'm not familiar with the methodologies used for making these samples, but I was wondering, could this be a source of error that could be the cause of a lot of controversy or concern in sizing fibers that seems to be going on in ths area of research?

MRS. HUBERT (Fairleigh Dickinson University): It may be a factor. However, we count and size a large number of fibers. In some cases, we may only examine 250 but it's better to examine at least 1000 fibers, and then we eliminate those that are not at least 3 times as long as their diameter. They are not really fibers. You want to be sure you are really only counting true fibers so that all lows would be eliminated. If the majority of the sample was cross sectionally oriented, then you really wouldn't have a representative sample. I should think you could see it.

DR. BERNSTEIN (Brookhaven National Laboratories): In response to that question, we found that if we looked at animal lungs exposed to fibers, in the histologic sections the fibers were cut up and no longer randomly orientated. The only way the fibers could be sized properly was to dissolve away the tissue and put the fibers on a planer surface and size them then.

MAJOR MAC NAUGHTON (Engineering and Services Laboratory): Has anybody conducted any research on the carbon fiber composites that are being used more and more these days?

DR. CULVER: The discussion document that I referred to this morning specifically excludes carbon fibers and boron fibers from the consideration of effects of man-made mineral fibers on the biological system.

DR. CULVER: Mrs. Hubert, are the mesotheliomas that you see in your intrapleural studies located on the visceral or parietal surface?

MRS. HUBERT: Most of them occupy a good part of the intrapleural space so it really would be hard to say. They sometimes even invade the rib cage. They are apt to start in the right costophrenic angle. The live hamster stands and walks mostly on its legs, and most

of the injected material is sitting there spread along the pleural surfaces. I was interested in that question when you asked it of Dr. Gross, but I'm really not sure that I could discern a difference. We've had some cases where we've had multiple sites. The example that I showed you was a papillary mesothelioma in a hamster injected with one milligram of crocidolite. It had little patches that looked almost like pleural plaques, but they looked different to me. So I collected some of these little bits that grossly looked like tumors to me and injected them into some other hamsters. The tissue transplants have grown, and we still maintain this was a transplantable mesothelioma.

TOXICOKINETICS OF INHALED GASES AND VAPORS

Chairman

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CLOSED ATMOSPHERE GAS UPTAKE STUDIES AND THEIR VALIDATION BY DIRECT METABOLITE DETERMINATION

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INTRODUCTION

Inhalation of vapors of industrially important chemicals represents a significant potential hazard of the work environment in both private industry and government. To adequately control workplace chemicals, their toxicological properties must be evaluated in appropriate animal studies and, based on the results of these studies, sound, defensible limits can be set for controlling vapor concentrations within these working environments. With many volatile chemicals, the primary toxic sequelae of exposure are caused by the metabolites formed and not by the parent chemical. In order to predict the amount of metabolite formed in any exposure, a complete toxicological evaluation of these materials should include estimation of the kinetic parameters for metabolite formation in vivo. This paper reviews our studies on the metabolism of inhaled 1,1-dichloroethylene[1] (1,1-DCE) in the rat (Andersen et al., 1979a,b) and the development of an indirect technique - referred to as gas uptake - for the determination of the kinetic constants of 1,1-DCE metabolism in vivo. In addition, a variety of experiments on the metabolism of inhaled halothane[2] (HAL, 2-bromo-2-chloro-1,1,1-trifluoroethane) are described. The studies with HAL

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[1]K&K Laboratories, Plainville, New York.
[2]Halocarbon Laboratories, Inc., Hackensack, New Jersey.

included both indirect determination of kinetic constants by gas uptake methods and their direct determination by quantitation of the amount of metabolite formed after a conventional 4-hour HAL inhalation exposure. This work, which is reported here for the first time, was undertaken to demonstrate the validity of the gas uptake method.

Certain basic aspects of the physiology of inhalation exposures bear review before describing our results with 1,1-DCE and HAL. Following exposure to a chemical with a low blood:air partition coefficient, equilibrium between the gas and the blood (and richly perfused tissues) is quickly achieved. The circulating concentration during the course of exposure is determined by the partition coefficient and the concentration in the atmosphere. Then at the end of exposure, blood levels fall rapidly, as long as the chemical is not stored to any great extent in secondary depots. This inhalation exposure regimen with low solubility materials produces at the organ the equivalent of a step infusion. The duration of maintenance of constant blood concentration is equal to the duration of exposure, and the blood concentration is proportional to the atmospheric concentration. For chemicals of only moderate lipid solubility and high volatility, the amount of metabolite formed during exposure is assumed to be very large compared to the amount formed after cessation of exposure. The rate of metabolite formation during exposure is approximated by the Michaelis-Menten equation:

$$\text{rate} = \frac{V_{max} \cdot ppm}{K_m + ppm},$$

where V_{max} is the maximum rate of metabolism and K_m, the apparent inhalation Michaelis constant, is that atmospheric concentration of material at which metabolism proceeds at half maximum. As with any enzymatically catalyzed reactions in vivo, the rate will become pseudo-zero order at sufficiently high concentrations. The amount of metabolite produced under these conditions will be independent of concentration and depend instead on the duration of the reaction. Because the amount of toxic metabolite produced will no longer be proportional to concentration at sufficiently high concentrations, saturable metabolism may be reflected in vivo by the existence of unconventionally shaped dose-response curves (Gehring et al., 1978; Andersen et al., 1979a).

METHODS

ANIMALS

The gas uptake studies of 1,1-DCE utilized fasted male Holtzman rats, strain designation HOT:(SD)BR, weighing between 200 and 300 grams. The rats were fasted overnight and exposed in groups of 6 or 9. During all exposures, rats did not have access to either food or

water. Rats for gas uptake were returned to commercial rat chow and water ad libitum immediately following exposure. For the gas uptake and bromide production studies of HAL, fed male Fischer (F-344) rats were used weighing between 200 and 300 grams. The rats for gas uptake were exposed in groups of 9, and for production of bromide, in groups of 4 to 9. Rats for plasma bromide determinations were sacrificed immediately after exposure for the collection of plasma samples.

GAS UPTAKE EXPOSURE SYSTEM

Exposures were conducted in 31-liter battery jar chambers (Figure 1), modified from the original apparatus described by Leach (1963). Water vapor was removed by means of a cold water trap maintained at 4°C and CO_2 was removed with Baralyme[1]. Oxygen was constantly monitored and maintained between 19 and 21% with gaseous oxygen. A diaphragm pump was used to recirculate the atmosphere at a flow rate of 9 liters/minute. The pump was fitted with needle valves at both intake and exhaust to regulate flow rate and indirectly control chamber pressure. A T-fitting with septum immediately up-stream of the chambers used for the introduction of toxicant was fitted with heating tape for facilitating vaporization. At the initial set-up and following reassembly after cleaning, the chamber was pressurized to 10 inches of water and all connections checked for outward leaks with a detergent solution. The total volume of the system was deter-mined by first making standards in aluminized Mylar® bags. Known volumes of liquid 1,1-DCE were injected into the bag with 30 liters of air that was measured through a wet test meter. Atmospheres from the standard bags were analyzed with a gas chromatograph equipped with a hydrogen flame ionization detector and a gas sampling valve with 0.5 ml sampling loop. Flow through the sampling loop was ∿ 28 ml/minute. The total system volume was estimated by injecting known volumes of 1,1-DCE into the empty chamber and extrapolating the curve of observed chamber concentration back to zero time. Based on the mass of 1,1-DCE injected and the resulting zero time concentration, the total volume of the entire system was estimated at 32 liters. For both 1,1-DCE and HAL, a 30 foot, 1/8 inch stainless steel column with DC-200 as the stationary phase was used[2]. Injection temperature was 250°C, flame ionization temperature was 300°C, oven temperatures were 90°C for 1,1-DCE and 85°C for HAL, carrier flow (N_2) was 33 ml/minute. Under these condi-tions, the retention time for 1,1-DCE was 4.8 minutes and for HAL, it was 5.5 minutes. For the animal exposures, measured amounts of liquid 1,1-DCE or HAL were injected through the septum in the T-fitting. Samples were taken 5 minutes after injection and every 10 minutes thereafter. At each sampling, chamber pressure and O_2 concentration were recorded and the O_2 flow or pump output valve adjusted accord-ingly. The chamber pressure was maintained at a negative 1-3 inches of water.

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[1]Chemetron Medical Products Division, Stuyvesant Falls, New York.
[2]Analabs "Hi Plates," high efficiency packed column, Analabs, Inc., New Haven, Connecticut.
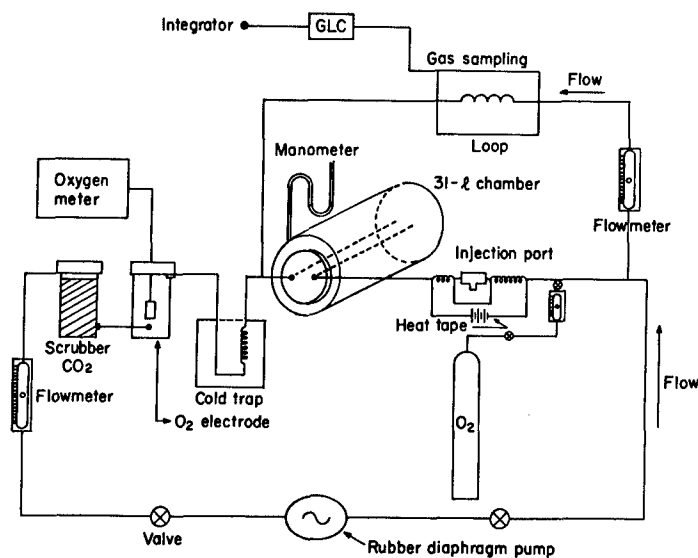
Figure 1. Schematic of the closed recirculating atmospheric chamber system used in gas uptake studies.

CONVENTIONAL EXPOSURE SYSTEM

For constant concentration exposures to HAL, a 31-liter battery jar chamber was used (Leach, 1963). A 9.5-liter stainless steel sampling cylinder with valves on both ends was charged with 100 ml of liquid HAL and then pressurized with N_2 to 72 psig. A low pressure regulator was attached to one end of the cylinder and HAL-containing gas was bled off through the regulator, monitored with a flowmeter, and mixed with chamber input air from a diaphragm pump flowing at 9 liters/minute. On the effluent side of the chamber, a second diaphragm pump was used to maintain a slightly negative chamber pressure (1-2 inches of H_2O). During animal exposures, samples were collected in 30-liter Mylar® bags every 30 minutes and analyzed by gas chromatography under the conditions described. Exposures were carried out at various concentrations for 4 hours.

PLASMA PREPARATION AND BROMIDE DETERMINATIONS

Immediately following the conventional 4-hour exposure to HAL, the test and control rats were killed with an intraperitoneal injection of a lethal dose of sodium pentobarbital, the abdomen was rapidly opened, and the rat exsanguinated by withdrawing blood from the portal vein into a heparanized syringe. The plasma was harvested by two separate 10 minute centrifugations at 4000 rpm and then decanted to a clean glass tube. Bromide determinations were carried out using a bromide specific electrode, reference electrode and Ionalyzer[1]. Our procedure followed methods supplied by the manufacturer with slight modifications. These included a 1:10 dilution of the plasma with 10% trichloroacetic acid to produce a protein-free filtrate then subsequent 1:10 dilution with deionized H_2O to obtain a sufficient volume for measurement. All standard solutions used in the procedure were appropriately balanced with 10% trichloroacetic acid and sodium chloride solution before analyses. This compensated for interference due to chloride ion and trichloroacetic acid. The measured levels are expressed as mean group values, reported as millimoles of plasma inorganic Br^- produced per liter per 4 hours minus the corresponding mean control values (ΔBr^- as mM). Bromide ion concentrations in control plasma samples were between 0.067 and 0.128 mM, with an average value of 0.110 mM.

RESULTS AND DISCUSSION

REVIEW OF RESULTS ON GAS UPTAKE OF 1,1-DCE

Approximately three years ago, our laboratory at the Naval Medical Research Institute became interested in developing convenient methods for estimating the rate of metabolism of inhaled chemicals. At that time, we were studying the toxicity of inhaled 1,1-dichloroethylene (1,1-DCE). The concentration-response curves for mortality (Figure 2) were unconventional, appearing to be concentration-independent above 200 ppm. Each point on the curve was obtained by exposing a group of six rats to a particular concentration of 1,1-DCE for 4 hours, and then noting the observed percent mortality at each concentration. There was a rapid increase in percent mortality between 100 and 200 ppm. But, above 200 ppm, the curve leveled off abruptly and percent mortality remained constant over a wide range of concentrations. With this steep increase, and the abrupt and persistent plateau, it was essentially impossible to estimate an accurate LC_{50}. Based on these data (Andersen et al., 1979a) as well as a variety of other experimental results (Andersen and Jenkins, 1977), it seemed that the toxicity of 1,1-DCE must be caused by metabolites and not by 1,1-DCE itself. The question faced then was to quantitate the kinetics of metabolism in vivo in order to relate observed mortality with the amount metabolized instead of with the exposure concentration. Since the presumed expoxide

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[1]Orion Research, Inc., Cambridge, Massachusetts.

metabolite formed in the oxidation of 1,1-DCE is extremely reactive and short-lived (Greim et al., 1975; Gay et al., 1976; Hull et al., 1973), it would have been technically difficult and very time consuming to develop an in vitro method. In addition, the constants determined in vitro would not necessarily provide information relevant to the in vivo conditions. Previous work had been done estimating the rates of metabolism of vinyl chloride monomer by gas uptake from a recirculating atmosphere (Hefner et al., 1975; Bolt et al., 1977). It was our intention to measure rates of gas uptake for 1,1-DCE as a function of its concentration and from these data to determine the kinetic constants, Km (as atmospheric ppm) and V_{max} (the maximum rate of metabolism), for 1,1-DCE metabolism in the rat. The disappearance of 1,1-DCE from an unoccupied chamber was accurately represented by a single exponential function whose rate constant was essentially independent of concentration and varied between 0.088 hr^{-1} and 0.092 hr^{-1}. The rate constant was unchanged after dismantling, cleaning, and reassembling the chamber. In exposures involving live animals, the observed data were corrected on a point to point basis by subtracting the contribution of this non-specific "loss" rate. A typical uptake curve with point to point corrections is shown in Figure 3. Rats in groups of six were exposed to various initial concentrations of 1,1-DCE ranging from 10 to 2000 ppm. The corrected curves were markedly biphasic, containing a rapid phase that was complete in 60 to 80 minutes and a slow phase that was nearly linear after this time. Pretreatment of the rats with microsomal inhibitors essentially abolished the slow phase uptake. Under these conditions, the rapid phase was studied without significant interference from the slow phase. The contribution of the rapid phase was calculated by subtracting the corrected data points from the extrapolated line along the slow phase at the same time (Figure 4). The rate constant of the rapid phase was independent of chamber concentration and unaltered by pretreatment with inhibitors of microsomal function. The rate constant for the rapid phase was 2.2 hr^{-1}. While the rate constant was independent of the number of animals, the extent of its contribution to the overall reaction was dependent on the number of animals in the chamber. These characteristics suggested that the rapid phase was due to tissue equilibration. The slow phase rate constant in naive rats diminished with an increase in concentration and increased with the number of animals present in the chamber. A plot of the percent of initial concentration remaining with respect to time (Figure 5) shows a decrease in the rate constant as concentration increases. These results were consistent in suggesting that the slow phase was due to metabolism of 1,1-DCE and that the reaction involved was saturable. Eight separate gas uptake curves were obtained at various initial concentrations. A tangent to the corrected curve was taken at 60 to 80 minutes and the rate of decrease, in ppm per kilogram per hour, was estimated. A plot of the instantaneous rate of uptake versus concentration was constructed (Figure 6). The points were represented accurately by a rectangular hyperbola, again supporting the assumption that the rate was due to metabolism with a saturable
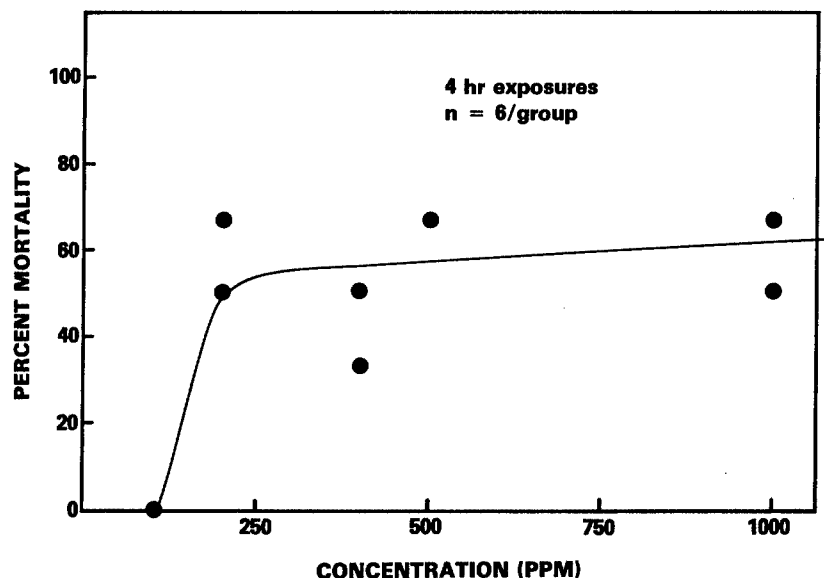
Figure 2. Effect of increasing concentration of 1,1-dichloroethylene in mature male rats. Each point represents percentage mortality of a group of six animals exposed for four hours. (Reprinted by permission of Academic Press, Inc., from Andersen et al., 1979a).
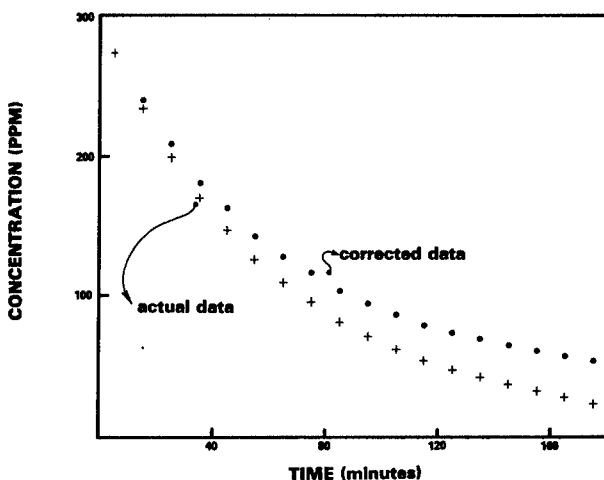
Figure 3. Typical uptake curve of 1,1-dichloroethylene by 9 control rats. Total rat weight was 2.33 kg. (Reprinted by permission of Academic Press, Inc., from Andersen et al., 1979b).

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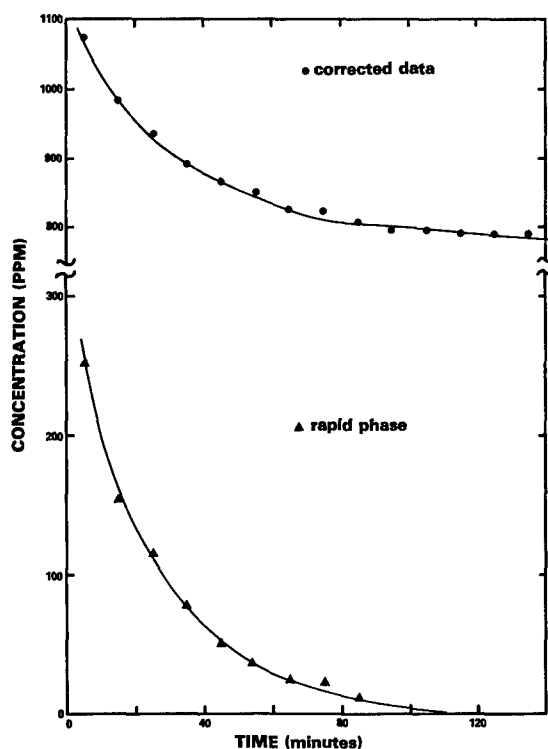
Figure 4. Uptake of 1,1-dichloroethylene by male rats pretreated with pyrazole. Nine rats of total weight 2.12 kg were used 1 hour after they received 320 mg of pyrazole/kg i.p. The rapid phase, $k = 2.2$ hr^{-1} was determined by subtracting the extrapolated slow phase from the corrected data points. (Reprinted by permission of Academic Press, Inc., from Andersen et al., 1979b).
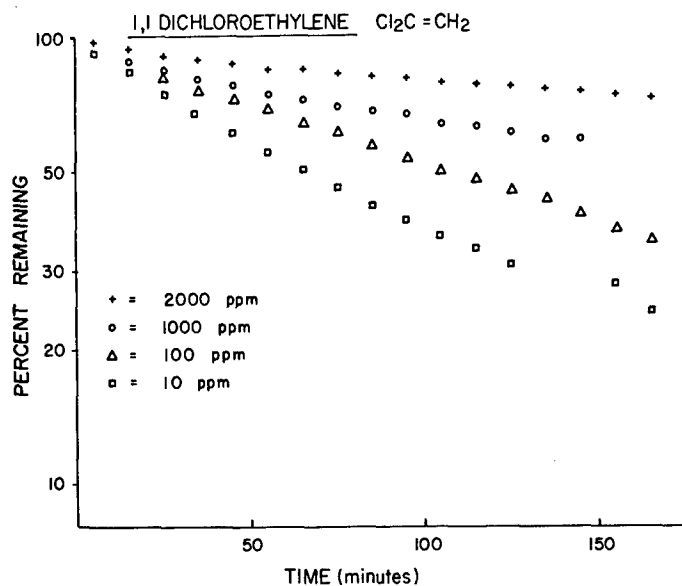
Figure 5. Uptake of 1,1-dichloroethylene by male rats at four concentrations. Plotted data were derived from corrected uptake curves and expressed as percent initial concentration remaining at each sampling time. Six rats were used in each exposure and the plot is semi-logarithmic.

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dependence. By linearizing the data using a modified Eadie-Hofstee plot (Laidler and Bunting, 1973), the maximum rate of metabolism was determined to be 132 ppm/kg/hr (15.87 mg/kg/hr) with an apparent inhalation Michaelis constant of 335 ppm. The smooth curve shown in Figure 6 represents an unweighted least squares ($r^2 = 0.988$) best-fit Michaelis-Menten curve. As described earlier, it had been impossible to derive a meaningful estimate of the LC_{50} from the concentration-response curves of 1,1-DCE because of the rapid increase in mortality between 100 and 200 ppm and the plateau that existed between 200 and 1000 ppm. Even though it was not possible to determine a 4-hour LC_{50} for 1,1-DCE, it was possible to determine LT_{50}s at various concentrations (Table 1). An LT_{50} is the time required to kill half an exposed population at a constant concentration. Using the LT_{50} of 4.1 hr at 200 ppm as a reference point, the expected LT_{50}, assuming a concentration x time dependence, would have a 5-fold increase from 1000 to 200 ppm. This expectation did not correlate well with the observed LT_{50} data that showed less than a 2-fold increase over the same concentration range. By utilizing our estimated kinetic constants of 1,1-DCE metabolism and the Michaelis-Menten equation:

$$\text{rate} = \frac{V_{max} \cdot ppm}{Km + ppm},$$

we were able to calculate the amount of metabolite formed at each concentration and its corresponding LT_{50}. The calculation was accomplished by multiplying our estimated V_{max} of 15.9 mg/kg/hr times the LT_{50} concentration and dividing this product by the sum of our estimated Km of 335 ppm plus the LT_{50} concentration. This rate, as mg per kg per hr, was then multiplied by the corresponding LT_{50} to arrive at the estimated amount of metabolite formed during the exposure, which is expressed as mg per kg. While the LT_{50} varied by almost a factor of two between 200 and 1000 ppm, the estimated amount of metabolite formed over this range of concentration was found to be essentially constant, only ranging between 24 to 30 mg/kg (Table 2). This suggested that the toxicity was not dependent on concentration, but instead on the amount of metabolite formed during an exposure.

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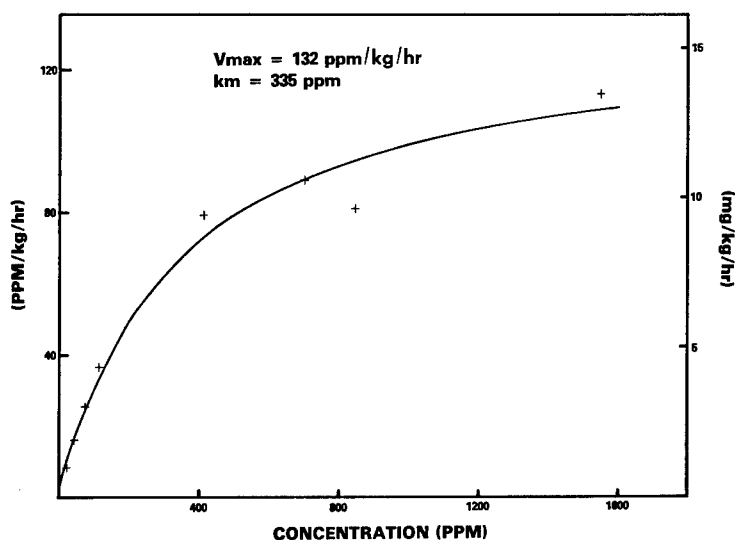
Figure 6. Dependence of the slow phase rate on 1,1-dichloroethylene concentration. The smooth curve is the best-fit Michaelis-Menten rectangular hyperbola. (Reprinted by permission of Academic Press, Inc., from Andersen et al., 1979b).

TABLE 1. DURATION OF 1,1-DICHLOROETHYLENE EXPOSURE
REQUIRED TO KILL HALF THE EXPOSED POPULATION
(LT_{50}) OF MATURE MALE RATS

1,1-DCE Concentration (ppm)	LT_{50} (hr) (95% C.I.)	Expected LT_{50}*
100	>8 hr**	--
200	4.1 (4.5-3.7)	4.10
400	3.6 (4.3-3.0)	2.05
500	3.0 (3.8-2.3)	1.64
1000	2.4 (3.2-1.7)	0.82

*Expected LT_{50} assuming C x t = k is valid and using the 4.1 hr LT_{50} at 200 ppm as the basis for calculation.

**Mortality in an 8-hour exposure to 100 ppm was 1/9.

TABLE 2. THE THEORETICAL AMOUNT OF METABOLITE FORMED DURING EXPOSURE OF RATS TO 1,1-DICHLOROETHYLENE FOR TIMES EQUAL TO THE LT_{50} AT VARIOUS CONCENTRATIONS

Concentration (ppm)	LT_{50} (95% Confidence Interval) (hr)	Metabolite Formed (mg/kg)
200	4.1 (4.5-3.7)	24.2 (26.5-21.9)
400	3.6 (4.3-3.0)	30.0 (36.0-24.9)
500	3.0 (3.8-2.3)	26.6 (34.1-20.0)
1000	2.4 (3.2-1.7)	28.1 (37.5-19.9)

HAL STUDY

Since animal gas uptake determinations are indirect and only infer metabolism, it was necessary to validate this technique by a more direct method. To do this, we studied the metabolism of an organic bromide and estimated the kinetic constants by both gas uptake and inorganic bromide. Halothane (HAL) was chosen for this study. HAL is metabolized to inorganic bromide which is retained in the extracellular fluids. Inorganic bromide has a volume of distribution of 0.26 liters/kg in the rat (Woodbury, 1966), and it is slowly excreted with a $t^{\frac{1}{2}}$ of 2.8 days (Gargas et al., 1979). The rate of metabolism of HAL can be estimated by calculating the rate from the endpoint determination of plasma inorganic bromide produced during a conventional exposure.

GAS UPTAKE RESULTS OF HAL

The rate constant of the nonspecific "loss" rate of HAL in an unoccupied chamber varied between 0.087 hr^{-1} and 0.100 hr^{-1}. A loss rate was determined prior to each individual animal run and was used in correcting the observed data. Rats in groups of 9 were exposed to initial concentrations of HAL ranging from 30 to 3000 ppm. The uptake curves were similar in appearance to those obtained with 1,1-DCE (Figure 3), possessing both a rapid and slow phase. Plots of the percent of initial concentration remaining with respect to time (Figure 7) were similar to the percent remaining plots of 1,1-DCE. The rate constant decreased with increasing concentration. The equilibration (fast) phase was essentially complete in 75 to 90 minutes. A tangent to the corrected curve was taken at 75 to 90 minutes and the rate was estimated as ppm/kg/hr. As with 1,1-DCE, an instantaneous rate versus concentration plot was constructed (Figure 8). The points were best represented by a complex dependence, containing contributions from both a saturable component and an apparent first order component. Since HAL is known to be taken up in poorly perfused tissues (Holaday, 1977), it was assumed the apparent first order component was not due to metabolism. An unweighted least squares line ($r^2 = 0.95$) was fitted (Figure 8) using the points obtained above 200 ppm from which the first-order rate constant was determined to be 0.051 kg^{-1} hr^{-1}. The contribution of this

apparent first order process was calculated at each data point and subtracted from the observed rate. The corrected rate was then plotted against concentration (Figure 9). The best fit straight line ($r^2 = 0.97$) to the modified Eadie-Hofstee plot had a Km of 81 ppm and a V_{max} of 36 ppm/kg/hr (8.9 mg/kg/hr).
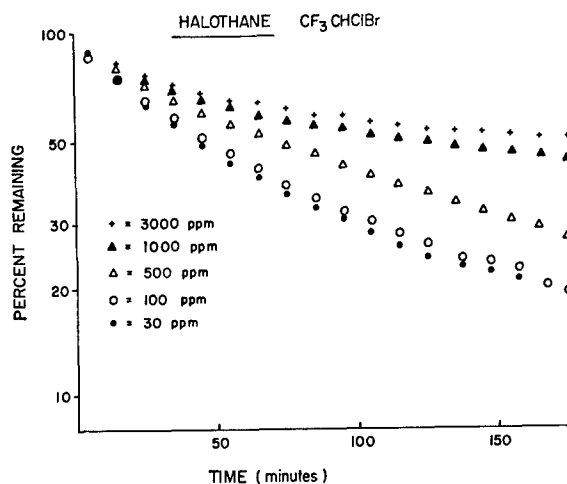
Figure 7. Uptake of halothane by male rats at 5 initial concentrations. Plotted data were derived from corrected uptake curves and expressed as percent initial concentration remaining at each sampling time. Nine rats were used in each exposure and the plot is semi-logarithmic.
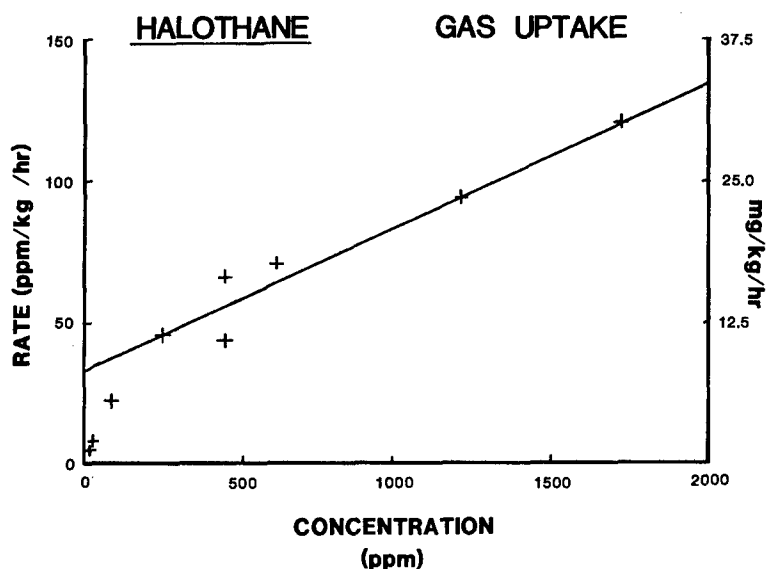
Figure 8. Dependence of the rate of uptake of halothane on its ambient concentration. The best-fit least squares ($r^2 = 0.95$) line represents the contribution to the overall reaction of the first order component with a rate constant of 0.051 kg^{-1} hr^{-1}.

HALOTHANE GAS UPTAKE

ESTIMATED $K_m = 81$ ppm
ESTIMATED $V_{max} = 36$ ppm kg^{-1}hr^{-1}
(8.9 mg kg^{-1}hr^{-1})
$r^2 = 0.97$
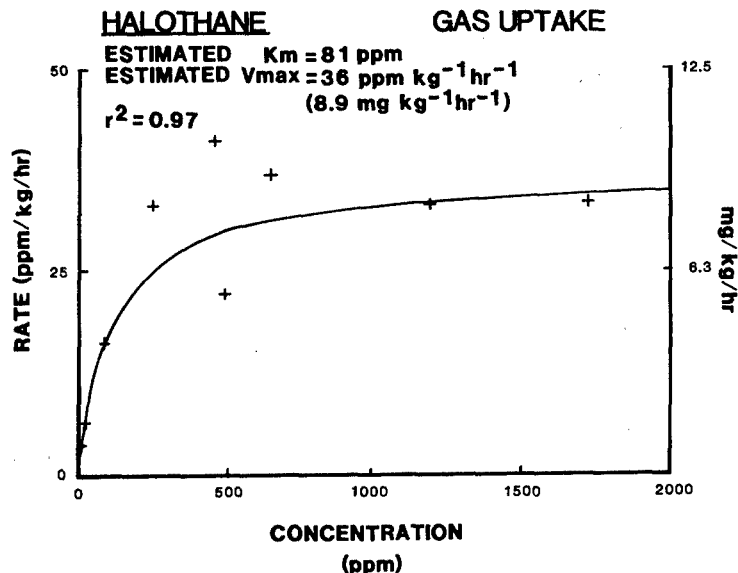
Figure 9. Dependence of the corrected rate of uptake of halothane on its ambient concentration. The data points were corrected by calculating the contribution of the first order phase at each point and subtracting this contribution from the observed data. The smooth curve represents the best-fit Michaelis-Menten rectangular hyperbola.

BROMIDE PRODUCTION DURING CONVENTIONAL EXPOSURE

Rats in groups of 4 to 9 were exposed to various concentrations of HAL for 4 hours. Nine separate concentrations were utilized ranging from 25 to 1666 ppm. Plasma inorganic bromide levels were determined and mean group values of ΔBr$^-$ (Test Br$^-$ minus Control Br$^-$ in millimoles per liter per 4 hours) were calculated and plotted against concentration (Figure 10). The data points were accurately described by a rectangular hyperbola consistent with a saturable Michaelis-Menten dependence. It should be noted here that no first order process was apparent at high concentrations as found with the gas uptake results. This supported the assumption that the first order process associated with gas uptake was not due to metabolism, but with tissue loading. In any event, no evidence of inorganic Br$^-$ production following first order kinetics at elevated concentrations was found. The data were once again linearized by a modified Eadie-Hofstee plot ($r^2 = 0.99$) and the best fit Michaelis-Menten curve constructed having a K_m of 73 ppm and a V_{max} of 0.7576 millimoles Br$^-$/liter/4 hr (Figure 10). This corresponds to a maximum rate of metabolism for HAL of 9.7 mg/kg/hr.

HALOTHANE   Br- PRODUCTION

ESTIMATED   Km = 73 ppm
ESTIMATED Vmax= 9.72 mg kg^{-1}hr^{-1}
r^2=0.99
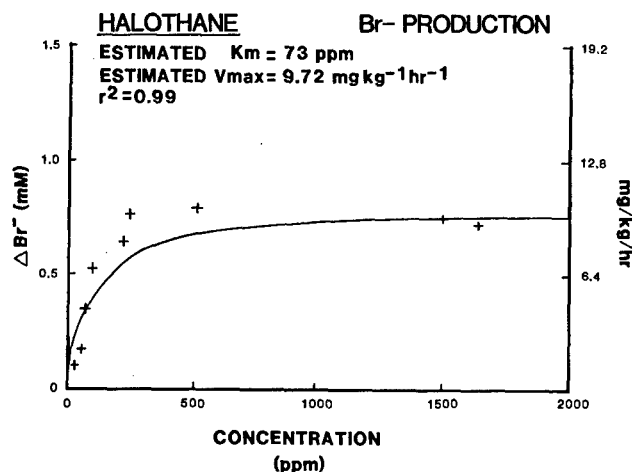
CONCENTRATION
(ppm)

Figure 10.   Dependence
of the rate of production
of plasma inorganic
bromide of halothane on the
ambient concentration after
a 4-hour exposure.   Smooth
line   represents best-fit
Michaelis-Menten rectangular
hyperbola.

CALCULATED AND OBSERVED RATES OF METABOLISM FOR HALOTHANE

The amount of plasma inorganic bromide released was determined as
a function of duration of exposure by exposing groups of 4 animals to 200
ppm of HAL for 1, 2, and 4 hours and performing endpoint plasma bromide
determinations.   The amount of bromide produced (ΔBr$^-$) was plotted
against the appropriate time of exposure (Figure 11).   A best fit line was
constructed (r^2 = 0.98) with a resulting slope of 0.1567 millimoles/liter/
hour.   To express the observed rate of production in consistent terms,
this slope was multiplied by the volume of distribution of bromide in the
rat (0.26 liters/kg) and then by the molecular weight of HAL (197.4
mg/millimole).   The observed rate for the metabolism of HAL at 200 ppm
was determined to be 8.0 mg/kg/hr.   A theoretical determination of the
expected rate of metabolism at 200 ppm can be calculated from estimated
inhalation kinetic constants (Km and V_{max}) and the Michaelis-Menten form:

$$\text{rate} = \frac{(V_{max}) \cdot (ppm)}{(Km) + (ppm)}$$

Based on the estimated kinetic constants, the calculated rates of metabo-
lism of HAL from gas uptake and bromide production studies were 6.3 and
7.1 mg/kg/hr, respectively.   A comparison of the kinetic constants and
rates of metabolism for HAL is summarized in Table 3.   This comparison
shows excellent correlation between results obtained by indirect gas uptake
techniques and direct measurements of plasma bromide production.
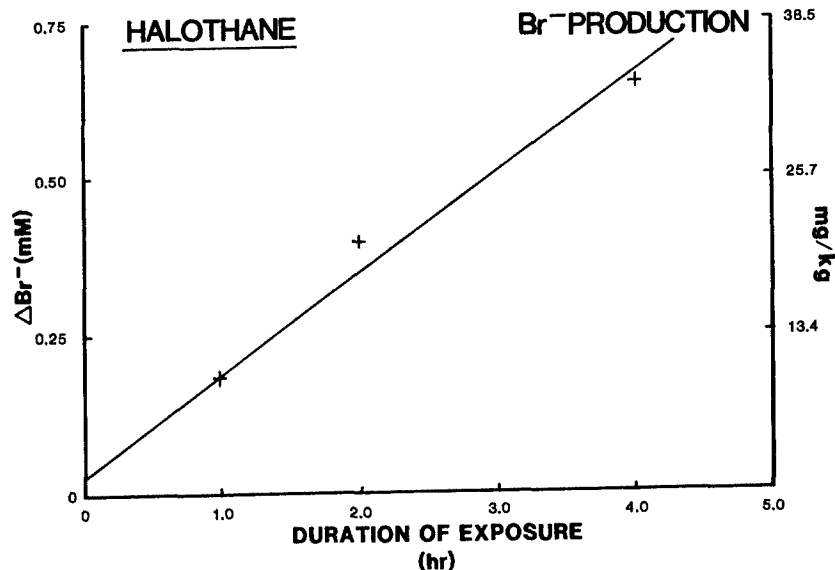
Figure 11. The observed production of plasma inorganic bromide of halothane after exposure to 200 ppm for various times. The line represents a best-fit least squares line ($r^2 = 0.98$) with a slope of 8.0 mg/kg/hr.

TABLE 3.   HALOTHANE RESULTS

Estimated Kinetic Constants

Method	Km (ppm)	V_{max} (mg kg^{-1} hr^{-1})
Gas Uptake	81	8.9
Br^- Production	73	9.7

Calculated and Observed Metabolism of HAL at 200 ppm

Calculated by Gas Uptake (mg kg^{-1} hr^{-1})	Calculated by Br^- Production (mg kg^{-1} hr^{-1})	Observed HAL Metabolism (mg kg^{-1} hr^{-1})
6.3	7.1	8.0

METHODOLOGICAL CONSIDERATIONS

Gas uptake methods were developed to assess the kinetic constants of 1,1-DCE metabolism in vivo. They are convenient and straightforward and can readily be applied to a variety of volatile chemicals with partition

coefficients in the range of 0.5 to 20. Besides HAL and 1,1-DCE, the kinetic constants for metabolism of many other chemicals have been determined in our laboratories using these techniques. The chemicals include benzene, methyl bromide, vinylidene bromide, bromochloromethane, methyl chloride, trans-1,2-dichloroethylene, trichloroethylene, vinyl methyl ether, vinyl bromide, ethylene (Andersen et al., 1979c), cyclohexane, and methylcyclohexane (Woods and Andersen, unpublished work, 1979). Filser and Bolt (1979) have studied the uptake and metabolism of 9 halogenated ethylenes using these methods. Gas uptake studies will not be useful with chemicals which have a very small blood: air partition coefficient or for chemicals for which V_{max} is very small. For these materials, the amount of uptake is always small with respect to the amount of chemical in the atmospheric reservoir. Under these conditions, uptake rates will be small and difficult to distinguish from nonspecific "loss" from the chamber. The technique will also be of little value for chemicals with very large blood: air partition coefficients. For these materials, it will be impossible to establish equilibrium between the atmosphere and richly perfused tissues during short duration exposures. Despite these admitted limitations, there remains a large number of chemicals whose metabolism can be investigated by gas uptake methods.

Gas uptake is an indirect measure of chemical metabolism. It assumes that uptake which follows saturation kinetics represents uptake driven by metabolism of the inhalant. We have studied bromide release from a variety of organic bromine containing chemicals - HAL (as described here), methyl bromide, vinyl bromide, and bromochloromethane (Gargas et al., 1979). The kinetic curves for Br^- release for these 4 chemicals demonstrated diverse rate dependencies. With HAL, the rate dependence was described by a single, saturable component (Figure 10), while with vinyl bromide a complete description required two saturable components with widely separated values of Km. The rate curve for methyl bromide was essentially first-order, but that for bromochloromethane was complex, possessing both first-order and saturable components. In all cases, the estimations of the kinetic constants by gas uptake were accurately confirmed by determination of circulating bromide concentrations at the cessation of exposure. For vinyl chloride, the kinetic constants have been determined by measuring the total amount of radioactive metabolites formed from ^{14}C-VCM after 6-hour exposures to various concentrations of VCM (Gehring et al., 1978). Filser and Bolt (1979) also estimated them by gas uptake. These two independent estimates of the inhalation Km agreed to within a factor of 2. McKenna et al. (1978) have determined the total amount of ^{14}C-1,1-DCE metabolized in various inhalation exposures. The inhalation Km estimated from these data was essentially identical with the Km derived from our gas uptake work (M. J. McKenna, personal communication, 1979). Calculated inhalation Km values are useful for determining the concentrations of toxicant at which metabolism proceeds at given fractions of V_{max}. They are not to be directly correlated with the biochemical Km, which is that concentration of substrate at the enzymatic active site which

causes the reaction to proceed at half maximum velocity. All that can be said is that the concentration at the enzymatic active site approximates the biochemical Km when the concentration of toxicant in the atmosphere is equal to the inhalation Km.

Presently, the most valuable alternative to gas uptake is determination of the kinetic constants by quantitation of the total amount of ^{14}C-metabolite produced from a parent compound during exposure. Most toxicology laboratories are not equipped to undertake such a study. In contrast, very little equipment other than that normally used for acute inhalation toxicity evaluations is required to conduct gas uptake studies. This equipment is available in most laboratories involved in toxicity evaluations of volatile chemicals. In addition, this method is rapid; 10 to 15 data points can be collected over a 2-week period. For accurate determination of both the shape of the rate curve (such as Figure 8) and the kinetic constants, it is important to have a liberal number of data points scattered relatively uniformly over the accessible concentration range. Reliance on only three or four data points and rigid specification of a model of metabolism can lead to misleading conclusions regarding the kinetic constants of metabolism. Based on such limited data, it would be extremely difficult to predict correctly the shape of the overall rate curve. The results with the various bromide containing organics demonstrated clearly the variety of curves which might be expected. Nonetheless, for chemicals with a very small V_{max}, determination of the amount of ^{14}C-metabolite produced during exposure is the only technically feasible approach for estimating kinetic constants. Regardless of the experimental demands of the procedure used to assess metabolism, sufficient data points must be collected to define with confidence the concentration dependence of the rate of metabolism.

An interesting potential application of gas uptake methods is in determination of the interdependence of the metabolic pathways for various volatile chemicals. Data have been reported, for instance, showing that exposure of rats to CCl_4 and 2,2-DCE, simultaneously, can essentially block production of the toxic metabolites of 1,1-DCE at sufficiently high concentrations of CCl_4 (Andersen et al., 1979b). These types of data are necessary to determine the hazards associated with exposure to mixed atmospheres. The presently accepted method for evaluating the TLV of a mixed atmosphere, where the contaminants have the same target organ, is to assume additivity of effect (American Conference of Governmental Industrial Hygienists, 1978). Additivity is not necessarily expected if the materials are metabolized to toxic agents along the same pathways and can inhibit each other's metabolism. To assess the hazard arising from exposure to a mixed atmosphere, quantitative knowledge of the kinetic constants of metabolism is required.

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DETERMINATION OF KINETIC CONSTANTS
FROM PULMONARY UPTAKE

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The adverse biological effect of an air pollutant is related to its concentration and/or time integral of concentration in the target organ. The passage of pollutant from the environment to the target organ is a dynamic process, determined by the physical and chemical properties of the pollutant and by the physiological parameters of the exposed subject (Eger, 1963). Inhaled, nonwater soluble vapors are removed from the body by pulmonary and metabolic clearance. Since pulmonary clearance takes place only after the end of exposure (or when exposure concentration decreases), the vapor is removed during exposure only by metabolic clearance (Fiserova-Bergerova et al., 1974).

Metabolic clearance in vivo is usually defined by the half-time of disappearance of the xenobiotic from plasma after bolus administration, or as the half-time of urinary excretion of xenobiotic metabolites. The determination of plasma clearance requires frequent blood sampling, which imposes stress on subjects. The urinary excretion of metabolites is affected by a variety of factors, such as distribution of metabolites in the body, binding, and renal clearance.

Inhalation administration makes possible a noninvasive, accurate measurement of the rate of overall metabolism of inhaled vapors (Teisinger and Soucek, 1952).

The pulmonary uptake rate, $\dot{u}$, is the sum of retention rates of vapor in tissues, $\dot{u}_{tis}$, and the rate of overall metabolism, $\dot{u}_m$:

$$\dot{u} = \dot{u}_{tis} + \dot{u}_m \tag{1}$$

The pulmonary uptake can be determined from the difference between vapor concentrations in inhaled and mixed-exhaled air multiplied by minute ventilation:

$$\dot{u} = (C_{inh} - C_{exh}) \, \dot{V} \tag{2}$$

where C_{inh} and C_{exh} are vapor concentrations (mg/liter) in inhaled air and in mixed-exhaled air, and $\dot{V}$ is minute ventilation (liter/minute).

We have presented a compartmental model which can be used to determine the retention rate of inhaled vapor in tissues. Using three compartments (VRG - vessel rich tissues, MG - muscles and skin, FG - fat and fat marrow), the retention rate:

$$\dot{u}_{tis} = C_{alv} \left[F_{VRG} \lambda_{bl/air} \ exp \left(- \frac{F_{VRG}}{V_{VRG} \lambda_{VRG/bl}} \ t \right) \right.$$

$$+ F_{MG} \lambda_{bl/air} \ exp \left(- \frac{F_{MG}}{V_{MG} \lambda_{MG/bl}} \ t \right) \qquad (3)$$

$$\left. + F_{FG} \lambda_{bl/air} \ exp \left(- \frac{F_{FG}}{V_{FG} \lambda_{FG/bl}} \ t \right) \right]$$

where F's are blood flows (liter/minute) through the compartments, V's (1) are their volume. λ's are the corresponding partition coefficients of inhaled vapor at 37°C, C_{alv} is the vapor concentration (mg/liter) in alveolar air, and exp is the natural logarithm.

Substituting from Equations 2 and 3 in Equation 1, the rate of overall metabolism can be determined. Since the retention of vapor in tissues is an exponential function, the retention rate diminishes with exposure duration. This determination is most accurate during apparent steady state, when retention by tissues is small ($\dot{u}_m \gg \dot{u}_{tis}$).

We demonstrated the effect of metabolism on pulmonary uptake in an informed volunteer patient who was anesthetized with fluroxene ($CH_2{:}CH.O.CH_2.CF_3$) in the presence of a small concentration of non-metabolized isoflurane ($CHF_2.).CHCl.CF_3$) (Fiserova-Bergerova and Holaday, 1979). The amount of metabolites accounts for less than 1% of isoflurane uptake (Holaday et al., 1975), and about 45% of fluroxene uptake (Gion et al., 1974).

During anesthesia, samples of inhaled gas and end-exhaled gas were drawn during the appropriate phase of respiration via a nylon cannula inserted in the endotracheal tube at 4 to 10 minute intervals. Mixed-exhaled gas was obtained at the same time at the outlet of a mixing chamber interposed in the expiratory breathing tube. Gas samples were collected in 20 ml glass syringes. At the same time,

minute ventilation was measured with a Wright respirometer. The cumulative uptake D was determined: (1) from the amount of anesthetics delivered by syringe in the closed anesthetic circuit, and (2) from the sum of the differences between concentrations of inhaled and mixed-exhaled air multiplied by minute ventilation, and time intervals between sampling (t in minutes)

$$D = \Sigma \, \dot{V} \, t \, (C_{inh} - C_{exh}) \qquad (4)$$

The cumulative uptake predicted by integration of Equation 3 as retention in tissues correlates with the measured uptake of isoflurane (calculated by Equation 4), but the measured fluroxene uptake greatly exceeds the calculated fluroxene retention in tissues (Figure 1). The difference accounts for fluroxene metabolism.
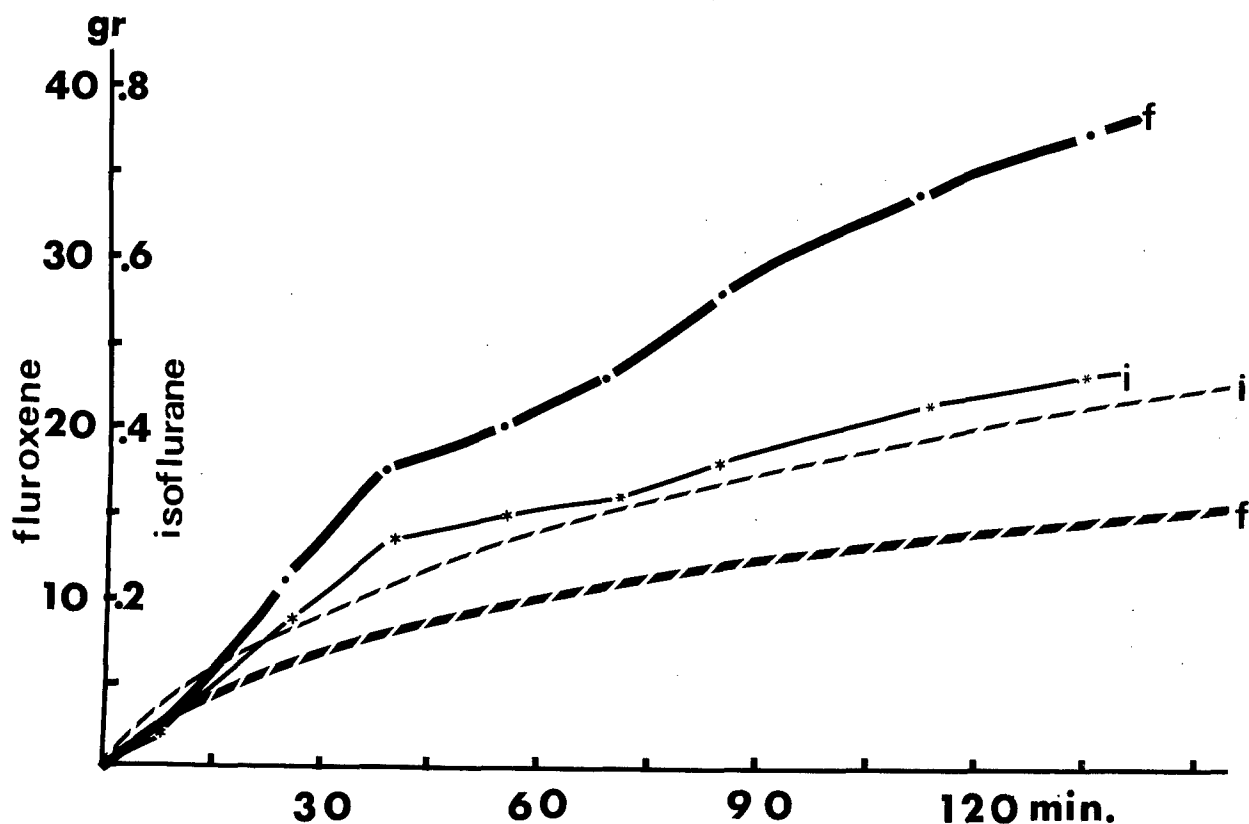
Figure 1. Cumulative uptake of isoflurane (i) and fluroxene (f) administered simultaneously to a surgical patient. Cumulative uptake is plotted against the time after the start of anesthesia. The dashed lines are uptake curves predicted by integration of Equation 3 for alveolar concentrations of fluroxene (154 mg/liter) and isoflurane (3 mg/liter).

To study the effect of exposure concentration on pulmonary uptake, the following assumptions were made: (1) the retention of vapor in tissue is a first order process, which means its rate constant is concentration independent; and (2) metabolic clearance is a limited-capacity process described by Michaelis-Menten kinetics.

To determine the Michaelis-Menten constants in vivo, we exposed male rhesus monkeys (approximately 3 kg) consecutively to three concentrations of one of the following compounds: benzene, halothane $(CF_3 \cdot CHClBr)$, methylene chloride, or trichloroethylene. Concentrations were in the range of TLV during the first exposure; equal to five times TLV during the second exposure; and equal to 25 TLV during the third exposure. In order to reach apparent steady state, each exposure lasted approximately two and one-half hours. Vapors were administered in light sernylane anesthesia via endotracheal tube. The following parameters were measured: (1) vapor concentrations in inhaled air (C_{exp}), mixed-exhaled air (C_{exh}), and end-exhaled (C_{alv}) and arterial blood (C_{art}); (2) blood-gas partition coefficients $(\lambda bl/air)$; (3) minute ventilation; (4) blood pressure and pulse rate; and (5) blood gases and PCO_2 in mixed-exhaled air. Uptake rate, metabolic rate, and alveolar ventilation were calculated from the measured data. Apparent Michaelis-Menten constants of overall metabolism in vivo (K_m) were calculated from double reciprocal plots of metabolic rate versus C_{alv}, C_{exp}, or $C_{art}/\lambda bl/air$ (measured at a steady state), and versus calculated concentrations in tissues.

In Figure 2, the double reciprocal plots from benzene and methylene chloride are presented. K_m values related to the concentrations in alveolar air at steady state for all four studied compounds are in Table 1.

Figures 3 and 4 demonstrate double reciprocal plots of metabolic rates of trichloroethylene and halothane versus exposure concentration, alveolar concentration, arterial concentration, and tissue concentration. The data indicate that K_m values depend on the site in which the concentration is measured, but the maximum metabolic rate (V_{max}) is the same regardless of whether it is derived from concentration in inhaled air, alveolar air, arterial blood, or tissue.
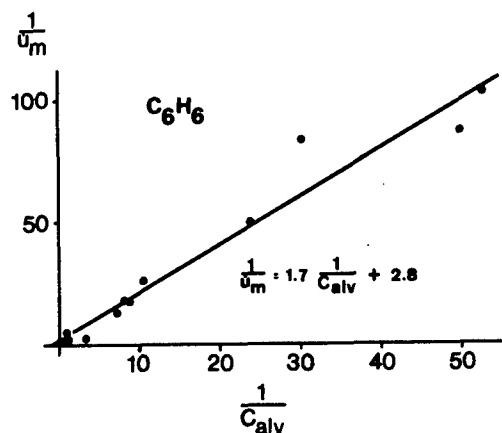
Figure 2. Double reciprocal plot of uptake rates of benzene and methylene chloride versus alveolar concentration. The lines represent optimum fit to experimental data obtained in rhesus monkeys (3 kg males).
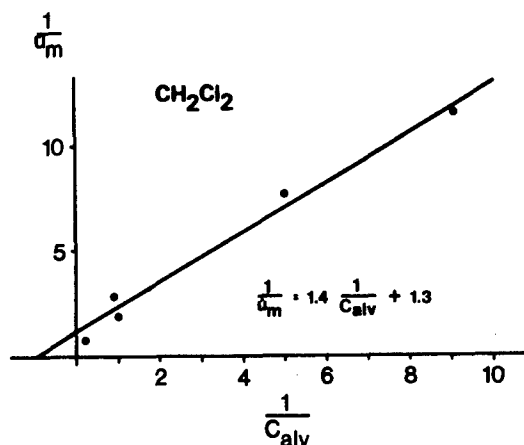
TABLE 1. APPARENT MICHAELIS-MENTEN CONSTANTS IN VIVO
(3 KG MALE RHESUS MONKEY)

Compound	V_{max} (mg/min)	K_m* (mg/liter)	TLV** (mg/liter)
Benzene	0.3	0.6	0.03
Trichloroethylene	0.7	1.0	0.27
Methylene Chloride	0.7	1.1	0.36
Halothane	1.2	7.4	0.40

*Related to C_{alv}

**Threshold limit values recommended by ACGIH in 1979 as TWA.
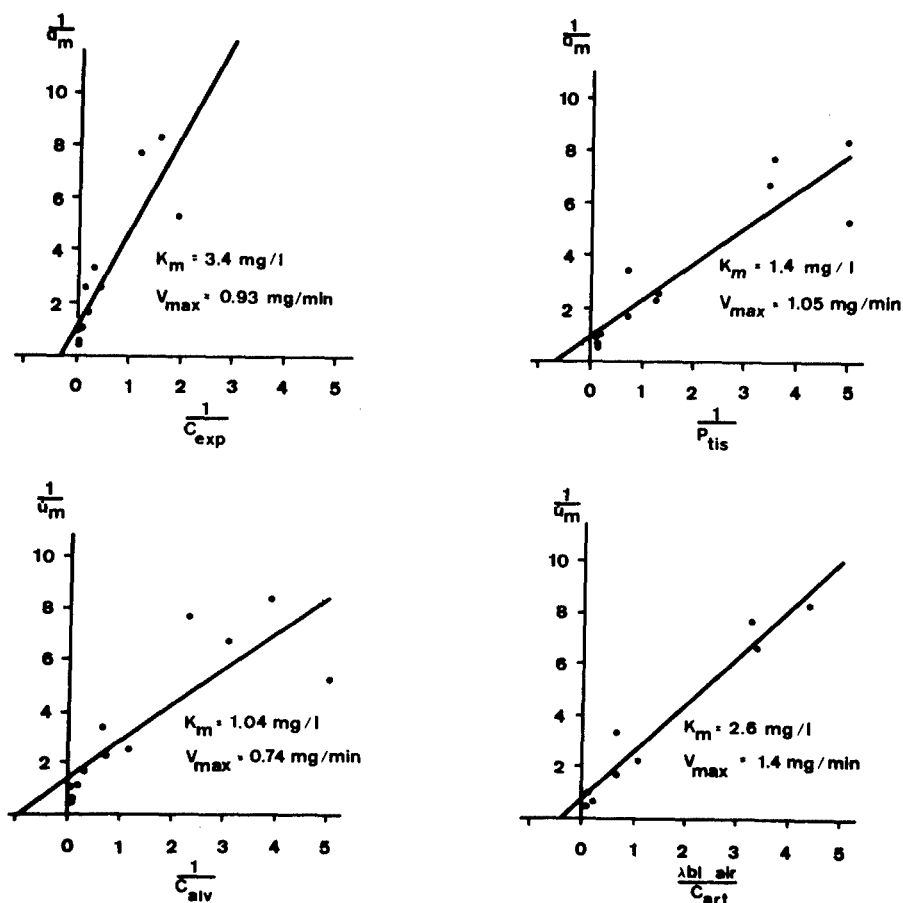
Figure 3. Double reciprocal plot of uptake rates of trichloroethylene versus measured concentrations in inhaled air (C_{exp}), end-exhaled air (C_{alv}), arterial blood (C_{art}), and calculated concentrations in tissues.

$$P_{tis} = \frac{C_{tis}}{\lambda_{tis/air}} = \left(-\frac{\dot{u}_m}{F_{VRG}} + C_{art}\right) \frac{1}{\lambda_{bl/air}}$$

The lines represent optimum fit to experimental data obtained in rhesus monkeys (3 kg males).
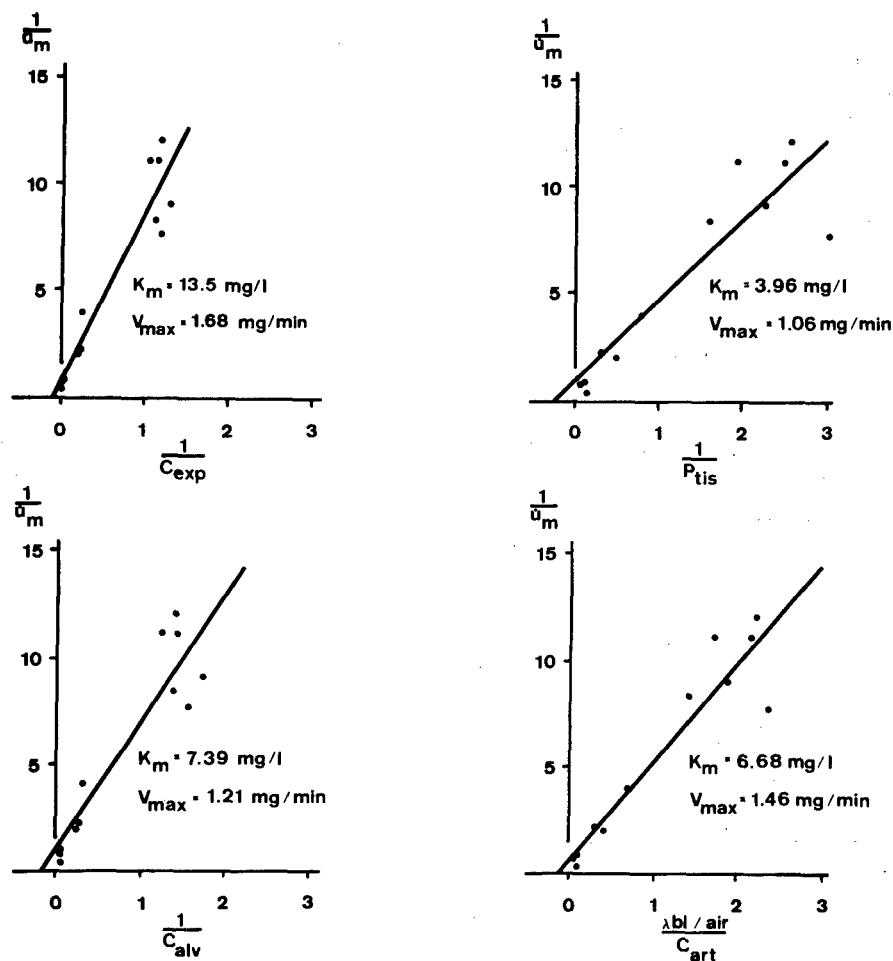
Figure 4. Double reciprocal plot of uptake rates of halothane versus measured concentrations in inhaled air (C_{exp}), end-exhaled air (C_{alv}), arterial blood (C_{art}), and calculated concentrations in tissues.

$$P_{tis} = \frac{C_{tis}}{\lambda tis/air} = (- \frac{\dot{u}_m}{F_{VRG}} + C_{art}) \frac{1}{\lambda bl/air}$$

The lines represent optimum fit to experimental data obtained in rhesus monkeys (3 kg males).

The differences in K_m values might be explained by the three concentration gradients on the pathway of the vapor from the environment to the metabolic site: (1) Vapor concentration entering the lung with each breath is smaller than the exposure concentration because of the dilution by alveolar air from deadspace; (2) When the air reaches the alveoli, the partial pressures in alveolar air and arterial blood are readily equilibrated. Uptake of vapor by arterial blood reduces the concentration further. The concentration decreases, depending on the cardiac output and alveolar ventilation, on the solubility of vapor in blood, and on the concentration of vapor in mixed-venous blood; (3) Arterial blood transfers the vapor to the tissues, where it is retained to the extent that partial pressures in tissue and venous blood are equilibrated. The concentration gradient C_{art}/C_{ven} in blood which supplies metabolic sites is further increased by metabolic clearance.

At steady state, the partial pressures of nonmetabolized vapor equilibrate, and the vapor concentrations in tissues equal the exposure concentration multiplied by the appropriate partition coefficient. If the vapor is metabolized, the concentrations are reduced.

Employing our nonlinear model (Fiserova-Bergerqva et al., in preparation), we examined the conditions under which this method for K_m and V_{max} is applicable, and found the following limitations:

(1) Metabolism must be concentration dependent. According to Michaelis-Menten kinetics, this requirement is met if substrate concentrations are smaller than 10 K_m. This means that the studies must be performed in the range of exposure concentrations which are smaller than 10 K_m.

$$C_{exp} < 10 K_m$$

(2) The system cannot be flow-limited. This requires that transportation rate of vapor from environment to the metabolic site is larger than metabolic rate. This condition is met if:

$$\frac{V_{max}}{K_m} < \frac{F \, \lambda bl/air \, \dot{V}_{alv}}{\dot{V}_{alv} + F \, \lambda bl/air}$$

This expression can be rearranged:

$$\frac{V_{max}}{K_m} < F \, \lambda bl/air \, \frac{1}{1 + \dfrac{F}{\dot{V}_{alv}} \lambda bl/air}$$

Optimum conditions for determination are: (a) the vapor is highly susceptible to biotransformation, and (b) the metabolite sites are well perfused or the vapor is well soluble in blood.

We analyzed the tissues of rats and monkeys exposed to different concentrations of halothane and trichloroethylene to determine the effect of exposure concentrations on concentrations of these vapors in tissues. When exposure concentrations were larger than K_m, the metabolic clearance diminished and the concentration ratios C_{exp}/C_{tis} increased (Fiserova-Bergerova, unpublished data). The same conclusions were drawn using our nonlinear mathematical model (Fiserova-Bergerova et al., in preparation).

The determination of metabolic rate from pulmonary uptake is also suitable for studying the effect of modifiers on metabolism of inhaled vapors. In experiments similar to those described above, we administered two vapors simultaneously to monkeys. One vapor was administered at a low constant concentration; the concentration of the other vapor – the modifier – was increased in three steps. Data from these experiments are in Figure 5. As the concentration of modifier increased, the pulmonary uptake of the studied vapor decreased. The decrease is probably caused by competitive inhibition of metabolism of the inhaled vapors.
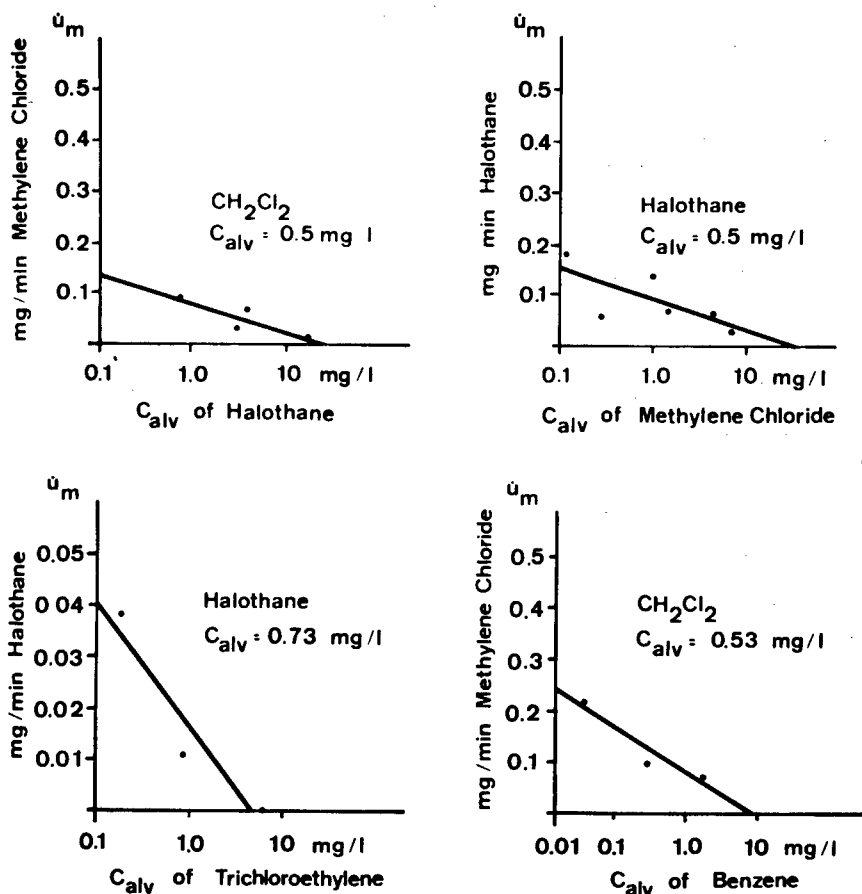
Figure 5. Effect of increasing exposure concentration of vapor-modifier (abcissa) on metabolic rate of the vapor inhaled at constant concentration.

CONCLUSIONS

We have presented rationales for the determination of metabolic constants in vivo from pulmonary uptake rate, and briefly described the procedures and some applications.

The determination of the extent of vapor metabolism from the uptake rate has an advantage over making this determination from excreted metabolites, in that the effect of metabolite distribution and binding in the body, and the effect of renal clearance, are eliminated. Since air sampling is a noninvasive procedure, samples can be collected continuously, or as frequently as needed.

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COMPARATIVE PHARMACOKINETICS OF INHALED STYRENE IN RATS AND HUMANS*

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INTRODUCTION

Estimation of the potential toxic hazard of a chemical to humans in both the environment and in the industrial work place requires extrapolation of toxicity data observed in laboratory animals at relatively high doses to the expected response at much lower dose levels of the chemical which may be encountered by humans under realistic conditions. This extrapolation is in reality a two-dimensional one since it encompasses both (a) projection of the dose versus response curve into a lower range of dose levels where the observable toxic response may vanish into the normal background incidence of the lesions, and (b) interspecies extrapolation of the projected response from laboratory animals to humans.

The most rational extrapolation of toxicity data to humans integrates observations of chronic toxicity with knowledge of the pharmacokinetic profile and probable mechanisms of toxicity such as macromolecular interactions and the role of metabolic transformations (Gehring and Blau, 1977; Reitz et al., 1978). This communication is primarily concerned with the application of pharmacokinetic principles in aiding the projection of the dose-response curve to lower dose levels and the interspecies extrapolation of toxicity data.

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PHARMACOKINETIC PRINCIPLES

Pharmacokinetics defines and quantifies the dynamics of absorption, distribution, biotransformation, and excretion of a chemical in the body. When the rates of these processes are directly proportional to the concentrations (or amounts) of the reacting entities, then the pharmacokinetic model will be described by first-order (linear) kinetics. Under these conditions, the time-related concentrations of the chemical and its metabolites in the tissues and organs of the body will be directly proportional to the administered dose level. Since the time-related concentration of a chemical in the vicinity of receptors usually governs toxicity, the toxic response may also be expected to remain proportional to the administered dose level, enhancing confidence in the projection of toxicity to even lower dose levels.

However, when the dose level of a chemical is raised sufficiently to overwhelm or saturate any of the biological processes governing its pharmacokinetic profile, then the direct proportionality maintained at lower doses will be destroyed. Since the tissue distribution, metabolism, and excretion under these conditions are distorted relative to those at lower (nonsaturating) doses, any toxic response observed at these high levels will no longer be proportional to that attained at lower doses. Thus, toxicity data obtained at saturating dose levels are rendered virtually worthless for the purpose of direct extrapolation to lower dose levels (Gehring and Young, 1977; Gehring et al., 1976).

If the pharmacokinetic profile of a chemical is similar between an animal test species and humans, then it is reasonable as a first approximation to infer that the animal species may be a reasonable model for toxicity of the chemical in humans. Conversely, significant qualitative or quantitative differences in the pharmacokinetic profile of a chemical between two species suggest that differences in toxic susceptibility can be expected, and this animal species should be considered a poor model for humans.

STYRENE-BACKGROUND INFORMATION

Styrene monomer is an industrial chemical for which sufficient data are available to provide an example of application of the foregoing pharmacokinetic principles. Styrene is used worldwide in the plastics and reinforced fiberglass industries. Small quantities of styrene monomer have been detected in some foods packaged in polystyrene containers (Withey and Collins, 1978), but most human exposure to styrene probably occurs via inhalation in the industrial work place. The current 8-hour time-weighted-average exposure is limited to 100 ppm, and 50 ppm has recently been proposed as the new limit (ACGIH, 1979).

The acute toxicity of styrene is low, the single oral dose LD$_{50}$ in rats being approximately 5000 mg/kg (Wolf et al., 1956), and a recent chronic toxicity study revealed no evidence for carcinogenicity in Fischer 344 rats or B6C3F1 mice (National Cancer Institute, 1979).

The metabolism of styrene has been studied in rats and humans (Ohtsui and Ikeda, 1971; Hake et al., 1977; Bardodej and Bardodejova, 1970; Ikeda et al., 1974) and the general metabolic scheme of this compound is shown in Figure 1. Although styrene oxide has been proposed as an intermediate in the in vitro oxidative metabolism of styrene (Liebmand and Oritz, 1970), it has not yet been isolated following the administration of styrene to intact animals. The time course of blood concentration of styrene has been studied following intravenous administration in rats (Withey and Collins, 1977) and following inhalation exposure in humans (Stewart et al., 1968; Hake et al., 1977; Astrand et al., 1971). In general, these studies and others have shown that styrene is cleared by both animals and humans mostly by metabolism and to a much smaller extent by expiration of the parent compound. The studies reported here were conducted to define more completely the pharmacokinetic profile of inhaled styrene in both rats and humans to enable direct comparison between the two species, as well as to investigate the impact of changing exposure levels.
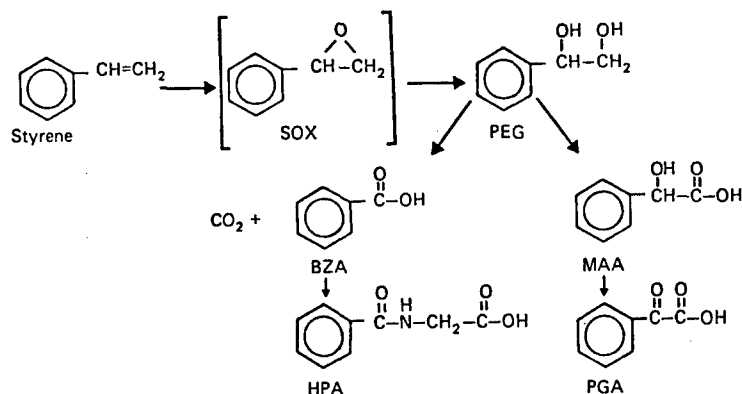
Figure 1. Probable pathways of styrene metabolism. Styrene oxide (SOX) is a proposed intermediate; it has not yet been isolated in vivo following administration of styrene. PEG = phenylethylene glycol, BZA = benzoic acid, HPA = hippuric acid, MAA = mandelic acid, PGA = phenylglyoxylic acid.

EXPERIMENTAL

RATS

Groups of 51 male Sprague-Dawley rats were exposed to either 80, 200, 600, or 1200 ppm styrene in stainless steel inhalation chambers under dynamic airflow conditions. The chamber concentration of styrene was monitored continuously with a Miran IR analyzer at 11 microns. Twenty-one of the rats in each experiment were removed from the chambers at 6 hours and killed in groups of 3 at selected time intervals for 18 hours thereafter to determine the clearance kinetics of styrene. The remainder of the rats were left in the chamber for periods of up to 24 hours to determine plateau blood levels. At each sampling interval, 3 rats were killed and samples of whole blood and epididymal fat were obtained for styrene analysis.

HUMANS

Four human male volunteers were exposed to 80 ppm styrene for 6 hours in a polyethylene enclosed room under dynamic airflow conditions. The chamber concentration of styrene was monitored continuously with a Miran 1A IR analyzer at 11 microns. Venous blood samples were obtained at selected time intervals during the 6-hour exposure period, and for 41 hours thereafter. In addition, expired air samples (approximately 10 liters) were obtained at selected timed intervals following the exposure. Urine samples were collected at 6, 12, 18, 24, and 48 hours following the start of the exposure period and were stored frozen until analyzed for hippuric acid, benzoic acid, mandelic acid and phenylglyoxylic acid.

ANALYTICAL PROCEDURES

The concentration of styrene in blood and fat samples was determined by gas chromatography/mass spectrometry of a hexane extract of the samples (Karbowski and Braun, 1978). The concentration of styrene in expired air was determined by forcing the expired breath through two traps containing hexane cooled to dry ice temperature, and subsequent analysis of styrene trapped in the hexane by gas chromatography/mass spectrometry. Urine samples were analyzed for metabolites of styrene by extraction, derivatization, and gas chromatographic detection (Matsui et al., 1977; Guillemin and Bauer, 1976).

RESULTS

RATS

Figure 2a is a semilogarithmic graph of the concentration of styrene in the blood and fat of the rats exposed to 80 ppm. The blood concentration rose quickly and maintained a maximum concentration of approximately 0.8 $\mu g/g$ blood throughout the 24-hour exposure period. The fat concentration of styrene rose more slowly and attained a maximum of approximately 34 $\mu g/g$

at 24 hours. In the rats that were removed from the chamber at 6 hours, the blood concentration of styrene exhibited a biphasic log-linear decline typical of the central compartment of a two-compartment linear pharmaco-kinetic model, and the fat concentration exhibited a monophasic log-linear decline typical of the peripheral compartment of a linear two-compartment model.

Figure 2a (80 ppm)   Figure 2b (200 ppm)

Figure 2a & 2b. Concentration of styrene in blood and fat of rats exposed to 80 or 200 ppm for periods of up to 24 hours. A group of rats was re-moved from the chamber at 6 hours to obtain the clearance data. Each data point is the mean ± standard deviation for 3 rats. Solid lines are predicted concentrations calculated with the best pharmacokinetic parameter estimates at each exposure level (pharmacokinetic model of Figure 3).

Figure 3 shows a diagram of a two-compartment linear pharmacokinetic model describing the uptake, distribution, and clearance of inhaled styrene, and the differential equations that describe the rates of change of styrene in both compartments. The observed concentrations of styrene in both blood and fat for the 6-hour exposure period and for the subsequent clear-ance phase shown in Figure 2a were fit to the concentrations predicted by the model shown in Figure 3.

$$C_2(t)=A_2(t)/V_2$$

k_{12} k_{21}

k^o $C_1(t)=A_1(t)/V_1$ k_{1c}

Styrene uptake   Metabolic and Respiratory clearance

$$\frac{dA_1(t)}{dt} = \begin{Bmatrix} k^o, 0<t\leq t^* \\ 0, t>t^* \end{Bmatrix} + k_{21}A_2(t)-(k_{12}+k_{1c})A_1(t)$$

$$\frac{dA_2(t)}{dt} = k_{12}A_1(t)-k_{21}A_2(t)$$

Figure 3. Pharmacokinetic model and differential equations describing the uptake, distribution, and clearance of inhaled styrene in rats and humans. k^o = zero order input rate; k_{12} and k_{21} = first order mass transfer coefficients; k_{1c} = apparent first order rate constant for combined metabolic and respiratory clearance; $C_i(t)$ = concentration of styrene in compartment i at time t; $A_i(t)$ = amount of styrene in compartment i at time t; t = total elapsed time from start of exposure; t^* = time at end of exposure; V_i = apparent volume of distribution of compartment i.

The time-dependent values for each variable were determined by numerical integration using the Continuous System Modeling Program (CSMP), (IBM, 1972). Best estimates of the pharmacokinetic parameters of the model were obtained by the method of least squares using an iterative search routine. For this purpose, the input rate of styrene was set at 3160 µg/hr based on a ventilation rate of approximately 14 liters/hour (for a 300 g rat), and assuming that 66% of the inhaled styrene was absorbed (Astrand et al., 1971; Bardodej and Bardodejova, 1970).

The solid lines in Figure 2a are the predicted concentrations of styrene in the blood and fat, calculated from the best pharmacokinetic parameter estimates of the model. An excellent fit to the observed data was obtained during the 6-hour exposure period and during the subsequent clearance phase. Furthermore, the pharmacokinetic model accurately predicted the plateau concentrations of styrene in both blood and fat throughout the 24-hour exposure period. The half-life values obtained for the rapid (α) and slower (β) clearance phases were 0.24 hours and 3.49 hours, respectively. The apparent volumes of distribution for the central (blood) and peripheral (fat) compartments were 5100 g/kg and 155 g/kg, respectively.

Figure 2b is a semilogarithmic graph of the observed concentration of styrene in the blood and fat of rats exposed to 200 ppm. In this case, the plateau concentrations of styrene in blood and fat were approximately 1.5 and 120 µg/g, respectively. These data were also typical of the two-compartment linear model shown in Figure 3, and an identical procedure was used to obtain the best pharmacokinetic parameter estimates of the model based on both the blood and fat concentrations at an exposure concentration of 200 ppm. The solid lines of Figure 2b are the theoretical values predicted by the model, for which the half-life values of the α and β phases were 0.27 and 2.94 hours, respectively.

The concentrations of styrene in the blood and fat of rats exposed to 600 and 1200 ppm are shown in the semilogarithmic graphs of Figures 4a and 4b, respectively. At these exposure concentrations, the clearance phase of styrene in blood no longer exhibited the typical characteristics of the linear pharmacokinetic model, but were more representative of a saturated clearance process. The plateau blood concentrations attained at the 600 ppm and 1200 ppm exposure levels were 25 µg/g and 64 µg/g, respectively.
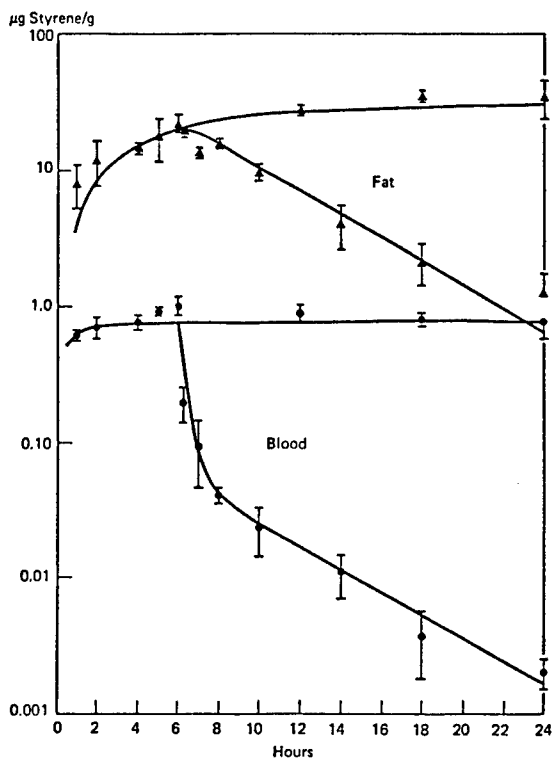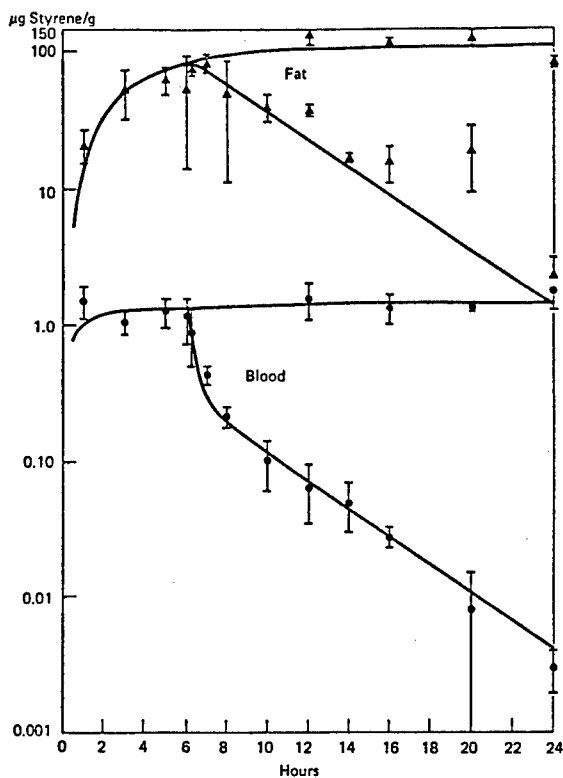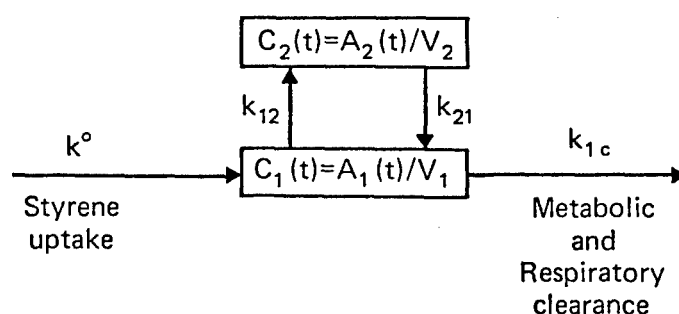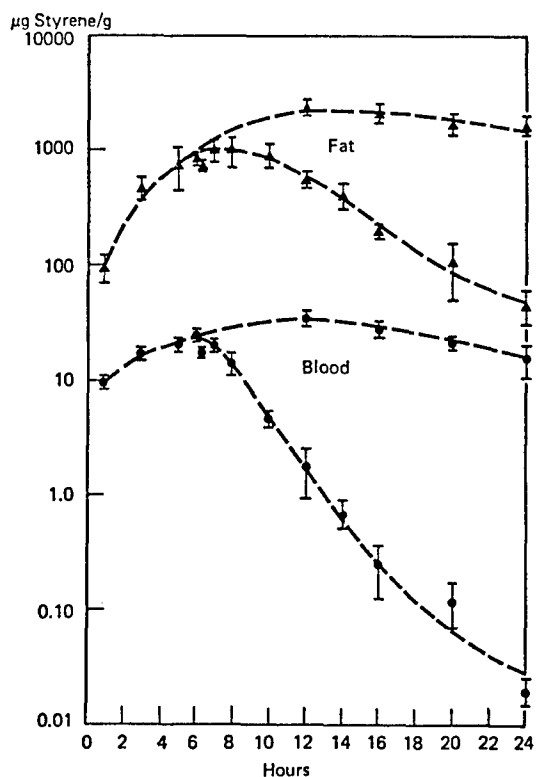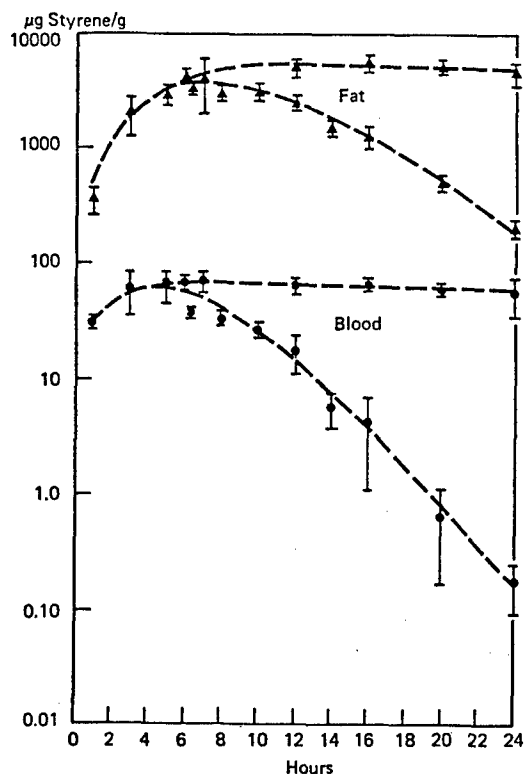

Figure 4a (600 ppm)   Figure 4b (1200 ppm)

Figure 4a & 4b. Concentration of styrene in blood and fat of rats exposed to 600 or 1200 ppm for periods of up to 24 hours. A group of rats was removed from the chamber at 6 hours to obtain the clearance data. Each data point is the mean ± standard deviation for 3 rats. Dashed lines were drawn by inspection.

The areas under the blood concentration versus time curves (AUC) were calculated by the trapezoidal rule during the 6-hour exposure period and the subsequent clearance phase for the rats at all four exposure levels. The results are shown in Table 1, in which the last 2 columns are the exposure concentration and the AUC normalized to the 80 ppm level. With an increase of 2.5 in exposure level, from 80 to 200 ppm, the AUC increased by a factor of 1.5, somewhat less than the expected value of 2.5. However at 600 ppm, where the exposure level was increased from 80 ppm by a factor of 7.5, the AUC increased by a factor of 28, almost 4 times more than expected. And at 1200 ppm, an increase of 15 times in exposure level, the AUC increased 95 times, more than 6 times the expected value.

TABLE 1. AREAS UNDER BLOOD CONCENTRATION CURVES (AUC)
0-24 HOURS IN RATS EXPOSED TO 80, 200, 600 OR 1200 ppm
STYRENE FOR 6 HOURS

Exposure Concentration (ppm)	AUC (μg·hr/ml)	Normalized to 80 ppm	
		Exposure Concentration	AUC
80	5.8	1.0	1.0
200	8.6	2.5	1.5
600	160	7.5	28
1200	550	15	95

HUMANS

The volunteers took complete physical examinations before the exposure, and 1 and 14 days after the exposure. The results were normal in every respect, and revealed no exposure-related effects.

The concentration of styrene in the blood of the human volunteers during and after the 6-hour exposure to 80 ppm styrene is shown in Figure 5. The blood concentration of styrene rose rapidly to a maximum of approximately 0.9 μg/g at the end of the exposure. The concentration then declined in a biphasic log-linear manner consistent with the pharmacokinetic model of Figure 3. For humans, the input rate of styrene was set at 9.96×10^4 μg/hour, based on an average ventilation rate of 7.4 liters/minute and assuming that 66% of the inhaled styrene was absorbed (Astrand et al., 1971; Bardodej and Bardodejova, 1970). The best parameter estimates for each of the four volunteers were obtained by fitting the blood concentration data to the pharmacokinetic model of Figure 3 as described previously. The solid line in Figure 5 is the predicted blood concentration calculated with the average pharmacokinetic parameters for these volunteers, demonstrating an excellent fit to the observed data. The average half-life values for the α and β clearance phases of styrene in blood were 0.58 ± 0.08 hours and 13.0 ± 0.7 hours, respectively, and the average apparent volume of distribution for the central (blood) compartment was 1350 ± 360 g/kg.
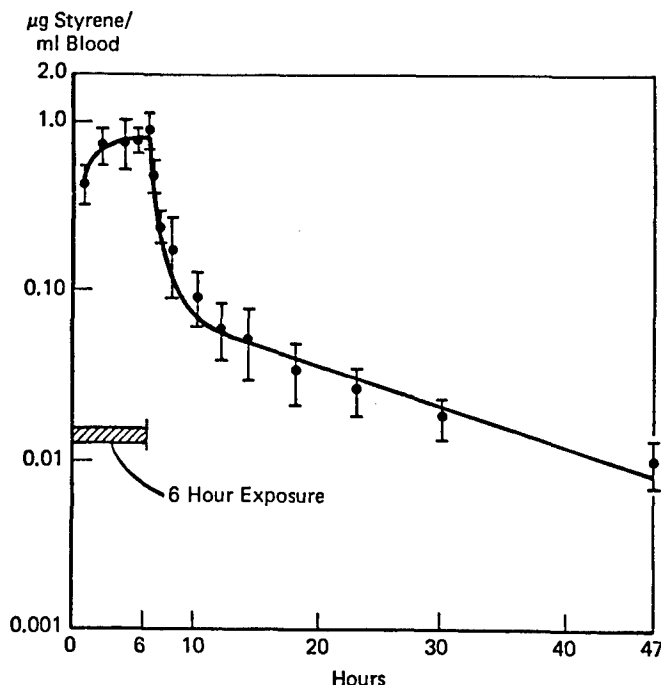
Figure 5. Concentration of styrene in blood of 4 human volunteers exposed to 80 ppm for 6 hours. Data points are mean ± standard deviation. Solid line is predicted concentration calculated with the average of the pharmacokinetic parameter estimates for each individual (pharmacokinetic model of Figure 3).

The concentration of styrene in expired air of these volunteers following the 6-hour exposure to 80 ppm styrene is shown in the semilogarithmic graph of Figure 6. The concentration of styrene in expired air followed a biphasic log linear pattern very similar to that in blood. These data were fit to a biexponential equation which allowed computation of the average AUC for the volunteers, 14.03 ± 3.9 $\mu g \cdot hr/\ell$ of air. This value was multiplied by the average ventilation rate of 444 liters/hour to calculate that approximately 60 μmole of styrene were cleared from the body by expiration during the postexposure period.

Benzoic acid levels in the urine were quite low, and hippuric acid levels varied so widely that neither of these acids could be correlated with styrene exposure. The average cumulative amount above background levels of mandelic and phenylglyoxylic acids detected in voided urine was 2.32 ± 0.80 mmole, of which 2.02 ± 0.69 mmole were excreted in the postexposure period*. When this quantity is compared with the 60 μmole of unchanged styrene that were excreted in the expired air during the postexposure period, it is evident that a total of only 2 to 3% of styrene cleared from the body was exhaled as styrene and the remainder was cleared by metabolism.

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*The urine samples were frozen at the end of each collection interval and stored for several months before being analyzed for phenylglyoxylic and mandelic acids. Since phenylglyoxylic acid exhibits some instability under these conditions (D. Bauer, personal communication), the quantity of phenylglyoxylic acid determined in this experiment must be considered a minimum.

μg Styrene/ ℓ Air
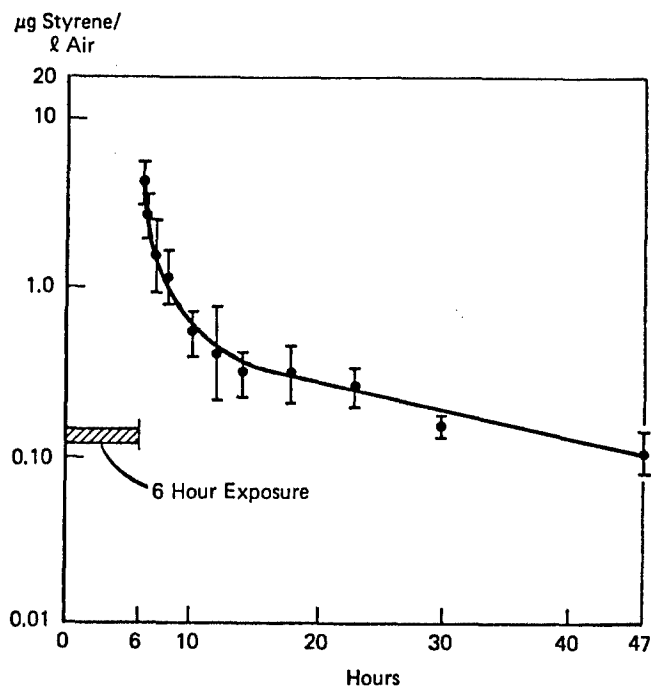
Figure 6. Concentration of styrene in expired air of 4 human volunteers following a 6-hour exposure to 80 ppm. Data points are mean ± standard deviation. Solid line calculated from the best fit of the data points to a bi-exponential equation.

DISCUSSION

These studies demonstrate that, at concentrations of up to at least 80 ppm, inhaled styrene is cleared from both rats and humans in a manner that is consistent with a linear two-compartment pharmacokinetic model. The linear kinetic characteristics of this model predict that at any rate of uptake (below that rate at which the clearance kinetics become saturated) the blood and tissue concentrations of styrene will remain proportional to the exposure level. This proportionality will be maintained for either continuous or intermittent exposure. The curves shown in Figure 7a represent predicted blood and fat concentrations of styrene in rats exposed to 80 ppm for 6 hours/day for 5 successive days, followed by a 2-day withdrawal period. These predictions are based on the best pharmacokinetic parameter estimates for the model shown in Figure 3. Both blood and fat concentrations have nearly reached their maximum concentrations after only one exposure, and subsequent exposures attain no greater concentrations.

A similar prediction based on the best pharmacokinetic parameter estimates for inhaled styrene in humans is shown in Figure 7b. In this case as well, maximum concentrations are reached after only a few exposures, and subsequent exposures do not continue to increase the styrene concentration.

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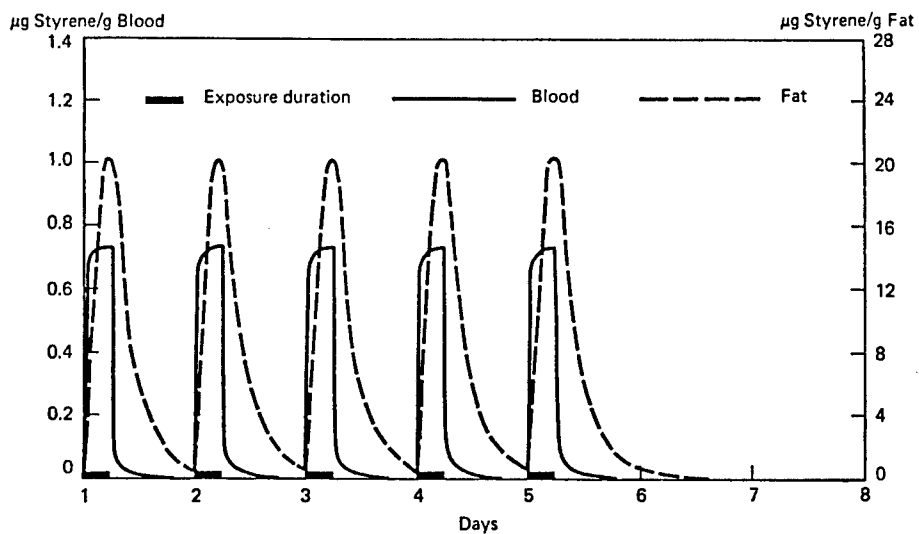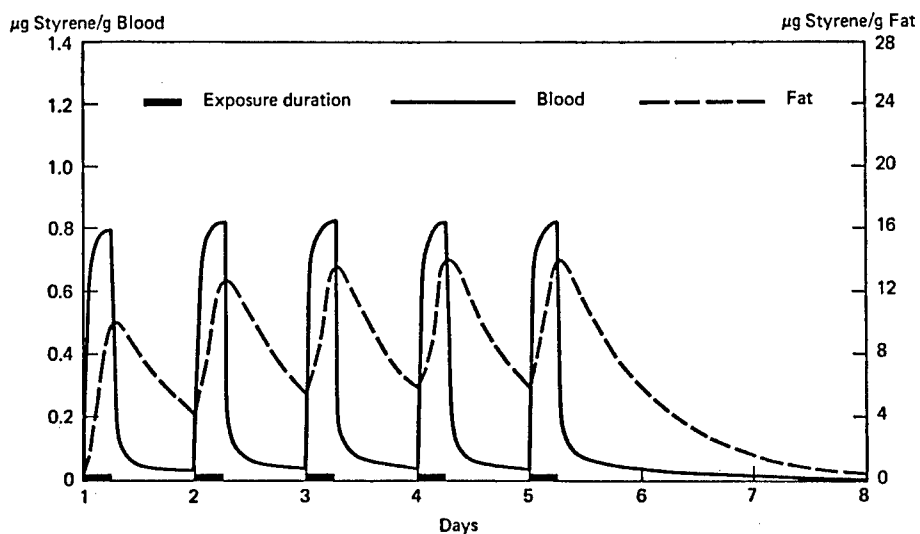
Figure 7a (rats)

Figure 7b (humans)

Figure 7a & 7b. Predicted concentration of styrene in blood and fat of rats (a) and humans (b) exposed to 80 ppm 6 hours per day for 5 days, followed by a 2-day clearance period. Predicted values calculated from the best pharmacokinetic parameter estimates for rats and humans (pharmacokinetic model of Figure 3).

The foregoing examples are typical of the linear pharmacokinetic characteristics of a chemical which lend confidence to the extrapolation of available data to lower dose (or exposure) levels, since these lower levels can be expected to result in proportionately lower tissue and blood concentrations.

The blood clearance curves of styrene in rats shown in Figures 2a, 2b, 4a, and 4b indicate that there is a transition from linear to non-linear clearance of styrene somewhere between exposure levels of 200 and 600 ppm. Furthermore, the maximum blood (and fat) concentrations relative to the exposure level increase with increasing exposure level above 200 ppm. Also, as shown in Table 1, the AUC for the blood concentration curves when normalized to the 80 ppm exposure level increase disproportionately at the 600 and 1200 ppm exposure levels. These data are all indicators of nonlinearities in the pharmacokinetic profile with increasing exposure level. Therefore, toxicity data observed at these saturating levels may no longer be proportional to the toxic response at much lower levels of exposure.

The similarities between the pharmacokinetic models of inhaled styrene in rats and in humans at lower levels of exposure are characteristic of the types of comparison which lend confidence to interspecies extrapolations at and below these levels. The degree of similarity in the time course of blood concentration of styrene upon repeated inhalation at 80 ppm by rats and humans is shown in Figure 8 (these simulated blood concentrations are those shown in Figure 7a and 7b superimposed on one graph). From these comparisons, the rat appears to provide a reasonable pharmacokinetic model for inhaled styrene in humans.
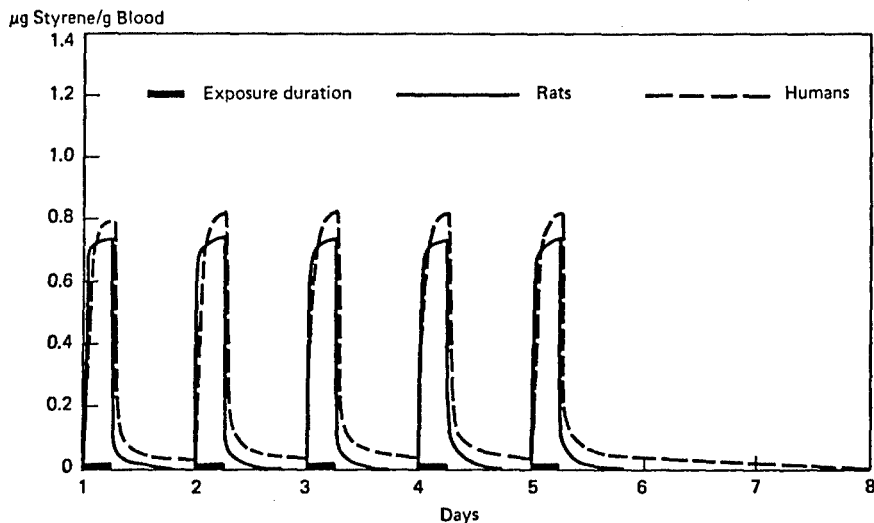
Figure 8. Predicted concentration of styrene in blood of rats and humans exposed to 80 ppm 6 hours per day for 5 days, followed by a 2-day clearance period. (Predicted values taken from Figures 7a and 7b).

SUMMARY

The data presented here, using inhaled styrene as an example, show that the chemical is cleared from the body of both rats and humans in a nearly identical manner, and that the rat provides a reasonable pharmacokinetic model for inhaled styrene in humans at exposure levels of up to at least 80 ppm. In both species, styrene is cleared from the body at a rate that is directly proportional to the blood concentration. This kinetically linear clearance predicts that time-related tissue and blood concentrations will maintain this proportionality at lower exposure levels, lending confidence in the extrapolation of toxicity data to lower exposure levels. On the other hand, when the uptake rate of styrene is high enough (which occurs at exposure levels between 200 and 600 ppm in the rat), the clearance of styrene apparently becomes saturated and the tissue and blood concentrations are disproportionately high. At these excessive exposure levels of a chemical, the toxic consequences may no longer be proportional to the difference in exposure levels alone, negating the possibility of projecting these results to lower exposure levels.

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PHYSIOLOGICAL FACTORS IMPORTANT IN CONTROLLING
THE METABOLISM OF INHALED GASES AND VAPORS

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INTRODUCTION

Once in the body most nonpolar, nonionizable xenobiotics are metabolized by one or more biotransformation pathways. The liver is the primary organ involved in xenobiotic metabolism, and the kidney is the primary organ involved in excretion of the metabolites produced within the liver. After transport from the lung to the liver, many low molecular weight, lipid soluble gases and vapors are also metabolized by these biotransformation reactions. Even the so-called inert anesthetic gases and vapors have now been shown to be rather extensively metabolized in vivo (Vaughan et al., 1978). Transport of inhaled chemical from alveolar air to the hepatic cytosol entails a number of distinct physiological processes. Upon inhalation, the gas or vapor is transferred from alveolar air into capillary blood by passive diffusion in response to an existing concentration gradient. It is transported in a dissolved form to the heart by way of the pulmonary vein and then distributed throughout the body by systemic circulation. About 25% of the cardiac output reaches the liver. In the liver, chemicals diffuse or are transported from the blood across various cellular membranes into the hepatocytic milieu. Hepatic biotransformation reactions are catalyzed either by enzyme systems located in the cytosol or, usually, by those associated with the membranous structures of the endoplasmic reticulum. Potentially, any one of these various steps - uptake from the lung, delivery to the liver via the circulation, diffusion into the liver from the blood, or hepatic metabolism could be rate-limiting for the metabolism of a particular inhalant.

Previous attempts to describe the concentration-dependence of the rate of metabolism of inhaled chemicals have relied solely on models which assume, either tacitly or explicitly, that enzymatic metabolism is rate limiting (Gehring et al., 1978; Andersen et al., 1979a, b; Bolt et al., 1977). For these models to be appropriate, the following three conditions must apply. First, the time required to reach equilibrium between the

blood and gas phase must be short compared to the duration of the exposure. Second, circulating concentrations of the chemical must be directly related to atmospheric concentration. And third, the rate of metabolism at the enzymatic active site must be described by the Michaelis-Menten equation,

$$v = \text{rate} = \frac{V_{max} [S]}{K_m + [S]} \qquad (1)$$

where V_{max} is the maximum velocity, K_m is the concentration of substrate, S, at which the rate is half V_{max} and [S] is substrate concentration (Riggs, 1963). Since the circulating substrate concentration in this model is assumed to be proportional to atmospheric concentration, equation (1) can be rewritten as,

$$v = \text{rate} = \frac{V_{max} [ppm]}{K_m + [ppm]} \qquad (2)$$

Here, [ppm] is the inhalant concentration in inspired air and K_m now represents that atmospheric concentration of inhalant at which the rate is half V_{max}. Basically, this is a simple Michaelis-Menten saturation model. It predicts that at sufficiently high inhalant concentration the rate will be pseudo-zero order and that the complete rate curve will have the shape predicted by the Michaelis-Menten equation.

Despite the apparent successes of a simple Michaelis-Menten model in describing the metabolism of various inhaled chemicals (Gehring et al., 1978; Andersen et al., 1979a, b; McKenna et al., 1979), it is excessively restrictive in assuming that only metabolism can be rate-limiting. The crucial role of physiological processes in delivering inhaled chemicals to the metabolizing organ(s) is completely ignored. Unfortunately, a critical, physiologically-based, pharmacokinetic model describing inhalant metabolism has not yet been developed. Without such a model, it is impossible to determine whether the success of the simple Michaelis-Menten model is coincidental or whether it has some firm basis in pharmacokinetic theory. This paper describes various data on the rates of metabolism of inhaled gases and vapors, especially halothane and benzene, which suggested that a simple, Michaelis-Menten model was not sufficient to describe inhalant metabolism. In addition, a preliminary comprehensive, pharmacokinetic model of inhalant metabolism is presented. It incorporates both physiological and biochemical constants into a unified description of the biological factors which control the metabolism of inhaled gases and vapors in vivo.

METHODS

HALOTHANE

Conventional, dynamic inhalation exposures were conducted with halothane (bromochlorotrifluoroethane). Rats were exposed for 4 hours to various concentrations of halothane in a 31 liter battery jar chamber.

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Details of the chamber design and atmosphere generation procedures are outlined in an earlier paper in these proceedings (Gargas and Andersen, 1979). At the cessation of exposure, rats were removed from the chamber, injected with an overdose of phenobarbital, and exsanguinated via the portal vein after opening the abdomen. Plasma was prepared from heparinized blood and inorganic bromide determinations were conducted with an ion-specific bromide electrode using standard methodology as modified by Gargas and Andersen (1979).

BENZENE AND OTHER CHEMICALS

The kinetics of metabolism of chemicals other than halothane were inferred using gas uptake techniques (Andersen et al., 1979a) in a 31 liter chamber. In these studies, rats were exposed to a closed recirculated atmosphere containing various concentrations of the test chemical. Atmospheric O_2 was maintained between 18 and 22% and expired CO_2 was removed by absorption on a solid substrate. Nine rats were used in each exposure. Each exposure was initiated by injecting a measured amount of volatile liquid or gas into the chamber atmosphere. Contaminant concentrations were monitored by gas chromatographic methods 5 minutes after injection of the test chemical and every 10 minutes thereafter for 180 minutes. For each chemical, the rate of gas uptake (i.e., the rate of disappearance of test chemical from the atmosphere) was determined for a number of initial chamber concentrations. In each individual uptake experiment, the rate was determined at 80-100 minutes after initiating the exposure. This timing was used to insure that the initial blood: air equilibration was complete and that the observed uptake was due to metabolism. Michaelis-Menten kinetic constants were then determined by analysis of the concentration dependence of the rate of gas uptake from the closed atmosphere. Gas uptake techniques are discussed in an earlier paper in these proceedings (Gargas and Andersen, 1979). Variations on these basic procedures have been used in other laboratories for pharmacokinetic analysis of inhalant metabolism (Hefner et al., 1975; Bolt and Filser, 1977). When inhalant metabolism proceeds according to equation (2), information regarding the two Michaelis-Menten kinetic constants can be obtained by examining the extremes of the rate curve. From equation (2), it is apparent that the rate will approach V_{max} as concentration approaches infinity. This is the pseudo-zero order portion of the curve. Conversely, as the concentration approaches zero, the rate equation becomes first-order,

$$\text{rate}_{ppm \to 0} = \frac{V_{max} \text{ [ppm]}}{K_m} \tag{3}$$

Under these conditions, the apparent maximum first-order rate constant is V_{max}/K_m.

RESULTS AND DISCUSSION

HALOTHANE METABOLISM

The curve relating the rate of bromide production from inhaled halothane to the exposure concentration (Figure 1) had an anomalous shape. A true first-order dependence extended to about 75% V_{max} and abruptly converted to a pseudo-zero order dependence. For a Michaelis-Menten dependence the rate constant at 75% V_{max} is expected to be only one-fourth of the limiting maximum first-order rate constant. The best-fit Michaelis-Menten curve for the halothane data is given by the curved line in Figure 1. Application of a simple, Michaelis-Menten model was clearly inappropriate with this data. It overestimated the points at the low concentrations and severely underestimated points at the higher concentrations. Errors about the best fit line should be more randomly distributed for an appropriate model. In this figure, the rate is expressed as both the change in plasma bromide during the 4-hour exposure (ΔBr) and as mg of halothane metabolized per kg per hour. In converting plasma bromide to the rate of halothane metabolized, a value of 0.26 liter/kg was used as the volume of distribution of inorganic bromide (Woodbury, 1966).
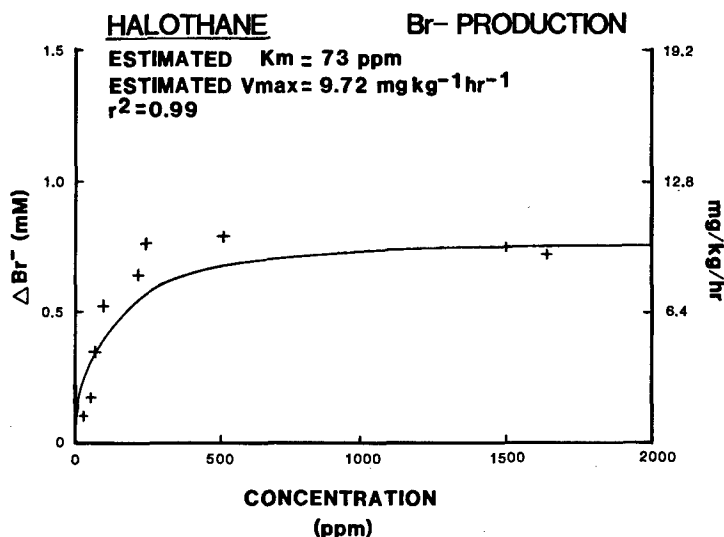
Figure 1. The rate of metabolism of inhaled halothane at a variety of atmospheric halothane concentrations. Rats were exposed to halothane for 4 hours. The rate of metabolism is given as the change in plasma inorganic bromide during the exposure on the right-hand side, as mg of halothane metabolized/kg/hr. The curve is the best fit Michaelis-Menten curve to these points and the Michaelis-Menten constants of the best fit line are given in the figure.

GAS UPTAKE STUDIES

Experiments were conducted with a variety of inhaled chemicals to determine the maximum first-order rate constant for gas uptake (as $hr^{-1} kg^{-1}$) from the 31 liter chamber (Table 1). As noted in METHODS, the maximum value of the rate constant is found at concentrations much below the K_m. For these chemicals, the maximum rate constants clustered over a very narrow range, suggesting that a common physiological process was rate-limiting at low concentrations of these inhalants. An alternate explanation was that the ratio of V_{max} to K_m was approximately equal for all these chemicals, a highly unlikely possibility.

TABLE 1. THE MAXIMUM FIRST-ORDER GAS UPTAKE RATE CONSTANTS FOR VARIOUS CHEMICALS STUDIED IN A 31 LITER BATTERY JAR CHAMBER

Chemical	Maximum Gas Uptake Rate Constant ($hr^{-1} kg^{-1}$)
Halothane	0.33
Benzene	0.50
Vinyl Bromide	0.34
Bromochloromethane	0.48
1,1-Dichlorethylene	0.39
Trichloroethylene	0.45
Vinyl Methyl Ether	0.37
Methyl Bromide	0.44

With benzene, the full uptake curve was complex. It possessed a saturable component which predominated at low atmospheric concentrations and a first-order component which predominated at high concentrations. As with halothane, the saturable component (Figure 2) was more accurately represented as an abrupt first-order to zero-order transition than by the smooth Michaelis-Menten curve. Metabolism of several other inhaled chemicals such as bromochloromethane and vinyl bromide was better represented by an abrupt, rather than smooth, first-order to zero-order transition (Gargas et al., 1979). In a study of the metabolism of nine halogenated ethylenes by gas uptake, Filser and Both (1979) also found that their data were best represented by assuming two distinct portions to the rate curve, one first-order and the other zero-order, with a sharp break at what they termed the saturation point. This accumulation of evidence prompted critical reevaluation of the assumption that biochemical reactions alone were rate-limiting for the metabolism of inhaled gases and vapors. The pharmacokinetic model that resulted from this reevaluation is described in the remainder of this paper.
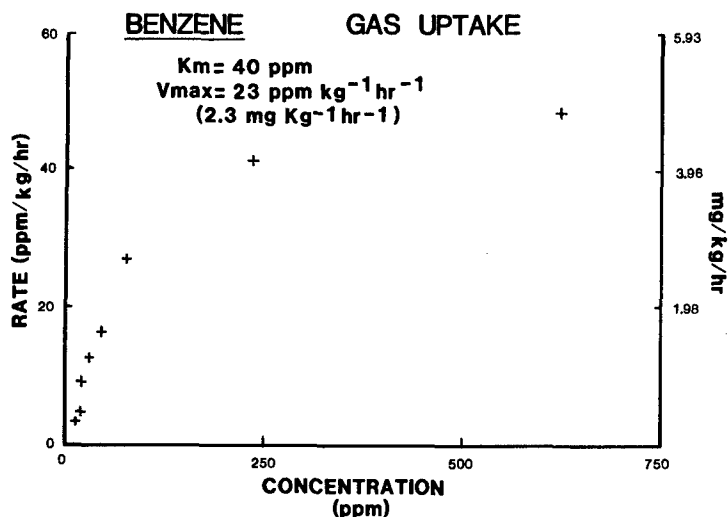
Figure 2. The rate of metabolism of inhaled benzene at various atmospheric benzene concentrations determined by gas uptake studies. The complete uptake curve had a persistent first-order phase with a calculated y-intercept of 23 ppm/kg/hr. The saturable component was determined by subtracting the contribution of the first-order component from the overall rate curve. The kinetic constants of the saturable component are given in the figure. The rate is given as both the observed loss (ppm/kg/hr) and as the amount of benzene which must have been metabolized to account for the observed loss from the chamber.

PHYSIOLOGICAL CONSIDERATIONS

A schematic of the circulation of the rat (Figure 3) for the purposes of this model includes two important interfaces: one between the blood and the enzymatic sites of metabolism in the liver and one between the inspired air and the capillary blood in the lung. Inhaled chemicals are absorbed from the lung until whole body equilibrium is attained. If a substance were neither metabolized nor excreted by organs other than the lung, there would be no net uptake of the chemical at equilibrium.

For a chemical metabolized by enzyme systems located within the liver, continued uptake occurs even after whole body equilibrium is achieved. Quantitatively, an amount of chemical equal to that removed by hepatic clearance must be replaced by pulmonary uptake. Any physiological model of inhalant metabolism then must consider both regulation of hepatic metabolism at the organ level and control of chemical absorption in the lung.

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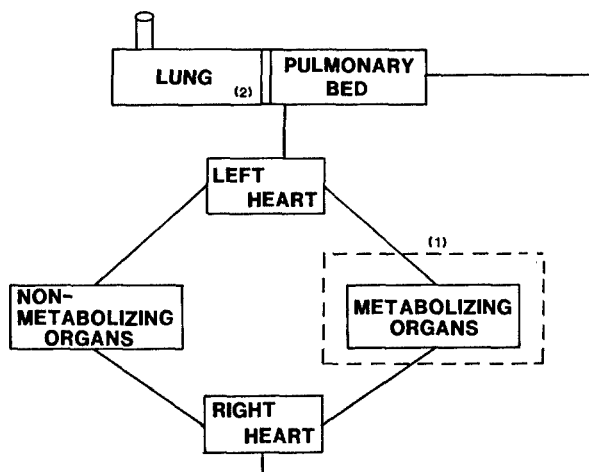
Figure 3. A schematic of the circulation of a rat.

HEPATIC METABOLISM

In this preliminary model, metabolism is assumed to occur only in the liver by way of a single uni-enzyme pathway. When the entire liver is considered, the rate of reaction at the entrance to the organ can be related to the arterial concentration of chemical by the Michaelis-Menten relationship, but the blood concentration of chemical should fall progressively as it courses through the hepatic sinusoids. The proper measure of liver function is its ability to remove, i.e., to clear, circulating xenobiotics (Wilkinson and Shand, 1975) and not the rate of reaction at entry into the organ. Two primary models of hepatic function have been used in recent years. They are the "Well-Stirred" model and the "Parallel Tube" model (Pang and Rowland, 1977). The analysis of hepatic function used herein is based on the parallel tube description. The liver is conceptualized as a group of cylindrical tubes in parallel. Hepatocytes are regarded as being attached to the inside of these tubes with blood flowing unidirectionally over the cells (Figure 4). The rate of reaction at any point within the tube is given by the Michaelis-Menten equation, but the concentration of chemical in the blood is variable.

The velocity of metabolism is given by the amount cleared, i.e., the input concentration (C_{in}) minus the output concentration (C_{out}), times hepatic blood flow, Q (Figure 5). Another useful relation is extraction, E, which is given by:

$$E = \frac{(C_{in} - C_{out})}{C_{in}} \qquad (4)$$

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METABOLIZING ORGAN
(LIVER)

BLOOD

In C_{blood}

time

At any point within liver:

$$\frac{-d\,C_{blood}}{dt} = \frac{Vmax \cdot C_{blood}}{Km + C_{blood}}$$
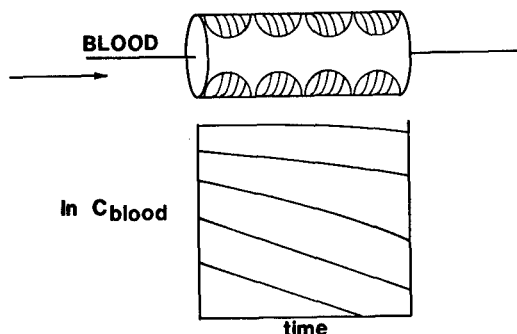
Figure 4. Essential points of the "Parallel Tube" description of hepatic function.

LIVER CLEARANCE

VELOCITY = Q(Cin-Cout)

**DETERMINE (Cin-Cout) BY INTEGRATING
RATE EQUATION.**

$$-\int_{Cin}^{Cout} \frac{(Km + C_{blood})dc}{C_{blood}} = \int_{o}^{t_r} Vmax\, dt$$

Figure 5. Mathematical description of liver clearance of a chemical metabolized by a a single hepatic enzyme system.

$$\Delta Ci\text{-}o = Cin - Cout = \Delta Cmax - Km\ln\frac{Cin}{Cout}$$

$$\frac{\Delta Ci\text{-}o}{\Delta Cmax} = 1 - \frac{Km}{\Delta Cmax}\ln\frac{Cin}{Cout}$$

According to a parallel tube description, the value of $(C_{in} - C_{out})$ can be determined by integrating the rate equation as in Figure 5. The term ΔC_{max} in the integrated equation is the maximum decrease in concentration in mg/ℓ which can occur across the liver at a given value by Q. The term ΔC_{max} is equal to the ratio V_{max}/Q (Pang and Rowland, 1977).

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Even though C_{out} appears on both sides in the last equation in Figure 5, the relationship can be solved reiteratively to calculate $(\Delta C_{i-o}/\Delta C_{max})$ as a function of C_{in}. Two curves were calculated in this way; one curve was constructed for the case where $K_m > \Delta C_{max}$ (Figure 6), and one for $K_m < \Delta C_{max}$ (Figure 7). For the former case the curve, % ΔC_{max} versus C_{in}, is accurately representative of a Michaelis-Menten dependence. This is the expected behavior when the affinity of the enzyme is low (high K_m) in relation to the maximum capacity of the enzyme, ΔC_{max}. On the other hand, when the affinity of the enzyme is high (low K_m) in relation to ΔC_{max}, a curve like that observed with halothane is predicted (Figure 7). The extent of the true first-order portion depends on the ratio of $\Delta C_{max}/K_m$. When the ratio is 10, the break occurs at 70% ΔC_{max}. When it is 5, it occurs between 40 and 50% ΔC_{max}. For halothane, then, the true molecular K_m is expected to be approximately 1/10 ΔC_{max}. When chemicals possess an extended first-order portion, the apparent K_m will differ substantially from the molecular K_m and will approach $\Delta C_{max}/2$. (This can be shown mathematically by setting $C_{in} = K_m$, apparent and $(C_{in} - C_{out}) = \Delta C_{max}/2$ in the last equation in Figure 5).
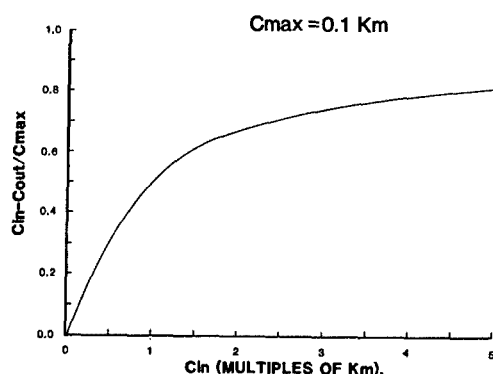
Figure 6. A theoretical curve of percent of maximum clearance versus input concentration to the liver for the case where ΔC_{max} is much smaller than K_m.
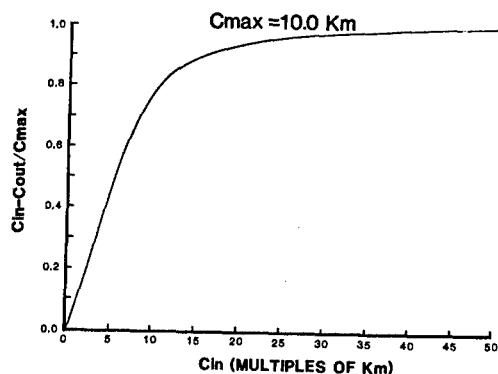
Figure 7. Theoretical curve of percent maximum clearance versus input concentration to the liver for the case where ΔC_{max} is much larger than K_m.

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From this same equation the extraction ratio can be calculated as a function of concentration for various ΔC_{max} to K_m ratios (Figure 8). These curves clearly demonstrate the reason for the first-order portion of the rate curves. With chemicals with a large $\Delta C_{max}/K_m$ ratio, extraction is essentially complete over a large range of input concentrations. Since the circulating concentration is proportional to the inhaled concentration and all the chemical presented to the liver is metabolized, the amount metabolized increases in direct proportion to the atmospheric concentration of chemical until the enzyme system is nearly saturated.

CALCULATED HEPATIC EXTRACTION
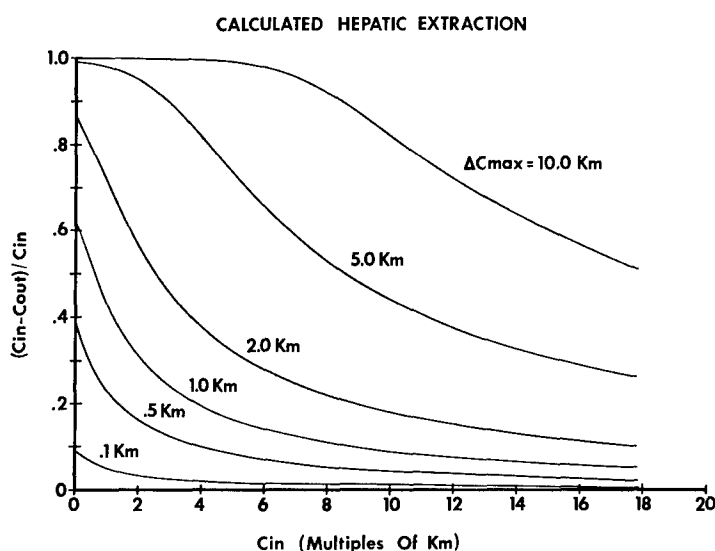
Figure 8. Theoretical hepatic extraction curves for enzyme systems with various ΔC_{max} to K_m ratios. Values of ΔC_{max} as multiples of K_m are shown in the figure.

PULMONARY UPTAKE

For highly extracted chemicals at low concentrations, blood returning to the lung will be 75% saturated. The 25% of the cardiac output going to the liver is completely cleared while the other 75% remains saturated. In general, if the percent of cardiac output to the liver is $[C\ell_h]$, blood returning to the lung will only be $(1 - [C\ell_h]) \times 100\%$ saturated. At equilibrium, the amount metabolized must be replaced by pulmonary uptake denoted by the heavy arrow in Figure 9. A simplistic algebraic analysis of this uptake can be made once an assumption is made concerning the ventilation ($\dot{V}$): perfusion ($\dot{Q}$) ratio in the rat lung. For the present, it is assumed that the $\dot{V}/\dot{Q}$ ratio is 1.0. If the partition coefficient for a test chemical is N, then,

$$\frac{C_{blood}}{C_{alveolar\ (out)}} = N, \tag{5}$$

by definition. But the concentration in the expired air is less than that in inspired air by an amount, $\Delta Conc_{(a)}$, as shown in equation 6.

$$C_{alveolar\ (out)} = C_{alveolar\ (in)} - \Delta Conc_{(a)} \tag{6}$$

This term, $\Delta Conc_{(a)}$, is equivalent to the ratio of the uptake rate (mg/kg/hr) divided by ventilation (ℓ/kg/hr), i.e.,

$$\Delta Conc_{(a)} = \frac{Uptake\ Rate}{Ventilation} \tag{7}$$

In the blood, the increase in concentration is $\Delta Conc_{(b)}$ and is given by:

$$\Delta Conc_{(b)} = \frac{Uptake\ Rate}{Blood\ Flow} \tag{8}$$
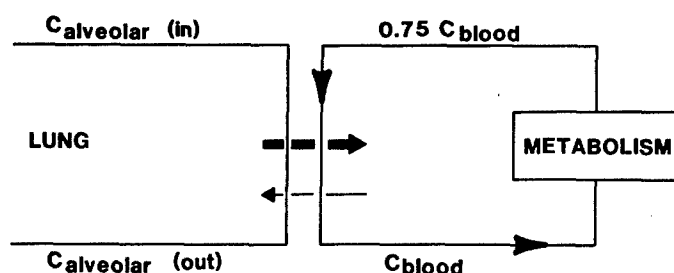
Figure 9. Continued alveolar uptake at equilibrium for a highly extracted chemical. In this figure, $C_{alveolar\ (in)}$ is equivalent to inspired air; $C_{alveolar\ (out)}$ is expired air.

Since $\dot{V}/\dot{Q}$ ratio is unity, the absolute value of $\Delta Conc_{(a)}$ and $\Delta Conc_{(b)}$ are identical. The change in blood concentration is $[C\ell_h]C_{blood}$ which is, by the arguments presented above, equal to $\Delta Conc_{(a)}$. Equation 5 can be rewritten as:

$$N = \frac{C_{blood}}{C_{alveolar\ (in)} - [C\ell_h]\ C_{blood}} \qquad (9)$$

Rearranging, we have

$$\frac{C_{blood}}{C_{alveolar\ (in)}} = \frac{N}{1 + [C\ell_h]N} = N_{eff} \qquad (10)$$

This ratio, the effective partition coefficient for alveolar air (N_{eff}), is significantly different from the partition coefficient in the absence of metabolism, N. If it is again assumed that $[C\ell_h]$ is 0.25, N_{eff} for halothane is only 1.46, compared to its true blood: gas partition coefficient of 2.3. In general, N_{eff} will approach $1/[C\ell_h]$ (or about 4), as the true value of N approaches infinity. During exposure to a chemical with a large partition coefficient at concentrations associated with high hepatic extraction, the circulating blood concentration will be significantly less than expected on the basis of the blood gas partition coefficient measured in vitro. With benzene, N is 7.3, but N_{eff} is calculated to be only 2.6.

A comprehensive rationale for describing the metabolism of inhaled halothane can now be developed (Figure 10). The curve of rate of metabolism as a function of atmospheric halothane concentration can be converted to rate versus C_{blood}. In the linear portion where extraction of halothane by the liver approaches 100%, C_{blood} is equal to $C_{atmosphere}$ times N_{eff}. Errors introduced in this step by using N instead of N_{eff} can be very large, depending on the magnitude of N. The slope of the linear portion of this curve is equal to the hepatic blood flow, and ΔC_{max} is estimated by dividing V_{max} by Q. Finally, in this preliminary analysis, the actual K_m can be estimated from the shape of the curve. The values of these parameters for halothane are given in Figure 11 and those for benzene in Figure 12. The estimates of Q from these metabolism studies were 64 ml/min/kg for benzene and 95 ml/min/kg for halothane. Recently, Pang and Gillette (1979) determined hepatic blood flow in rats by area under the curve analysis with acetoaminophen which is eliminated solely by hepatic metabolism. Their estimate for Q was 78 ± 16 ml/min/kg. The value of Q for benzene is within this range and that for halothane just outside it. Because a metabolite was measured directly and with good sensitivity, we are especially confident of the values obtained with halothane. The high value of Q for this anesthetic vapor, 94 ml/min/kg, suggests that tissues other than the liver are also active in its metabolism in vivo.

Metabolism of Inhaled Halothane

1. Shape of curve implicates perfusion limitation.

2. Calculate N_{eff} [use hepatic clearance].

3. Plot rate versus C_{blood}.

4. Slope of linear portion is:

$$\frac{mg \ (cleared)/kg/hr}{mg/liter \ (blood)} = liter \ (blood)/kg/hr$$

5. Estimate ΔC_{max}:

$$\Delta C_{max} = \frac{V_{max}}{blood \ flow} = mg/liter$$

6. Estimate K_m from shape of curve.

Figure 10. Rationale for a physiologically-based, pharmacokinetic description of the metabolism of inhaled halothane.

Halothane

1. $N_{eff} = 1.46$

2. Slope = 5.71 ℓ/kg/hr
   [95 ml/min/kg]

3. $\Delta C_{max} = \frac{9.72}{5.71} = 1.70$ mg/liter

4. $K_m \cong 0.1 \ \Delta C_{max}$
   $\cong 0.17$ mg/liter

5. $K_{m, app} \cong \frac{\Delta C_{max}}{2} \cong 0.85$ mg/liter

6. Inhalational $K_{m, app} \cong 72$ ppm

Figure 11. Calculated physiological and biochemical constants for halothane metabolism.

Benzene

1. N_{eff} = 2.58   (N = 7.3)

2. Slope = 3.81 ℓ/kg/hr
            = 64 ml/kg/min

3. ΔC_{max} = $\dfrac{2.3}{3.8}$ = 0.60 mg/liter

4. K_m ≅ 0.10 mg/liter

5. $K_{m, app}$ = 0.30 mg/liter

6. Inhalational K_m ≅ 36 ppm

Figure 12. Calculated physiological and biochemical constants for benzene metabolism.

Simple algebraic analysis of pulmonary uptake of gases and vapors for highly extracted chemicals can also be used to explain why values of maximum uptake rate constants (Table 1) clustered toward similar values but were not identical. The proportion of chemical taken up from the inspired air is given by:

$$\frac{[C\ell_h]\ C_{blood}}{C_{alveolar\ (in)}} = [C\ell_h]\ N_{eff} = \frac{[C\ell_h]\ N}{1 + [C\ell_h]\ N} \tag{10}$$

Calculations based on $[C\ell_h]$ = 0.25 for halothane predict that 37% of the inspired halothane will be retained, and for benzene 64% will be retained. Maximum theoretical uptake rate constants are those that would be observed if all inspired chemicals were retained. They are calculated by dividing the observed maximum rate constant by the proportion retained. For halothane, this value is 0.89 $hr^{-1}\ kg^{-1}$ and for benzene it is 0.75 hr^{-1} kg^{-1}. Even though the observed rate constant for benzene was more than 50% greater than that for halothane, the maximum theoretical rate constants are virtually the same (within 15%). With rats in the chamber, the available gas phase was approximately 30 liters. The exchangeable breathing rate for rats is volume times the maximum rate constant for uptake (MRCU). Averaging the values with benzene and halothane, the average MRCU is 0.84 $hr^{-1}\ kg^{-1}$, so the exchangeable breathing rate is 25.2 liters/hr/kg (30 x 0.84). Assuming a dead volume of 20%, the total breathing rate is 31.5 liters/hr/kg. The breathing rate calculated for a 250g rat based on equations described by Guyton (1947) was 32 liters/hr/kg. Again, as in the case of hepatic blood flow, the pharmacokinetic model is internally consistent in that physiological parameters derived from pharmacokinetic data are in agreement with published values for these parameters.

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SUMMARY

In the majority of cases studied to date, regulation of inhalant metabolism is much more complex than suggested by a simple Michaelis-Menten model. Since hepatic blood flow and pulmonary uptake determine the rate of presentation of inhalant to the liver, these physiological processes are rate-limiting for highly extracted chemicals at low concentrations. At higher concentrations, the enzymes involved in metabolism become saturated and the rate of metabolism is then limited by V_{max}. This behavior is reflected by rate curves with extended first-order dependencies and sudden conversion to pseudo-zero order dependencies. Biologically, this is a natural consequence expected when the Michaelis-Menten binding constant, K_m, is small compared to the maximum clearance capacity of the metabolizing organ, ΔC_{max}. A simple Michaelis-Menten model is valid for poorly extracted chemicals throughout the entire concentration range. Because K_m is much larger than ΔC_{max}, both biochemical constants are intimately involved in establishing the overall shape of the rate curve for poorly extracted chemicals.

While V_{max} is an intrinsic biochemical constant related to the number of enzymic centers available for metabolism, ΔC_{max} is a relative term inversely related to hepatic blood flow ($\Delta C_{max} = V_{max}/Q$). Changes in Q will alter the $\Delta C_{max}/K_m$ ratio and thereby change the shape of the rate curve and the extraction ratio. For highly extracted chemicals, blood flow may well limit the metabolism, since it determines inhalant delivery to the liver, but the total amount of inhalant in the blood is regulated by pulmonary uptake, which is dependent in turn on $\dot{V}/\dot{Q}$ ratio, functional whole body clearance of the metabolized chemical, and its partition coefficient, N.

More complete development of a physiologically-based, pharmacokinetic model of inhalant metabolism is amply justified. For instance, the present model is capable of predicting the effect of various parameters such as alterations in breathing rate and depth, changes in pulmonary $\dot{V}/\dot{Q}$ ratios, and redistribution of regional blood flow on the rate of metabolism of inhaled chemicals. Such information could be useful in assessing the hazards of a metabolized chemical under conditions of heavy work as compared to its hazards under sedentary conditions. Even in its rudimentary form, the model is capable of predicting in vivo kinetic behavior based on knowledge of biochemical and physiological constants of the test species. Perhaps the greatest potential of a fully developed physiologically-based model is to provide a better scientific basis for both interspecies comparison of metabolic data and for extrapolation of such data from experimental animals to man. Scale-up to man will be more reliable using a model which incorporates both biochemical and physiological parameters, especially at low concentrations, where physiological processes are more likely to be rate-limiting. Development of improved extrapolative procedures are especially important in cancer risk assessment for chemicals like vinyl chloride (Gehring et al., 1978) and trichloroethylene (Waters et al., 1977) where metabolites are presumed to be the active carcinogens.

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DR. NEWTON (University of California, Irvine): Dr. Ramsey, you mentioned that the expired breath sample that you took was after a forced expiratory maneuver. I'm used to getting alveolar samples after a breath hold and just normal expiration. Are we talking about the same type of sample and if not, why do you use forced expiration? What are the advantages or disadvantages of the two techniques?

DR. RAMSEY (Dow Chemical Company): I think it's probably different from the way you did it. I can't think of any specific advantages to the way we did it. The people were required to breathe out 10 liters of air which was measured in a spirometer after passage through two fritted bubblers containing hexane as a trapping agent for the styrene. There was a great deal of back pressure from the bubblers. This was the status of the method we had developed at the time we carried out the exposure, and we didn't further refine the method. The hexane was also cooled to dry ice temperature so water vapor formed in the sparging tubes. It was literally forced exhalation of mixed air, and I would hesitate to call it alveolar air.

DR. FISEROVA-BERGEROVA (University of Miami School of Medicine): I would also think if you take breath samples after an apneic interval, you would still have some air which was in dead space, and you have to separate that.

DR. BACK (Air Force Aerospace Medical Research Laboratory): Dr. Andersen, it seems to me that kineticists may be putting all their eggs in one basket by looking at only liver uptake and metabolism. Is anyone looking at the same thing in the lung because lung tissue may be the primary site of certain metabolic activities such as that of catecholamines? One pass through the lung and they are gone. Is anybody looking at blood flow through the lung and metabolic factors going on in the lung?

DR. ANDERSEN (Air Force Aerospace Medical Research Laboratory): What I have described is a preliminary model really. I'd like to look at metabolism in various other organs, and I'd like to find out how good the assumption that most of the metabolism is occurring in the liver actually is. I'd like to find out what the contribution of other organs is to clearance. Nonetheless, I think that the data themselves and then the attempts to understand the data show there has to be a certain amount of whole body clearance occurring in the liver. It turns out that this model, at least, is internally consistent when you assume a chemical is about 25-30% cleared. The lung does have microsomal oxidase activity, but it's not a good organ for metabolism of such things as halothane and many of the other materials which we've studied. These compounds are predominantly metabolized in the liver. However, with the metabolism of the halothane where we've measured bromide levels directly, the calculated blood flow exceeds what

you would expect for blood flow through the liver. I believe that indicates there is metabolism in other tissues that has to be considered when one develops a more comprehensive description of metabolism of inhaled gases and vapors. It will require other types of experiments than whole body experiments to completely resolve this point.

DR. FISEROVA-BERGEROVA: I have had some experience with tissue metabolism of halothane and found there was some metabolism of this material in lung and kidney tissue. The metabolism in liver tissue is ten times greater than in lung and kidney. You have to consider kidney metabolism. I would say metabolism takes place in all tissues that are well perfused with the possible exception of the brain.

DR. RAMSEY: I'd like to add a comment to the question about trapping styrene in expired air. One reason for requiring that volume of expired air was because it increased the analytical sensitivity for the method. We could detect about 20 nanograms per liter of air by collecting that large a volume.

DR. ANDERSEN: I have a question for Dr. Ramsey. Were you able to make estimates of the biochemical constants for styrene metabolism based on your pharmacokinetic data? Can you perform those calculations knowing the rate of excretion from the lung and the total rate of removal or clearance from the body?

DR. RAMSEY: Yes, we have calculated the value for the clearance rate constant. It is the metabolic clearance rate constant for styrene in the whole body minus several percent which are due to expiration of unchanged material.

DR. ANDERSEN: Is the amount of the expired unchanged material small when compared to the total clearance?

DR. RAMSEY: Yes, approximately 2%, and therefore the rate constant can really be considered as virtual metabolic clearance. We have also attempted to determine a k_m for that compound in terms of the concentration in inspired air. In other words, the concentration in inspired air which might represent the concentration at which the metabolic clearance is at half maximum velocity. This is a crude estimation. There's a lot of uncertainty in the number, but it's probably somewhere around 440 ppm.

DR. FISEROVA-BERGEROVA: I would like to ask one question, Dr. Andersen. When you calculate C_{max}, you divide V_{max} by blood flow through the liver. How would you compare it with classical Michaelis-Menten kinetics measured in vitro where the V_{max} is a certain way of showing the affinity between the enzyme and substrate?

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DR. ANDERSEN: Yes, C_{max} is the maximum change in concentration of substrate you can get across the liver. It's a function of V_{max}, the number of enzyme sites that are there and blood flow rate. At different flow rates, the amount of substrate metabolized will vary but it's still a function of enzymic centers for metabolism that are present in the liver.

MAJOR MAC NAUGHTON (Engineering and Services Laboratory): Dr. Ramsey, under some circumstances, it's difficult to get an accurate measure of exposure concentration. Do you think your methodology can be standardized enough so that a breath sample can be used as a real measure of the exposure of the individual and his real exposure dose?

DR. RAMSEY: Yes, I think it could be used that way with the caveat of the possible variability between people. If the permissible values or limiting values are set with a reasonable safety factor, then we would expect that the individual variations would still fall within an acceptable range of dose levels.

DR. REISCHL (University of California, Irvine): I would like to address this question to Dr. Fiserova-Bergerova. In many of your calculations, you start with the premise that the substance taken up by the animal is calculated from the difference in inspired minus the expired concentration times the minute ventilation. Do you account for a difference between inspired and expired volume due to inequalities of $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$?

DR. FISEROVA-BERGEROVA: Are you asking if I make a correction for the dead space?

DR. REISCHL: No. Do you correct for CO_2 production not equal to O_2 consumption when the respiratory quotient is not equal to one?

DR. FISEROVA-BERGEROVA: No, I don't make corrections for that.

DR. REISCHL: Could that possibly add error to your calculations? Have you done any error analysis in the overall mathematics to check for mathematical instabilities?

DR. FISEROVA-BERGEROVA: You mean like a second gas effect such as nitrous oxide when uptake increases because of greater oxygen consumption?

DR. REISCHL: Yes, in general, inspired ventilation would be more than expired ventilation. Although that difference is small in your case, it could play a significant role.

DR. FISEROVA-BERGEROVA: We are dealing with exposure concentrations lower than 1%, and I don't believe we need to be concerned with that problem. The error comes when you have gas exposure mixtures of 50% or more when you have to supplement oxygen for air.

DR. HODGE (University of California, San Francisco): Dr. Ramsey, if I understood what you said, the similarity in the pharmacokinetic patterns in the example shown was really quite close between rat and man. I believe that the deduction was made that on these grounds, the rat would be a useful animal in predicting what to do about man. Is that correct?

DR. RAMSEY: In general, yes, as long as there isn't any information to the contrary.

DR. HODGE: Don't you need to know the nature of the metabolites in order to make that kind of assumption?

DR. RAMSEY: You would be far better off if you did now, obviously. It's a stepwise thing with a continuum of gradations. I think the first step might be the clearance or disappearance of the parent chemical and secondly, the appearance in the metabolites that are formed, and then this can become more or less important depending upon the mechanism of toxicity. I consider a kinetic similarity between species only a general indication of similarity of response, and it doesn't necessarily mean that man has an absolutely identical response.

DR. FISEROVA-BERGEROVA: I also think that an important factor in the rat that the human doesn't have is that the rat equilibrates fast. If you want to reach steady state for a human, you have to go with an exposure of hours or days in order to reach equilibrium. With the rat, you can go fast because the perfusion and methylation is related to the surface area which is much greater in the rat. Equilibrium of partial pressures is established much faster in the rat than other species. One consideration you have to take is that a human has an average of about 10% adipose tissue. This adipose tissue is a big dump, and it takes about 48 hours for this fat soluble compound to reach partial pressure equilibirum. In rats, you do it much faster because the fat depot is very small compared to man, and most of the material is metabolized. The uptake rate is the sum of what is stored in the tissues, so if the fat depot is small, then you determine mainly the metabolic rate. From this point of view, a monkey or rat is much better than humans for such studies.

IMMUNOTOXICOLOGY

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INTRODUCTION AND SKIN HYPERSENSITIVITY

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Text of this presentation is not available for publication.

LUNG HYPERSENSITIVITY*

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INTRODUCTION

Passively sensitized human lung and human peripheral leukocyte preparations from allergic donors, when challenged with the appropriate antigens, release several chemical mediators including histamine, slow reacting substance (SRS), and eosinophil chemotactic factors (Lichtenstein, 1977). The release processes which are involved have been extensively described. In each instance, the mediators are actively secreted by a process which is temperature and calcium-dependent, requires metabolic energy, and is controlled by hormone-receptor interactions which influence the intracellular level of cyclic nucleotides (Lichtenstein and Margolis, 1968; Lichtenstein, 1971). While much attention has been given to the possible role of these small molecular weight mediators in inflammatory or allergic reactions, little data has been available pertaining to the possible role of high molecular weight mediators which are also actively secreted during the IgE-mediated response. Recent studies of mast cell systems have revealed that, during secretory events, large molecular weight complexes may be secreted which possess biologically active

molecules. Thus, heparin, bound to a large molecular weight proteogly-can, is secreted and is biologically active (Metcalfe et al., 1979). Our work, in the mast cell and basophil systems, also suggests that biologically active molecules are secreted bound to large molecular weight complexes (possibly fragments of granular matrices). We have described the IgE-mediated release of a high molecular weight complex from both human lung and peripheral leukocytes which has at least three activities associated with it: a tosyl arginine methyl ester (TAMe) esterase activity, a kinin-generating activity, and a Hageman factor cleaving activity (Newball et al., 1975, 1979a, 1979b, 1979c, 1978; Meier et al., 1979). In this discussion, we will address ourselves primarily to the newly described large molecular mediators which we have termed "lung kallikrein-like activities" (LK-A) and "basophil kallikrein-like activities" (BK-A) and only briefly review new information regarding the small molecular weight mediators. We will dis-cuss two models of immediate hypersensitivity: the chopped human lung model and the human peripheral leukocyte model. These two models com-plement each other, and data derived from the two models are generally similar.

EXPERIMENTAL STUDIES OF THE LUNG KALLIKREIN-LIKE ACTIVITIES

Specimens of macroscopically normal human lung that were recovered from lungs usually excised for carcinoma were dissected free of pleura, cartilage and large vessels, cut into fragments ($\cong 100$ mg), washed with tyrodes buffer, then passively sensitized with serum from a ragweed sen-sitive donor (Lichtenstein et al., 1979). Challenge of the sensitized lung fragments with ragweed antigen E (AgE) resulted in a dose response release of histamine (Figure 1); also released were other small molecular weight mediators such as prostaglandin F-2α (PGF-2α) (Adkinson et al., 1979, Figure 2); a prostaglandin with potent agonist effects on human airways (Newball and Lenfant, 1977). Antigen challenge of the lung fragments also led to the release of molecules with lung kallikrein-like activities (LK-A): a TAMe esterase activity (Figure 2), a Hageman factor cleaving activity, and a kinin generating activity (Meier et al, 1979, Figures 3-5).

ANAPHYLACTIC RELEASE OF HISTAMINE FROM HUMAN LUNG
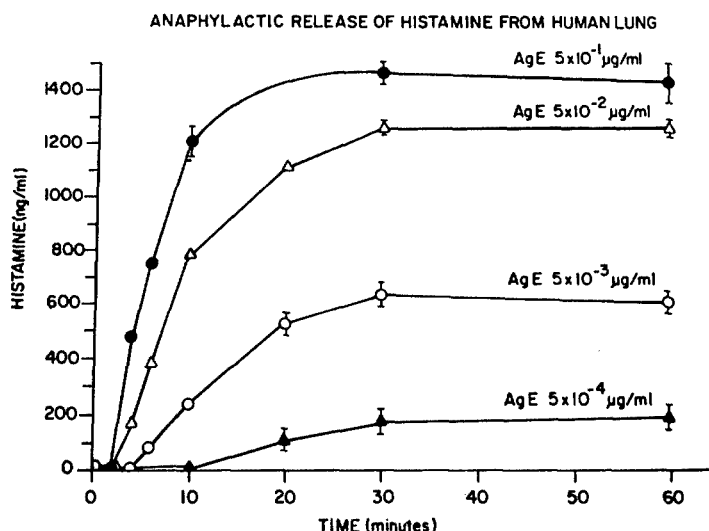
Figure 1. Dose-response and kinetics of AgE-induced hista-mine release from sensitized human lung fragments.

ANAPHYLACTIC RELEASE OF MEDIATORS FROM HUMAN LUNG
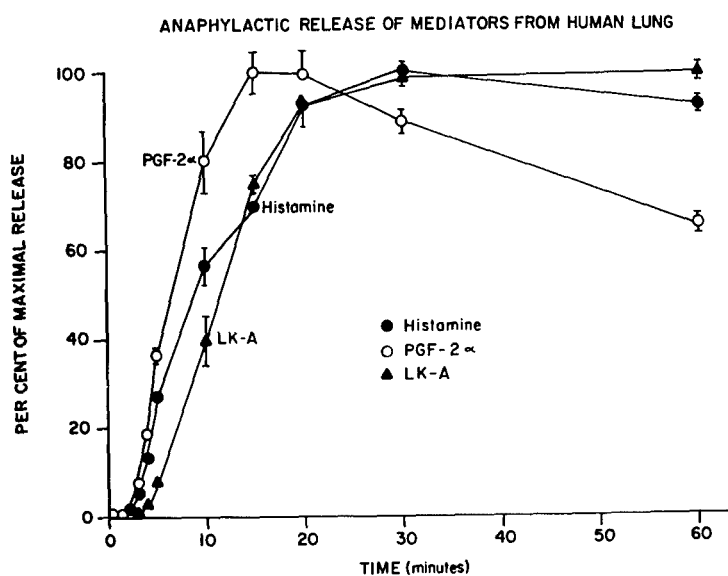
Figure 2. Kinetics of AgE-induced release of histamine, PGF-2α, and LK-A from sensitized human lung fragments.

CLEAVAGE OF SURFACE-BOUND 125I-HAGEMAN FACTOR (HF) BY PLASMA KALLIKREIN AND BASOPHIL KALLIKREIN OF ANAPHYLAXIS (BK-A)
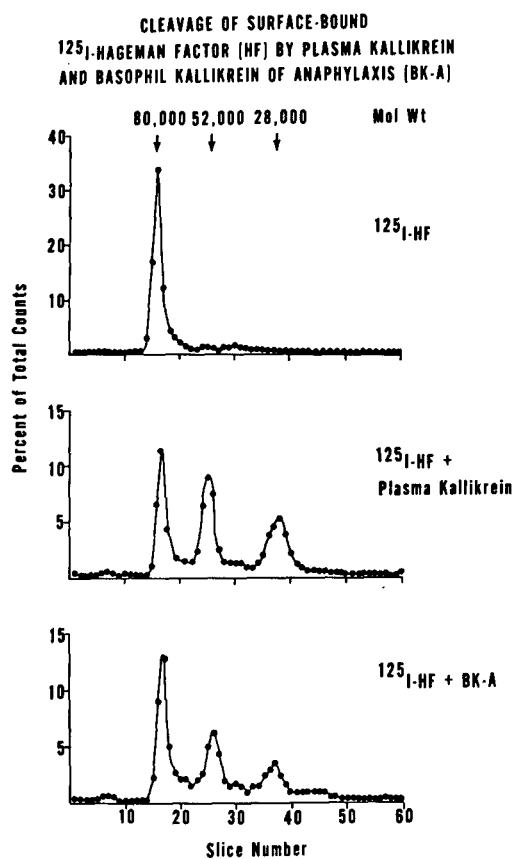
Figure 3. Cleavage of human Hageman factor by preparations of the basophil HF protease. Similar cleavage is obtained with LK-A.

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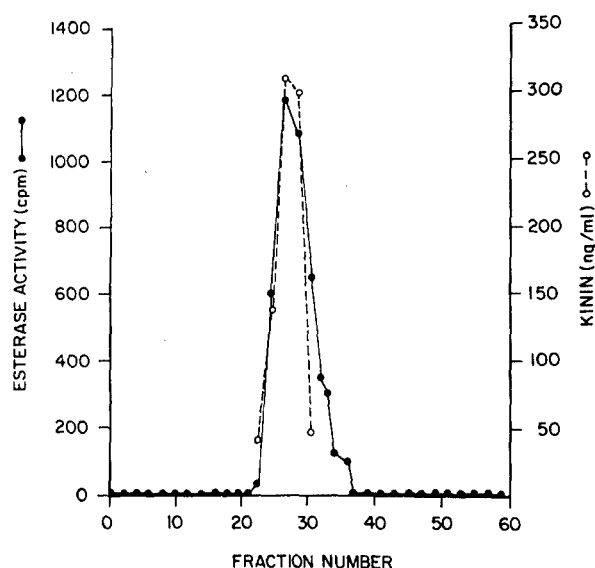
Figure 4. Arginine esterase activity and kinin generation by BK-A chromatographed on Sepharose 6B (cpm x 10^{-1}). Similar kinin generation is obtained with LK-A.
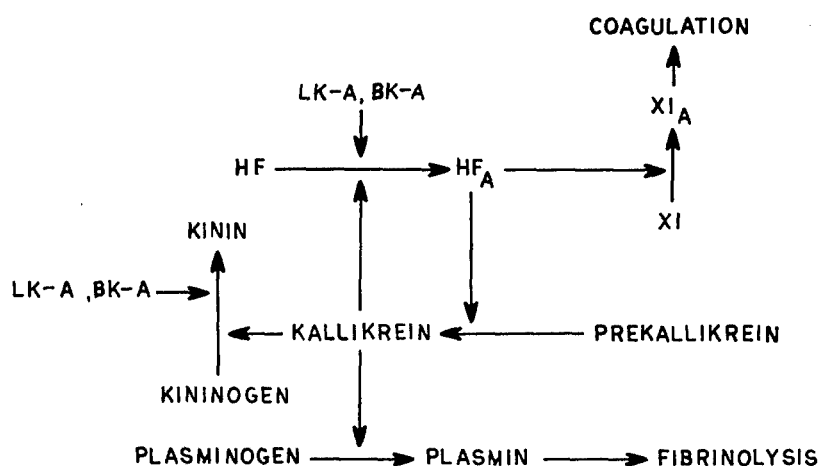
Figure 5. Activation of the Hageman factor dependent pathways of coagulation, fibrinolysis and kinin formation by LK-A and BK-A.

EXPERIMENTAL STUDIES OF THE BASOPHIL KALLIKREIN-LIKE ACTIVITIES

Human peripheral leukocyte preparations, challenged with either purified protein antigens, such as ragweed antigen E, or with highly specific anti-IgE, release a TAMe esterase activity. Our studies on the mechanism of the release of BK-A have primarily concentrated on this esterase activity since its measurement is relatively easy and precise. As will be indicated below, however, it cannot be assumed that TAMe esterase activity parallels each of the biological activities of BK-A.

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A typical dose-response curve for the release of TAMe esterase is shown in Figure 6a. In general, the pattern of histamine and TAMe esterase release is similar, whether the release is initiated by antigen or by anti-IgE. The maximal percentage of histamine or TAMe esterase release has, however, no fixed relationship. This differential release of histamine and TAMe esterase is similar to the observations of others relating to differential release of enzymes from the neutrophil (Wright et al., 1977). In this cell type, which shares a common cell of origin with the basophil, the release of beta glucuronidase from the azurophil granule and lysozyme from the specific granule are often quite different. There have also been reports showing differential release of histamine and SRS-A from mast cells (Orange and Langer, 1973).

While the quantity of histamine and TAMe esterase release may differ, the rate of release of the two mediators shows no significant difference (Figure 6b). Similarly, release of both histamine and TAMe esterase is temperature and calcium-dependent. Not only is the initiation of release of TAMe esterase temperature sensitive but decreasing the temperature at any time during the release process stops the reaction abruptly. The release is also absolutely dependent upon the extracellular calcium concentration and the addition of EDTA at any time during the process causes a similar rapid cessation of release (Newball et al., 1979b).
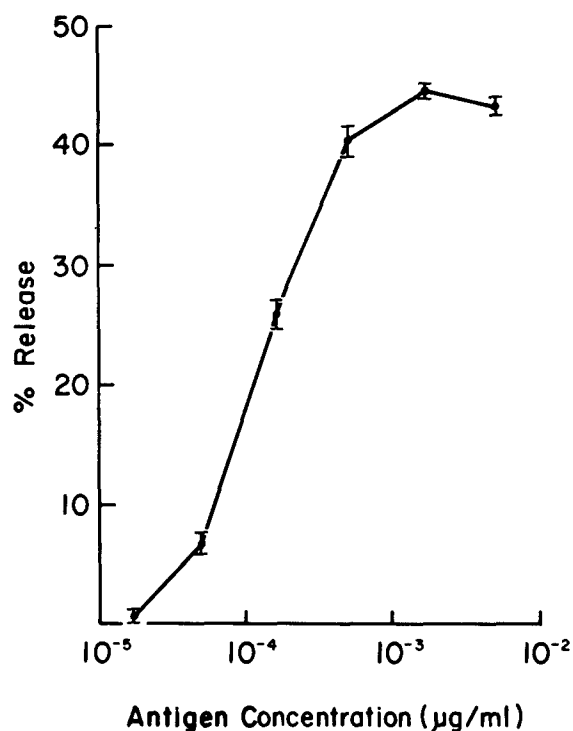
Figure 6a. Dose-response of Rye Group I antigen induced release of a basophil TAMe esterase.
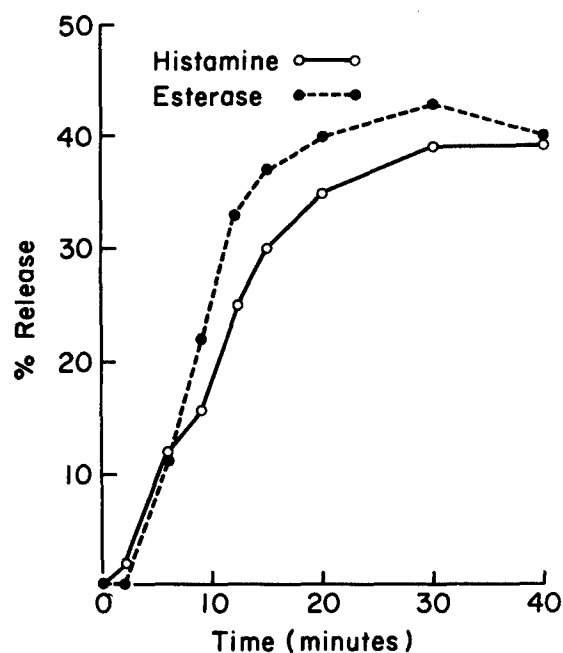
Figure 6b. Kinetics of AgE-induced (17 ng/ml) histamine and TAMe esterase release from basophils.

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Modulation of TAMe esterase release has been studied using the same series of agonists utilized to explore the control of histamine release. Agonists which act on specific receptors to activate adenylate cyclase inhibit the two processes similarly. For example, Figure 7a shows that prostaglandin E_1 inhibits both processes in a dose-dependent fashion. Similar experiments have been carried out with beta adrenergic agonists, histamine, and cholera enterotoxin. In each case, the inhibition dose-response curves were essentially identical. Agents, such as theophylline, which act by inhibiting the cAMP phosphodiesterase inhibit the release of histamine and of esterase with similar dose-response relationships (Figure 7b). Colchicine, which causes disaggregation of microtubules, is a potent inhibitor of histamine release while heavy water (D_2O), which favors microtubular aggregation, is a potent enhancer of this process; together, the two agents are antagonistic, so that their effects cancel. Similar studies with respect to esterase release showed that colchicine inhibited the process while D_2O caused a potentiation of release (Newball et al., 1979b). The need for metabolic processes in the release of TAMe esterase was studied with 2-deoxyglucose, an inhibitor of glucose phosphorylation. These studies indicate that the TAMe esterase release is entirely energy-dependent. The concentration of 2-deoxyglucose required for 50% inhibition of release is similar for the TAMe esterase (0.4 mM) and histamine release (0.3 mM) (Newball et al., 1979b).
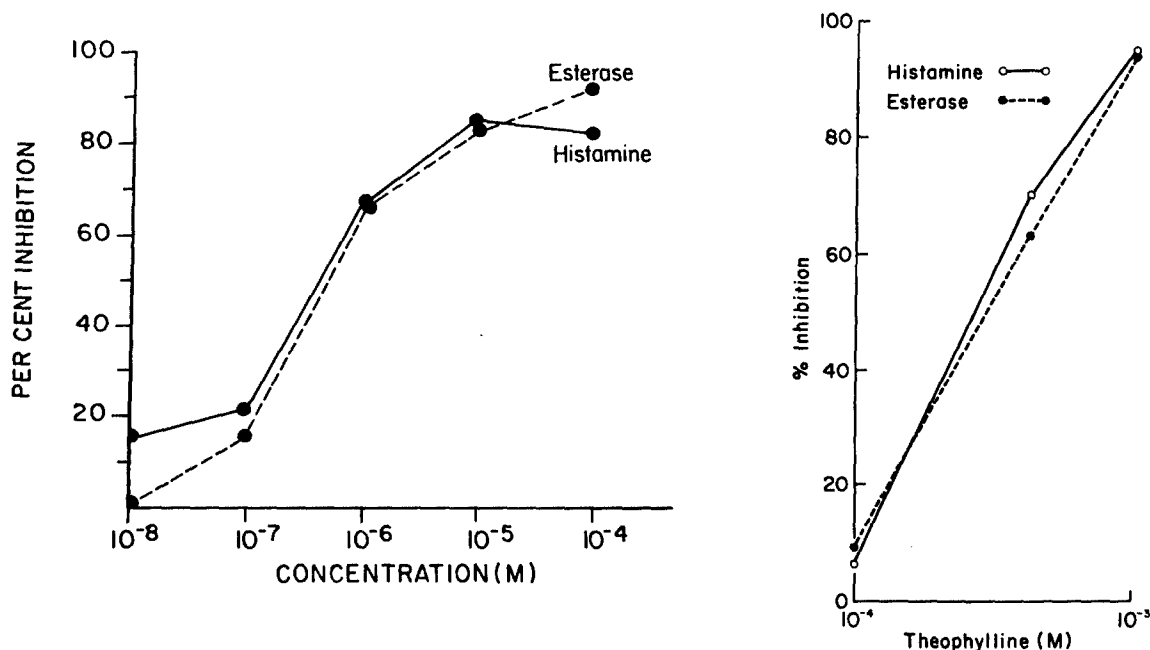
Figure 7. Inhibitory effects of PGE_1 (Figure 7a) and theophylline basophils. For each drug, the molar concentrations required for 50% inhibition of release are similar for histamine and the TAMe esterase.

After demonstrating that the release process and the pharmacologic control of esterase release was similar to that of the previously described mediators, we next attempted to purify the esterase and to ascertain its relationship to the biological activities (cleavage of kininogen to produce immunoreactive kinin and cleavage of Hageman factor) which had been observed in the crude supernatants. For this purpose, large quantities of the basophil supernatant were obtained by challenge of leukocyte preparations with anti-IgE or antigen E and these supernatants were sequentially chromatographed on Sephadex G-200, DEAE-Sephacel and Sepharose 6B. The chromatography on Sephadex G-200 yielded only one TAMe esterase-active peak, which was eluted in the void volume. When this was chromatographed on DEAE-Sephacel and eluted with a linear salt gradient, a single peak of esterase activity was again obtained. However, chromatography of this peak on Sepharose 6B led to a more complex pattern (Figure 8). A major peak of esterase activity was observed which coincided with the first absorption peak and eluted with an estimated molecular weight of 1.2 million. A second esterase peak was present, but was quantitatively of smaller magnitude and smaller molecular weight (c. 400,000). A third arginine esterase peak was variably present and was quantitatively of even smaller magnitude than the two other forms. This third form also had the smallest molecular weight, approximately 70,000.
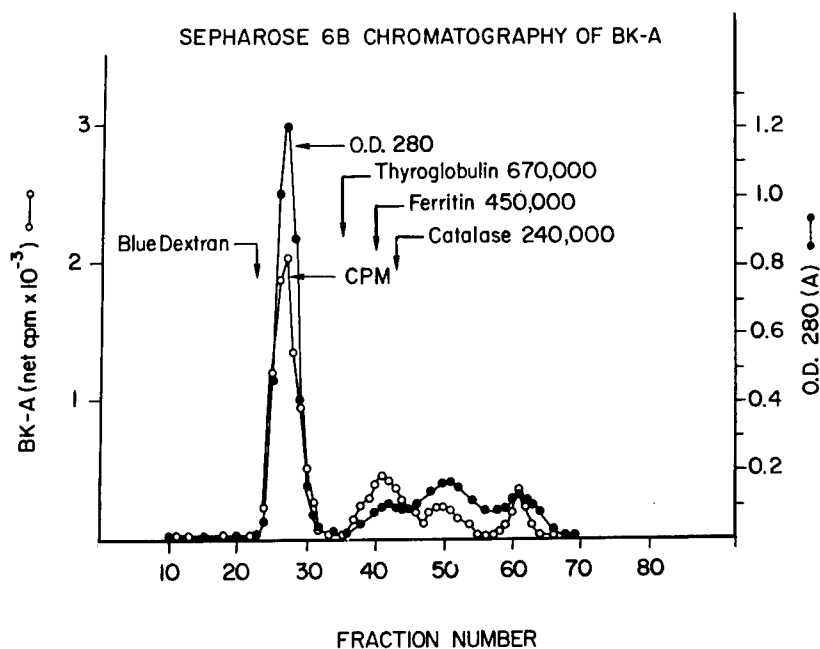
Figure 8. Sepharose 6B chromatography of TAMe esterase active fractions from DEAE-Sephacel.

These fractions were then examined for their ability to generate immunoreactive kinin and to cleave Hageman factor. Figure 4 shows the relationship between TAMe esterase activity and the generation of immuno-reactive kinin from the first, high molecular weight peak of the Sepharose 6B column. It is evident that the two activities co-chromatograph. This assay was carried out with acid treated, heat inactivated plasma. More recently, it has been shown that the same protease can cleave purified, high molecular weight kininogen to yield immunoreactive kinin (Newball et al., 1979a, 1979b).

Similar studies have not been completed with respect to the mediator which cleaves Hageman factor. It is clear, however, that while there is TAMe esterase activity associated with the first two peaks, there is little present in the last, small molecular weight peak; fractions from the last peak were, however, most potent in cleaving Hageman factor.

Activity of the basophil Hageman factor (HF) protease is shown in Figure 3. Radiolabeled Hageman factor was adsorbed to a glass surface after which it was cleaved by either plasma kallikrein or the basophil HF protease. After cleavage, samples were analyzed by electrophoresis on SDS-PAGE as previously described (Newball et al., 1979c, Revak et al., 1978). The top panel shows intact Hageman factor, with a molecular weight of 80,000. The middle panel shows the cleavage of Hageman factor by purified human plasma kallikrein. Part of the radiolabeled Hageman factor remains intact while the rest is cleaved into fragments of approximately 28,000 and 52,000 daltons. The lower panel shows cleavage of Hageman factor by the basophil HF protease. As with plasma kallikrein, part of the radiolabeled Hageman factor remains uncleaved while the rest is split into fragments of 28,000 and 52,000 daltons. Additional studies have determined that the 28,000 molecular weight fragment produced by the basophil enzyme has functional activity as assessed by its ability to activate prekallikrein to kallikrein (Newball et al., 1979c).

STUDIES WITH OTHER MEDIATORS

Perhaps the most interesting recent observation with respect to several of the mediators has to do with the role of arachidonic acid (AA) metabolism in the release process. With respect to histamine release, it has been shown that ETYA, which blocks both the lipoxygenase and cyclo-oxygenase pathways of AA metabolism, completely inhibits the release of histamine. On the other hand, the nonsteroidal anti-inflammatory drugs (NSAID) such as indomethacin, aspirin and meclofenamic acid, which are selective inhibitors of the cyclo-oxygenase pathway of arachidonic acid metabolism, potentiate the release of histamine. These observations suggest that a product of the lipoxygenase pathway is necessary for histamine release and that the NSAID act by shifting arachidonic acid metabolism to that pathway. In keeping with this suggestion was the fact that arachidonic acid itself also potentiated histamine release (Marone et al., 1979). A related observation was that indomethacin and other NSAID selectively block the inhibition of histamine release caused by each of the

agonists (isoproterenol, histamine, adenosine, prostaglandins) which act through specific receptors to increase adenylate cyclase activity. Arachidonic acid has a similar activity. In contrast, neither indomethacin nor arachidonic acid had any effect on the inhibition of release caused by drugs or agents which increase cyclic AMP by other mechanisms. Thus, they did not reverse the inhibition caused by theophylline or isobutyl-methylxanthine which are phosphodiesterase inhibitors; similarly, they had little or no effect on the inhibition caused by dibutyryl cyclic AMP itself (Marone et al., 1979). These data strongly suggest that there must be subcompartments of cyclic AMP which are differently affected by cyclase agonists and by other agents which increase cyclic AMP. It further suggests that not only is there a product of the lipoxygenase pathway necessary for histamine release but that another (or the same) product of that pathway modulates the effects of endogenous hormones or pharmacologic agents on the control of histamine release.

Also related to arachidonic acid metabolism is the recently described structure of slow reacting substance (SRS). Samuelson et al. (1979) have demonstrated that SRS is also derived from the lipoxygenase pathway of arachidonic acid metabolism, with an additional step which incorporates cysteine into the molecule to yield what has been designated leukotriene C. The precise role of SRS in inflammatory conditions is not fully elucidated but, with respect to smooth muscle, the duration of its action is known to be much longer than that of histamine. The definition of the structure of SRS should, for the first time, allow the synthesis of a series of antagonists which can delineate the role of this mediator in inflammatory processes.

Another interesting observation is that the major source of SRS in inflammatory reactions is probably not basophils or mast cells, but other cell types. This has been described by Bach in rat peritoneal cells (Bach and Brashler, 1978), and we have demonstrated that SRS is readily derived from human polymorphonuclear leukocytes (Conroy et al., 1976).

There are a number of eosinophil chemotactic factors (ECF) produced from mast cells and basophils. Two of these have been characterized as tetrapeptides by Austen and his colleagues (Goetzl and Austen, 1975). However, here as well, there is the recent observation that eosinophil chemotactic factors can readily be obtained from polymorphonuclear leukocytes by challenge with the calcium ionophore or as a result of a phagocytic stimulus. There is further evidence that arachidonic acid is either the precursor of the neutrophil ECF or enhances its production (Tesch et al., 1979). Platelet activating factor (PAF) has been described in rabbits as a basophil mediator which causes the secretion of serotonin from platelets. In primate species, however, this lipid mediator also appears to be derived primarily from nonmast cell or basophil sources (Clark et al., 1979).

It is possible to generalize, then, that the nonpreformed lipid mediators (SRS, ECF, PAF) which have previously been associated with IgE-mediated reactions in mast cells or basophils exist in and are commonly released from other cell types. It is likely that these mediators are generated as a result of the increase in phospholipid metabolism which occurs in activated cells, not only during the allergic response, but in other cell types activated by appropriate stimuli.

DISCUSSION

We have tried to point out that, in addition to the small molecular weight mediators of inflammatory reactions, there is a series of high molecular weight mediators which are released from mast cells and basophils following interaction of antigen and IgE antibody. These mediators have the ability to generate kinin from human plasma kininogen and to activate human Hageman factor. The importance of these mediators is illustrated in Figure 5. Thus, LK-A and BK-A, by acting on Hageman factor, are capable of yielding the biologically active 28,000 molecular weight fragment B-HF$_a$ which is capable of activating prekallikrein to kallikrein, which can either cleave plasminogen to produce plasmin or act on kininogen to generate kinin. Each of these activation steps is subject to control so that LK-A and BK-A's additional ability to generate immunoreactive kinin from kininogen is an important "feed forward" aspect of these reactions. These are the first mediators from mast cells and basophils which are capable of interacting with the coagulation and kallikrein-kinin systems to generate biologically relevant peptides. In fact, this represents the only known IgE-mediated pathway into the Hageman factor dependent systems.

We have previously suggested that certain of the chemical mediators, such as histamine, participate not only in immediate-type inflammatory reactions but also function in important aspects of the entire inflammatory process. While the role of LK-A and BK-A in immediate hypersensitivity reactions and in other inflammatory processes is not clear, they do represent a first and important interface between IgE-mediated reactions and the Hageman factor dependent pathways of the inflammatory response. By virtue of these activities, LK-A and BK-A may influence mechanisms which are involved in both subacute and chronic, cell-mediated inflammatory processes.

There has been recent interest in the involvement of IgE-mediated mechanisms in delayed reactions and in cutaneous basophil hypersensitivity, both of which represent subacute or chronic phases of inflammation. Dvorak and his associates have shown that, in these types of reactions, there is activation of the coagulation system (DeShazo et al., 1979). Perhaps, during an IgE-mediated lung reaction, mast cells and basophils secrete the above-described proteases which activate the Hageman factor dependent pathways, thus resulting in the inflammatory processes

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which are observed during delayed skin reactions. Studies by Dvorak and his associates and by Askenase also show the deposition of fibrin in and around inflammatory lesions associated with cutaneous basophil hypersensitivity (Dvorak and Dvorak, 1974; Askenase, 1977). It is possible that the basophils, through the mechanisms described above, activate the coagulation system and are responsible for the fibrin deposition. In addition to being involved in subacute and chronic inflammatory reactions, the mediators described here may be involved in more acute reactions. Thus, we have reported the consumption of coagulation factors during human anaphylaxis (Kaplan et al., 1977) and Pinckard and his associates have observed the same phenomenon in systemic anaphylaxis in rabbits (Pinckard et al., 1975). There has been clinical evidence that there are coagulation abnormalities in anaphylactic reactions, and LK-A and BK-A may well provide the mechanism by which this occurs.

The basic biochemistry of the coagulation system and the kinin generating system has been appreciated for a considerable period of time. How this is activated in vivo has not been clear, particularly from an immunologic point of view and the mediators we have described represent the first link between antigen activated reactions and the Hageman factor dependent systems.

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THE EFFECTS OF TOXIC CHEMICALS
ON THE IMMUNE SYSTEM:
THE INTERACTION OF MACROPHAGES WITH
GLUCOCORTICOIDS AS A MODEL SYSTEM

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INTRODUCTION

Mononuclear phagocytes form a first line of host defense, coming into contact with toxic chemicals, infectious agents, and foreign materials from the environment. Macrophages are not merely resident scavenger cells; these versatile cells are important in antimicrobial strategies, cell-mediated immunity, delayed hypersensitivity reactions, and destruction of neoplastic cells (North, 1978). They have extensive synthetic potential and may secrete such diverse molecules as lysosomal hydrolases, interferon, proteinases, complement components, and H_2O_2 (Table 1). These functions determine when these cells are helpful and when they produce damage. For example, airborne hazardous materials penetrate to varying depths in the lung depending to a great extent on their size and on the integrity of the ciliated epithelial lining of the bronchioles (Newhouse et al., 1976). Alveolar macrophages present in bronchioles may ingest these materials and then be cleared via mucociliary transport into the gastrointestinal tract. However, because alveolar macrophages present in terminal alveoli turn over more slowly, particulate materials reaching deep into the lung may remain fixed in alveoli and interstitial connective tissue for long periods of time (Sorokin and Brain, 1975).

There is a strong correlation between inhalation of materials and lung disease in man. It is well known, for example, that coal dust, asbestos, and silica, alone and in synergy with toxins from cigarette smoke, can lead to chronic obstructive lung disease (Doll, 1977; Higginson, 1977; Morgan, 1975; Selikoff et al., 1968; Werb and Dingle, 1976).

TABLE 1. SECRETION PRODUCTS OF MACROPHAGES*

Enzymes

Lysozyme	Plasminogen Activator
Arginase	Collagenase
Esterases	Elastase
Lysosomal Hydrolases	Other Neutral Proteinases

Proteins

Complement C2	Endogenous Pyrogen
Complement C3	
Complement C4	Interferon
Complement C5	
Properdin Factor B	α_2-Macroglobulin
Properdin Factor D	

Low Molecular Weight

Prostaglandin E_1	Thymidine
Prostaglandin E_2	$O_2 \cdot$
cAMP	H_2O_2

Factors Affecting Other Cells

B-Cell	Cytolytic
T-Cell	Angiogenesis
Fibroblast	Listericidal

*From Cohn (1978), Page et al. (1978), and Gordon (1977).

There are several potential mechanisms by which macrophages could participate in the response to environmental pollutants. Interaction with toxic chemicals may induce secretion of enzymes active in extracellular degradation of connective tissue matrix. Macrophages are known to secrete elastase (Werb and Gordon, 1975b), collagenase (Werb and Gordon, 1975a), plasminogen activator, and other proteinases in response to phagocytic stimuli. Because elastinolytic enzymes produce emphysematous changes in the lung (Kaplan et al., 1973), and because the number of macrophages increases in chronic lung disease, it is possible that macrophage elastase secretion may initiate irreversible damage to lung connective tissue.

Because macrophages have only a limited capacity for division in peripheral tissues such as lung, the effects of mutagenic substances such as benzo(a) pyrene acting directly on the macrophage genome are unlikely to be significant. However, alveolar macrophages contain inducible aryl

hydrocarbon hydroxylase (McLemore et al., 1977). By metabolizing and fixing mutagenic substances in foreign body granulomas, these long-lived cells may permit hazardous substances to accumulate and to persist for long periods of time where they may interact with other agents, e.g., from cigarette smoke, and thereby promote a multi-factorial disease process.

Macrophages activated by exposure to pollutants also may produce factors that promote the growth of other cells. Macrophages exposed to latex or silica particles have been shown to produce substances promoting fibroblast proliferation and collagen synthesis in vitro (Leibovich and Ross, 1975; Richards and Wusteman, 1974), and macrophages produce angiogenesis factors that promote the growth of blood vessels (Polverino et al., 1977). Although the nature of these substances has not been determined, proteolytic enzymes from platelets and plasma are known to be mitogens (Chen and Buchanan, 1975; Rutherford and Ross, 1976), and secreted proteinases from macrophages could have similar properties. Proteolytic enzymes such as macrophage plasminogen activator may activate other cells such as lymphocytes and fibroblasts. These macrophage factors could be responsible for granuloma formation (Adams, 1976) and initiation of lung fibrosis.

Macrophages may also be involved in host response to tumor growth. Although the mononuclear phagocyte system is involved in the killing of tumor cells (Currie, 1976), there is also recent evidence that macrophage granulomas may provide a conditioned environment that permits certain tumor cells to grow. Macrophages elicited by the alkane, pristane, permit growth of developing plasmacytoma cells both in culture and in vivo (Cancro and Potter, 1976).

The macrophage is a primary effector cell in cell-mediated and humoral immunity. Immunogenic compounds resulting from tissue injury by pollutants may be responsible for mobilizing the immune response that occurs in chronic environmentally produced lung disease. In addition to processing antigenic materials, macrophages become "activated" in immunological reactions (Cohn, 1978; Currie, 1976; Karnovsky and Lazdins, 1978). The ability of substances to mediate complement activation can be correlated with the intensity of the inflammatory response to these substances. For example, carrageenin, an activator of the alternative pathway of complement, is much more inflammatory than the equally indigestible polyvinylpyrrollidone, which does not activate complement (Werb and Dingle, 1976). Additionally, macrophage proteinases acting on complement components and connective tissue proteins produce chemotactic activity that could recruit more macrophages, and the local production of complement components by macrophages could exacerbate the local tissue response.

Because the effects of toxic chemicals on the mononuclear phago-cyte system can be complex, it is necessary to consider susceptible stages of differentiation and whether effects are directly mediated by macrophages as targets (Table 2). As a model for an immunotoxico-logical investigation, let us consider the interaction of dexamethasone, a glucocorticoid hormone, with macrophages in culture and in vivo.

TABLE 2. CONSIDERATIONS IN IMMUNOTOXICOLOGICAL INVESTIGATIONS ON MACROPHAGES

1. What is the evidence for macrophage-mediated response in an in vivo test system?
2. Are the responses the result of direct effects on macrophages or indirect effects via other cells such as lymphocytes, or via humoral factors such as complement?
3. Can these effects be monitored in a test system using isolated macrophages in culture?
4. What are the mechanisms of interaction of the chemical with macrophages, including dose responses, uptake, and metabolism?
5. What range of responses can be monitored in the test system in culture? Do all response systems monitored behave similarly or does the interpretation depend on test system?
6. How do analogues of the chemical act in this test system?
7. Which stages of differentiation and maturation in the mononulcear phagocyte system respond? Do stem cells and proliferative stages have sensitivities different from mature macrophages? Are re-sponses tissue-specific?
8. Can the response of macrophages in vivo be related to the test system in culture? Are responses species-restricted?

THE INTERACTION OF GLUCOCORTICOIDS WITH MACROPHAGES

Glucocorticoids have a major role in the therapy of inflammatory and immunologically mediated diseases. The precise mechanism of the suppressive and anti-inflammatory effects of these drugs is still unknown, but the functions of the mononuclear phagocyte system are generally believed to be sensitive to glucocorticoid action. During glucocorticoid administration in vivo, monocytopenia occurs (Thompson and van Furth, 1970, 1973) and monocytes fail to accumulate at inflammatory sites (Can-cro and Potter, 1976; Leibovich and Ross, 1975). In the presence of glucocorticoids, macrophages do not respond to macrophage migration inhibitory factor (Balow and Rosenthal, 1973) and fail to become acti-vated (Balow and Rosenthal, 1973; North, 1971; Vassalli et al., 1977). In experiments in vivo, it is difficult to determine whether glucocorti-coids are acting directly on macrophages or indirectly on lymphocytes and other cells that produce mediators of macrophage function. Mono-cytes and macrophages from man and experimental animals are easily obtained in pure populations and can be maintained in culture for

extended periods. Because hormones regulate the availability and several basic functions of macrophages, such studies with glucocorticoids also give insight concerning the participation of macrophages in inflammation and cellular immunity.

Macrophages may serve as a direct target for the therapeutic actions of anti-inflammatory steroids because they contain specific, high affinity receptors for glucocorticoids (Werb et al., 1978a, 1978b). Interaction of hormones with their specific receptors is the first step in their biological activity in virtually every target tissue. Hormones may act directly or via second messengers to regulate functions of membranes and proteins and to induce differential gene expression (Baxter and Funder, 1979). Thus, identification and characterization of hormone receptors is essential in the analysis of molecular mechanisms of hormonal regulation of cellular function.

Glucocorticoids permeate target macrophages by free diffusion and then combine with high affinity protein receptors in the cytoplasm (Table 3). The resulting hormone-receptor complex undergoes a temperature-dependent activation and then enters the nucleus, where it binds to chromatin acceptor sites. This view is supported by data indicating that in intact macrophages dexamethasone binds to cytoplasmic receptors at 3°C and shows temperature-sensitive translocation so that at 37°C the hormone-receptor complexes are predominantly associated with the nuclei (Werb et al., 1978a, 1978b). Glucocorticoid receptors in cell-free cytosol preparations of macrophages behave in a manner similar to receptors from intact cells. Dexamethasone added to cytosol at 3°C binds to the receptors, but these hormone-receptor complexes do not bind to nuclei until they are thermally activated when the cytosol is warmed to 25°C (Werb et al., 1978b). After activation, the macrophage glucocorticoid-receptor complexes bind equally well to nuclei of macrophages and thymocytes; it is likely that these receptors are identical proteins in many cell types. It now seems clear that the glucocorticoid receptor in the cytoplasm is itself taken up into the nucleus, where it is involved in specific interactions with chromatin sites. In the presence of glucocorticoids, the receptors remain in the nucleus, but on removal of steroid from the medium, the glucocorticoid-receptor complex dissociates and the original receptors recycle to the cytoplasm. This recycling does not require RNA or protein synthesis (Rousseau et al., 1973).

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TABLE 3. PROPERTIES OF GLUCOCORTICOID RECEPTORS OF MONONUCLEAR PHAGOCYTES

Cell Type	Species	Receptors Per Cell	Dissociation Constant, K_d (nM)	Reference
Monocyte	Man	7000	5.1	Lippman and Barr (1977)
	Man	9000	7.7	Werb et al. (1978a)
Peritoneal macrophage (resident)	Mouse	6500	1.9	Werb et al. (1978a)
Peritoneal macrophage (thioglycollate-elicited)	Rabbit	5700	2.3	Werb (1979)
Peritoneal macrophage (thioglycollate-elicited)	Mouse	4300	3.7	Werb et al. (1978a)
Peritoneal macrophage (endotoxin-elicited)	Mouse	5900	2.8	Werb (1979)
Pulmonary macrophage (resident)	Rabbit	5200	1.1	Werb (1979)
Pulmonary macrophage (adjuvant-elicited)	Rabbit	4600	1.8	Werb et al. (1978a)
P388D1	Mouse	4200	4.1	Werb et al. (1978b)

EFFECTS OF GLUCOCORTICOIDS ON MACROPHAGE FUNCTION

Glucocorticoids have effects on the growth and differentiation of macrophages and on their function in the inflammatory response. Both the catabolic and anabolic effects of glucocorticoids result from the binding of these hormones to their receptors.

During glucocorticoid administration in vivo, monocytopenia occurs (Thompson and van Furth, 1970, 1973) as well as reduced chemotaxis of monocytes to inflammatory sites (Leibovich and Ross, 1975). The formation of monocytic and granulocytic colonies from committed bone marrow precursors in vitro is decreased by dexamethasone (Figure 1), although it is unlikely that the precursor cells are killed by the steroid (Werb, 1978). Progesterone, which competes with dexamethasone for binding to the glucocorticoid receptor but does not itself bind, antagonizes the inhibitory effects of glucocorticoids on colony formation (Golde et al., 1976).
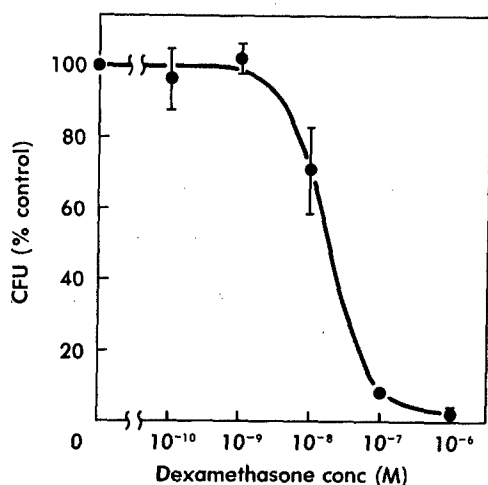
Figure 1. Dexamethasone inhibition of cell division during formation of monocytic and granulocytic colonies from mouse bone marrow precursor cells in culture. The formation of monocytic and granulocytic colonies from committed bone marrow precursor cells (CFU-C) was determined as described by Werb (1978). The effect of dexamethasone on the number of CFU-C that formed after 7 days of incubation was determined. Inhibition of CFU-C was seen at 20 nM dexamethasone, and very few colonies were found at 100 nM or higher concentrations. However, many clusters containing fewer than 50 cells were seen at several concentrations of the steroid (Werb, 1978), suggesting that the decreased number of colonies was due to a diminution in the rate of cell division, rather than to the lysis of the precursor cells.

Although the concentration of glucocorticoid receptors decreases with increasing states of macrophage differentiation (Werb et al., 1978a), stem cells, monocytes, resident macrophages, and inflammatory macrophages from various tissues all respond to glucocorticoids at physiological concentrations. The effects of glucocorticoids on macrophage function are summarized in Table 4. A wide variety of target molecules are modulated by glucocorticoids.

In most tissues, inhibition of glucose metabolism and alterations in adenosine triphosphate economy are early catabolic responses to glucocorticoids (Munck, 1968; Thompson and Lippman, 1974). Although glucose consumption by macrophages is reduced by dexamethasone, this step is probably not the primary site of glucocorticoid action because cytochalasin B-treated macrophages, in which glucose transport is inhibited, do not have the phenotype of steroid-treated macrophages and still respond to dexamethasone (Werb and Chin, manuscript in preparation).

Inhibition of macrophage proteinase synthesis and secretion is a specific action of glucocorticoids (Werb, 1978). Secretion of lysozyme is unaffected by as much as 1 μM dexamethasone, whereas secretion of plasminogen activator, fibrin-degrading neutral proteinase, collagenase, and elastase by macrophages in vitro is sensitive to 1 nM dexamethasone. Inhibition of elastase by glucocorticoids was seen by 6 hours after treatment and was maximum by 24 hours. After removal, the inhibitory effect of dexamethasone was reversed by 24 hours (Figure 2). Secretion of elastase and plasminogen activator occurs independently in other cell systems, but they seem to be inhibited coordinately by glucocorticoids in macrophages. The inhibitory effects on the proteinases are detected at concentrations well below that required for the half-saturation of the hormone receptor. It is not yet clear whether the inhibitory effects on proteinase secretion by macrophages are due to decreases in proteinase secretion or

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activation, or to increases in the synthesis of specific inhibitors of the enzymes. At the same time that glucose consumption and proteinase secretion are inhibited, glucocorticoids induce angiotensin-converting enzyme (Friedland et al., 1977, 1978). These observations make it difficult to explain the alteration of gene expression by glucocorticoids as a single event.

TABLE 4. EFFECTS OF GLUCOCORTICOIDS ON MACROPHAGE FUNCTION

Physiological Function	Concentration Producing 50% Maximum Effect (nM)	Effect	Reference
Monocytic colony formation	10	Decrease	Golde et al. (1976); Werb (1978)
Secretion of neutral proteinases	10	Decrease	Vassalli et al. (1976, 1977); Werb (1978); Werb et al. (1978a)
Glucose metabolism	10	Decrease	Werb (1978)
Prostaglandin synthesis	10	Decrease	Bray and Gordon (1976); Tashjian et al. (1975)
Macrophage migration inhibition	1000	Decrease	Balow and Rosenthal (1973); Leibovich and Ross (1975)
Lysosomal enzyme secretion	100	Decrease	Ringrose et al. (1975)
Differentiation	100	Decrease	Viken (1976)
Pyrogen production	1000	Decrease	Dillard and Bodel (1970)
Angiotensin-converting enzyme	1	Increase	Friedland et al. (1977, 1978)
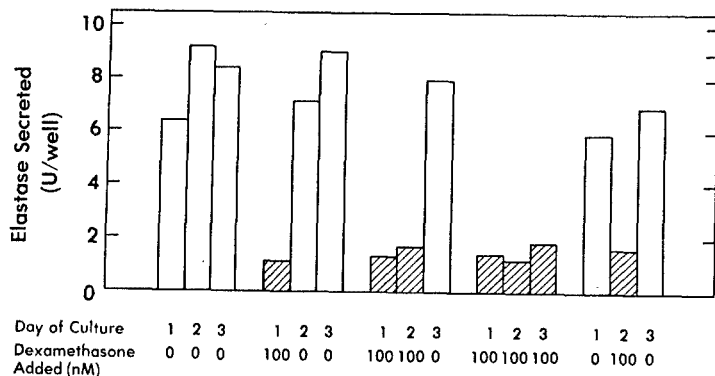
Figure 2. Reversibility of the inhibitory effects of dexamethasone on elastase secretion by thioglycollate-elicited mouse macrophages. Macrophages (2×10^6) were cultured as described by Werb (1978) in 35-mm-diameter plates in Dulbecco's modified Eagle's medium (DME) + 10% fetal calf serum for 48 hours, washed 3 times in DME, then placed in 1 ml of DME containing 0.2% lactalbumin hydrolysate (DME-LH) with or without dexamethasone. After 24 hours (day 1), conditioned medium was collected, the cultures were washed, and the cells were replaced in DME-LH with or without dexamethasone for a second 24-hour period (day 2); then the conditioned medium was collected, the cells were washed, and the cultures were replaced in the medium for a third 24-hour collection (day 3). The elastase activity of each collection was assayed in the ^{3}H-elastin-SDS assay (Werb, 1978). Each value represents the mean of 2 dishes. The effects of glucocorticoids occurred with 24 hours of treatment and were reversed in a similar time period.

It is generally accepted that the interaction of glucocorticoid-receptor complexes with the genome triggers the characteristic biological response. Productive gene activation is achieved when receptor molecules occupy multiple sites in the same region of the gene. The observation that the macrophage response to glucocorticoids may be both dose and time dependent (Werb, 1979) is difficult to interpret in view of the fact that steroid binding reaches equilibrium within minutes (Werb et al., 1978a). These data suggest that a certain threshold must be achieved before the glucocorticoid response can be triggered.

Glucocorticoids may have anti-inflammatory effects on macrophages by inhibiting prostaglandin or proteinase synthesis, pyrogen production, or response to macrophage migration inhibitory factor (Table 4), while other metabolic functions, including viability, are little affected (Table 5). Phagocytosis and intracellular killing by macrophages are unaffected by physiological and pharmacological doses of glucocorticoids (van Zwet et al., 1975; Weiner et al., 1972). Glucocorticoids may decrease the number of Fc receptors on macrophages at high concentratons of hormone (Atkinson et al., 1973; Hunninghake and Fauci, 1977; Rinehart et al., 1974; Schreiber et al., 1975).

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TABLE 5. METABOLIC FUNCTIONS OF THIOGLYCOLLATE-ELICITED
MOUSE PERITONEAL MACROPHAGES NOT AFFECTED
BY THE PRESENCE OF DEXAMETHASONE*

Metabolic Function	Dexamethasone Concentration (nM)	Activity (% Control)
[^{35}S]Methionine Incorporation	0.1	106 ± 7
	10	91 ± 0
	1000	96 ± 1
Phagocytosis (latex)	10	98 ± 7
	1000	91 ± 2
Phagocytosis (EIgG)	10	87 ± 11
	1000	92 ± 4
Viability	10	103
	1000	96

*[^{35}S]Methionine was measured for 5 hours after addition of the hormone; phagocytosis of latex particles and antibody-coated sheep erythrocytes (EIgG) was measured in a 30-minute pulse 5 hours after addition of the hormone. Viability was determined by trypan blue exclusion 24 hours after addition of dexamethasone. Values are mean ± S.D. for 3 replicate cultures.

The inhibitory effects on proteinase secretion described could be either direct effects of glucocorticoids on genetic loci that control synthesis and processing of these proteins by macrophages or indirect actions on loci that determine effector molecules, which then regulate proteinase secretion by macrophages. Prostaglandins, which are mediators of inflammation, are secreted by stimulated macrophages. In various cell types, production of prostaglandins is inhibited by glucocorticoids that block the release of arachidonic acid from phospholipids (Hong and Levine, 1976). Thus, it is possible that prostaglandins mediate some of the effects of steroids on macrophages; however, it is unlikely that they account for all the effects of glucocorticoids because exogenous prostaglandins do not overcome the inhibition by dexamethasone, and indomethacin, an inhibitor of prostaglandin synthetase, does not have the same effects as glucocorticoids on physiological functions of macrophages (Werb and Chin, manuscript in preparation). Other regulatory molecules that influence macrophage functions may also be mediators of glucocorticoid action. Lymphokines, cyclic nucleotides, or proteinases secreted by the macrophages themselves may mediate the inhibitory effects of glucocorticoids.

Because products of other cells, particularly lymphocytes, modulate macrophage functions, it is important to determine whether or not glucocorticoids act directly on mononuclear phagocytes. In vivo, lymphocytes may be glucocorticoid target cells for functions expressed by macrophages. Primary cultures of macrophages are contaminated by adherent lymphocytes and fibroblasts, which could be the target sites of hormone action. In the more homogeneous cultures of peritoneal and alveolar macrophages, it is likely that the glucocorticoids act directly, because populations of macrophages contaminated to various degrees by nonadherent cells do not respond differently to the steroids. Additionally, homogeneous populations of macrophages derived from tumors respond to glucocorticoids in many respects like inflammatory macrophages (Ralph et al., 1978; Werb et al., 1978b).

Mononuclear phagocytes treated with glucocorticoids phenotypically appear to be less differentiated or stimulated than untreated macrophages because of their decreased secretion of proteinases and their decreased metabolism (Cohn, 1978; Karnovsky and Lazdins, 1978; North, 1971, 1978). However, when the effects of glucocorticoids on macrophages are examined by studying biosynthesis and secretion of proteins labeled with [^{35}S] methionine (Figures 3 and 4), it becomes clear that the hormone-treated macrophages do not show a secretion phenotype of a less "activated" macrophage. Instead, glucocorticoid treatment results in the expression of a glucocorticoid-specific domain of response (Werb and Chin, manuscript in preparation). By using the glucocorticoid-induced secreted protein, D23, as a probe, it is clear that only glucocorticoids induce the specific phenotype (Figure 3). All mouse mononuclear phagocytes treated with glucocorticoids in culture show induced secretion of D23. Macrophages from animals treated with glucocorticoids in vivo also show induction of D23 (Figure 4). Therefore, our test system using cultured macrophages is related to some effects of the hormone in vivo.

CONCLUSIONS

Using dexamethasone as a model compound for investigating the effects of drugs on the mononuclear phagocyte system, I have been able to fulfill the criteria outlined in Table 2. I have demonstrated the uptake and metabolism of the hormone by macrophages in culture, that the effects occur directly on macrophages, and the mononuclear phagocytes from precursor stem cells to mature macrophages show some glucocorticoid-specific responses. Only glucocorticoids, and not sex steroids or chemical analogues, have glucocorticoid effects. Although a number of tests suggest that steroid-treated macrophages are less differentiated than control cells, in fact, the glucocorticoids induce unique phenotypic expression by the macrophages in protein biosynthesis studies. Using the labeling tests, I have shown that macrophages treated with glucocorticoids in vivo respond by inducing at least one specific new protein identical to that induced by treatment of cells in culture. These data show the importance of using several independent test systems. Similar studies with other chemicals should yield valuable data about changes in specific phenotypic expression of macrophages.
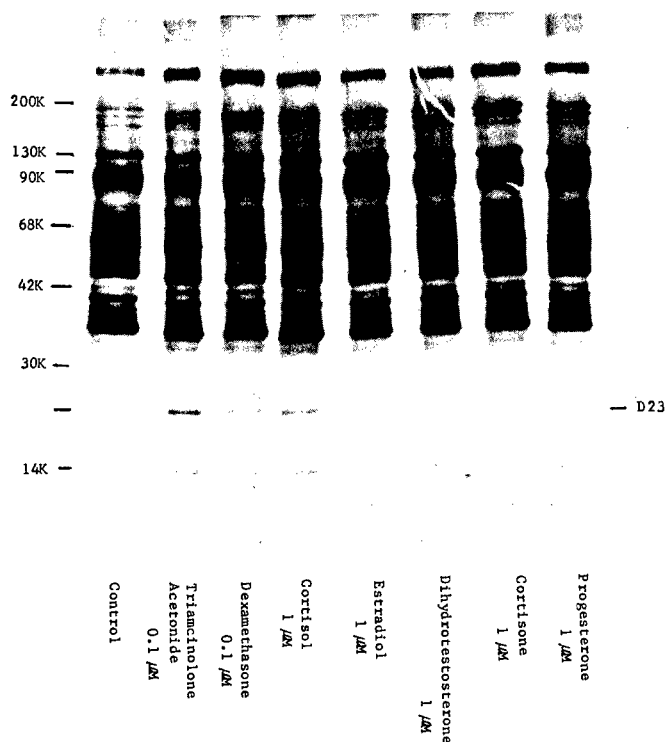
Figure 3. Induction of glucocorticoid-specific macrophage secreted proteins. Thioglycollate-elicited mouse peritoneal macrophages (1 x 10^6/well of a 12-well Linbro culture plate) were cultured in DME-LH for 24 hours in a CO_2 incubator at 37°C in the absence or presence of steroid hormones at the concentrations indicated. The cells were then washed and placed in 1 ml of methionine-free DME containing steroids and 25 μCi/ml [^{35}S]methionine (> 1000 Ci/mmol) for 4 hours. At the end of the labeling period, conditioned medium was collected in 1.5 ml microfuge tubes and centrifuged (10,000 x g for 5 minutes to remove any loose cells and debris). The medium was transferred to fresh 1.5 ml microfuge tubes and 150 µg Micrococcus lysodeikticus (75 µl of 2 mg/ml in saline) was added, then proteins were precipitated with 5% trichloroacetic acid (v/v). The precipitate was collected by centrifugation, washed, and dissolved in 50 µl 0.1% Trition X-100, followed by addition of 50 µl of Laemmli gel sample buffer (1% β-mercaptoethanol, 0.1% bromophenol blue, 25% stacking gel buffer [0.50 M Tris-HCl, pH 6.8], 50% glycerol, 2% sodium dodecyl sulfate [SDS]). The pH was adjusted if necessary by adding 1-3 µl of 1 N NaOH. Samples were boiled for 3 minutes and then electrophoresed on SDS½polyacrylamide gels (Laemmli, 1970) and patterns were developed by autoradiography (Bonner and Laskey, 1974). Molecular weight markers are shown on the left. The glucocorticoid-specific secreted protein D23 is marked. Only glucocorticoids (triamcinolone acetonide, dexamethasone, and cortisol) showed induction of D23 (Werb and Chin, manuscript in preparation).
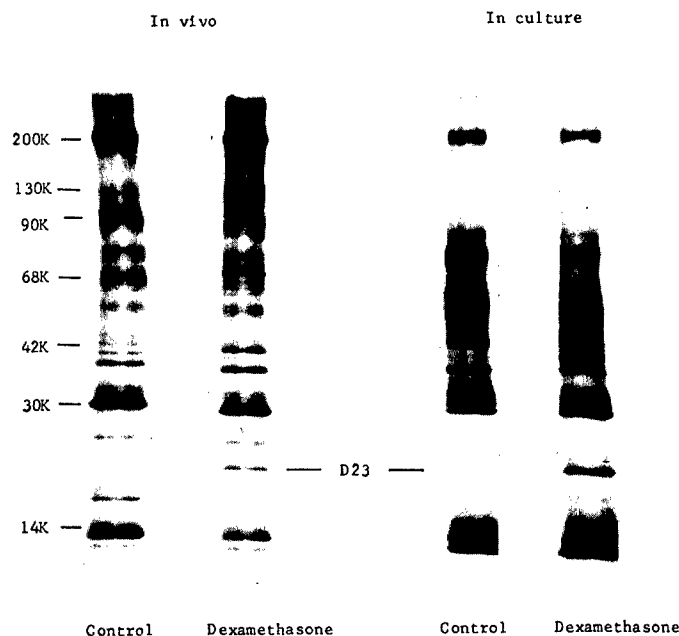
Figure 4.   Induction of glucocorticoid-specific macrophage-secreted proteins in vivo and in culture.   For macrophages treated in vivo, mice were injected with 1 mg dexamethasone suspended in 0.2 ml saline daily for four days.   Twenty-four hours after the last injection, resident peritoneal macrophages were harvested, plated for 1 hour in DME + 10% fetal calf serum, then washed and placed in methionine-free DME containing 25 μCi/ml [^{35}S]methionine for 2 hours.   Biosynthesized secreted proteins were then processed as in Figure 3.   For macrophages treated in culture, thioglycollate-elicited macrophages were incubated with 1 μM dexamethasone for 24 hours in DME-LH, then radiolabeled identically to the in vivo treated cells.   Note that the glucocorticoid-specific D23 protein is seen after exposure to dexamethasone both in vivo and in culture.   Also note that the secreted proteins of unstimulated resident peritoneal macrophages differ as much from the secreted proteins of the thioglycollate-stimulated macrophages as from the secreted proteins of dexamethasone-treated thioglycollate macrophages.   Therefore, the hormone treatment induces a secretion specific to glucocorticoids rather than reversion of the macrophages to a less differentiated phenotype (Werb and Chin, manuscript in preparation).

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DR. OLSEN (Ohio State University): One of the areas of immuno-toxicology that is applicable but which the speakers didn't have time to address is how a chemical may modulate the immune response. One of the things that we're doing in my laboratory is measuring the effect of several environmental toxicants, not as allergens but as agents for modulating the immune response. For example, one of the very common immunotoxicants that we hear a lot about today, methylnitrosourea, turns out to be immuno-suppressive even below carcinogenic levels. It seems to be very immuno-suppressive for the T cell lymphocyte. But more importantly, one of the derivatives of hydrazine is also a T cell suppressant. It's not toxic but it certainly interferes with suppressor T cell activity. This particular compound apparently eliminates suppressor cells and, in fact, enhances the immune response. It does just the opposite as one would think of complete immune suppression. This directs me to my question about the very interesting slide Dr. Maibach had on the angry skin syndrome. Is it possible that this particular reaction is really a loss of suppressor function? Each of these allergens that you mentioned are under T cell suppressor control; and the loss of that control would, in essence, enhance the response.

DR. WISTAR (Naval Medical Research Institute): May I ask you what the chemical you described was?

DR. OLSEN: It was asymmetrical dimethylhydrazine.

DR. WISTAR: I know nothing at all about that particular chemical. I think both Dr. Werb and I did make some effort to point out that immuno-toxicologists would be interested in studying the effect of the various compounds you deal with on specific cell populations. I quite expect that as you go through the galaxy of compounds that you deal with, you are going to find some, certainly those that we are already aware of, that affect the immune system. You will find a number of compounds that will affect B cells, T cells, and suppressor cells. I would be surprised if you didn't.

DR. OLSEN: I think the overall significance is that the exogenous substances we are dealing with can mimic intrinsic control factors which really takes us into the area of immunopharmacology. What we are dealing with is not just a problem of toxicology but it's only toxicity when we're dealing with doses that tend to override these control mechanisms. Indeed, all of these presentations have really dealt with problems in control mechanism which, I think, is a unique area of immunopharmacology.

DR. HENDERSON (Olin Corporation): Can you further identify that chemical? Do you mean UDMH?

DR. OLSEN: Yes.

DR. MAIBACH: It's an interesting hypothesis. I may try to follow it up.

DR. MILO (Ohio State University): Dr. Newball, you made a statement to the effect that 20:4 arachidonic acid did not apparently have effect with other smooth muscle cells in the human. I was wondering if you might care to expand on that a little bit and possibly interrelate it with what you or other people in your lab have possibly observed in regard to the effect of this compound on smooth muscle cells from other animal species.

DR. NEWBALL: Which arachidonic acid metabolite were you talking about?

DR. MILO: I'm talking about 20:4 itself.

DR. NEWBALL: The substrate arachidonic acid itself?

DR. MILO: Yes.

DR. NEWBALL: Would you repeat your question?

DR. MILO: You indicated during your presentation, if I understood correctly, that 20:4 had no effect on the smooth muscle cells.

DR. NEWBALL: I was just talking about the effect on contraction of muscle cells.

DR. MILO: Right. Well, we know there are other smooth muscle cells found throughout other organs in the body, and I was wondering if you meant that this 20:4 had no effect on these other smooth muscle cells in the human system?

DR. NEWBALL: If I made that comment, I did not mean to. I was referring specifically to muscle cells in human airways.

DR. MILO: Dr. Werb, the question I want to ask is in three parts. Could environmental effects affect your response in the colony proliferating assay for the different species that you are taking the macrophages from?

DR. WERB (University of California, San Francisco): We have only studied the proliferation of bone marrow cells in the mouse. There are studies like that in the human but not with glucocorticoids. It's possible to ask that question of stem cells in the humans as well. When we study the effect of glucocorticoids on stem cells, it looks like there are subpopulations that are differentially sensitive. For example, the precursor cell for erythrocytes, BFUE, is far more sensitive than the stem cell that is common to granulocytes and macrophages. If you use the real stem cell instead of the bone marrow precursors, you use only the proliferative capacity of the inflammatory macrophage tissue as the target which also requires the same macrophage gross factor. It's totally insensitive to

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glucocorticoids. The test system is dependent on the stage of the cell and the kind of cell. One cell population like the erythroid series is much more sensitive, but only the early precursors to the granulocytes and the mononuclear phagocyte series are sensitive. The local proliferation is totally insensitive to glucocorticoids.

DR. MILO: I'm glad to hear this because this is an area that Dr. Olsen and I are interested in. One of the problems that we're having is how to physically separate the subpopulations of cells. Are you really effecting the release or actually inhibiting the production of fibrinolysin or PGE_1?

DR. WERB: We didn't deal with PGE_1 except as a reference. The effect of prostaglandin on the production of plasminogen activator is on the production of functionally active enzymes. The problem with those systems is that we really don't have a handle on the earlier inactive or latent precursors of those enzymes. We know some things about the requirements for protein synthesis, but if there is a precursor which piles up in the cell, we don't know what it is because these proteins are present in such minute quantities that the probes such as mono-specific antibodies or messenger RNA probes don't yet exist.

DR. MILO: You suggested that the hormones are tied up by a serum protein and alluded to the fact that maybe some of these other substances may also be tied up by the serum protein found in circulating serum. And I thought you said that the free amount is the important compound and then you alluded to the fact that maybe other compounds are acting in the same way. Have you given any consideration to some of the new evidence that's coming out that maybe many of these chemicals are picked up by low density lipoproteins and they are the vectors or the vehicles rather than the specific proteins that we think of as acceptor protein complexes or transporting types of compounds?

DR. WERB: I don't know the answer. Of course, using the glucocorticoids as an example, it turns out the free material is what is important, but with other compounds, the reverse may be true. With environmental toxins, if they are hydrophobic, the LDL may be very important, and I think that's an area for some potentially valuable research.

DR. BACK (Air Force Aerospace Medical Research Laboratory): Last night, Dr. Wistar talked about phenotyping certain animals to produce certain effects that you wanted in the immuno system. It seems to me that someone has reported flight and fear with high IgE titers and flacidity with low IgE titers in certain animals. As a toxicologist, I'm beginning to find it difficult to know when I'm using the right strains and species. Is anybody working at systematically cataloging immunologic characteristics of various species and animal strains?

DR. MAIBACH: I think there are many people who are gravely concerned about some of the nuances of this matter. Dr. Werb was talking about it, and you heard Dr. Wistar talk about it last night, as it relates not only to animals but also to man.

DR. WISTAR: There are many dozens of different mouse strains at Jackson Laboratories with well defined genetic backgrounds. If you take those strains and start classifying them for a number of immunological responses, simple things like B cell levels, IgM levels, ability to respond to sheep cells, you can start seeing that there are genetically determined differences in a lot of these factors that you are looking for which sort of phenotype the mice. You can select strains that respond well to sheep cells and respond poorly to sheep cells. You can breed these, and they breed true. In the models that you are concerned with, the effect of environmental toxins on a mouse, you would do well to screen a number of strains before you really get going with the hope of finding the one that is the most suitable for whatever your particular interests are. All mice, obviously, as you now realize, are not the same. The available mouse strains have been carefully bred by a number of people over the last 40 years to produce specific factors. Many strains have been developed.

DR. WERB: May I make a comment? One of the other cell types that I've been interested in is the so-called natural killer cell which is a lymphoid cell which possibly is involved in immune surveillance of some sort against tumor cells. We have been looking at the effect of some compounds on the ability of those cells to kill T cells, lymphoma target cells. The compound of particular interest that I can tell you about is the tumor promoting phorbal esters. What is interesting, of course, is that the level of natural killer cells is different in various strains of mice, and they are also present in the peripheral blood of humans. We can actually do the experiments both in mice and man. What we find is that some compounds decrease the amount of killing of the tumor cell target. But interestingly, it looks as if what is happening is not so much that the ability of the natural killer cell to kill is being reduced as the target cell is becoming resistant to being killed. So, depending on how you design your experiments, you may be interpreting the results as a decrease in immune function, which, given one kind of target cell, may be true, but it may not be true for another target cell.

DR. BENZ (University of California, Irvine): Dr. Werb, you said you could treat animals in vivo and then take out the macrophages to look at them in vitro. Can you do that without harming the animals? More important, can you do this with humans?

DR. WERB: Yes, with humans, it's quite easy. We've actually done the experiments in humans but not directly yet. You can easily get blood monocytes, and you can get lung bronchoscopy specimens. Bronchoscopy is a routine procedure, and usually they throw the aspirated material away. You can get human cells to use that way.

In the human, we haven't actually looked at the function of the macro-phages from steroid treated patients. One of the people in our lab took a pretty hefty dose of methylprednisolone. We took a pretreatment blood sample and samples at various times after the methylprednisolone, and we showed that the pretreatment serum acted as if it had no effective glucocorticoid. About 4 hours after the methylprednisolone was taken, it started to be inhibitory and induce production of the steroid specific bands. So it's actually possible to do a test for this if you can show your substance will be present in plasma of humans. You can then take the plasma and put it into your test system and see if the plasma induces some functional change or a biosynthetic change which tends to be less effective by some of the other parameters.

MR. MOORMAN (National Institute of Occupational Safety and Health): I wonder if you have any precautions or comments regarding the use of skin testing to indicate hypersensitivity to an agent that has been administered by the inhalation route.

DR. MAIBACH: Would you clarify that?

MR. MOORMAN: In exposing an animal by inhalation, can you use skin tests to indicate when the animal has been immunized or hypersensitized?

DR. NEWBALL: I don't know of any study which specifically addresses that question; however, most individuals who are sensitized to antigens such as ragweed antigen are probably sensitized by the inhalation route. As you know, one can subsequently do skin testing and get a positive skin test. I would be surprised if one were not able to sensitize an individual by the inhalation route and then elicit a positive skin test.

DR. MAIBACH: I'll go one step further. We work with a skin syndrome that has a pulmonary component. The route of the exposure is the skin, but you get asthma and anaphylaxis as well. That's known as the contact or the carrier syndrome.

DR. HENDERSON (Olin Corporation): In regard to the inhalation response versus skin testing, toluene diisocyanate is an example where someone will show a bronchial asthmatic response and yet will not show a positive skin test. So there are both types of situations.

DR. STAAB (Exxon Corporation): Dr. Maibach, I'm concerned about your foot. Your foot should have reacted when you applied the known sensitizer. You did not fully explain it. You said it was confusing to you as well. If it is not a variation due to percutaneous penetration, what might you offer as an explanation?

DR. MAIBACH: For centuries, health care workers in industry have known that most of the allergic rashes are on the back of the hand and not the palm. The explanation had always been that the stratum corneum of the palm is thick and tough, and nothing goes through it. Then when radioisotopes became available and better studies were conducted, it was shown that there's not an enormous difference in penetration between the palm and the back of the hand. We're left with the biological observation that there's something immuno-logically different between these two sides, and we just don't know what. It's a very real phenomenon which is taken advantage of all the time in practical toxicology.

DR. WISTAR: Another comment in that same vein is that people doing passive cutaneous anaphylaxis studies in animals have known for a long time that there is a significant, repeatable difference in the area of the skin you use for the PCA test in the guinea pig. If you put it on the hind leg, stomach, face, or back skin, you get significantly different reactions in terms of the size of the wheal and flare which is probably related to the density of mast cells and tissue basophils in that area.

DR. MAIBACH: We could write a chapter for a book today on regional experimental dermatology because the exact same thing happens in man. I could place "X" number of micrograms of material on your forearm, forehead, back, and leg, and there would be a log difference in response. It is extremely difficult to produce a flare on your leg whereas they are readily produced on your arm.

ENVIRONMENTAL EFFECTS

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ATMOSPHERIC DEPOSITION OF PARTICULATE ORGANIC CARBON AND PCBs TO LAKE MICHIGAN

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INTRODUCTION

It is now well accepted that the major source of organic matter in lakes and oceans is via photosynthesis (Menzel, 1974; Duce and Duursma, 1977). However, certain stable and refractory organic compounds may have other sources. Organic matter input via rivers and the atmosphere has, therefore, recently received attention. This, in turn, has generated a renewed interest in the physical and chemical properties of atmospheric organic matter. Much of what is presently known about the input of organic matter to oceans has been reviewed by Duce (1979) and Duce and Duursma (1977). Little information on the relative importance of organic matter input via the atmosphere is available for fresh water systems such as Lake Michigan (Andren et al., 1975). Such a study is especially important since the atmospheric route seems to dominate other inputs for many inorganic trace constituents (Winchester et al., 1971; Klein, 1975; Gatz, 1975).

This paper presents several data sets of atmospheric particulate total organic carbon (TOC), both total and size fractionated. These samples were collected on Lake Michigan during several cruises during spring, summer, and fall from June 1975 to August 1977. The data are used to assess an annual atmospheric loading rate of TOC to Lake Michigan.

Since TOC does not give an indication as to the nature of the organic matter reaching the lake, we also present data for a specific set of compounds in the form of polychlorinated biphenyls (PCBs). These compounds are ubiquitous in the environment, and their global distribution is thought to be largely governed by atmospheric transport (National Academy of Sciences, 1979). Murphy and Rzesutko (1977) measured the annual wet flux of PCBs to Lake Michigan by determining a volume weighted mean concentration for a number of precipitation samples.

Doskey (1978) collected PCBs in air over Lake Michigan. Data from these collections are used in this paper to calculate the annual dry deposition of PCBs to Lake Michigan. The calculated atmospheric inputs of both TOC and PCBs are then compared with river inputs.

EXPERIMENTAL PROCEDURE

Atmospheric sampling was performed aboard three research vessels - the Laurentian, the Neeskay, and the Roger R. Simons - owned by the University of Michigan, the University of Wisconsin System, and the Environmental Protection Agency, respectively. Several cruises were conducted in the southern and central basins of Lake Michigan during the spring, summer, and fall seasons from June 1975 to August 1977 for TOC sampling and in the summer of 1977 for PCBs. Sampling was continuous while the ships were underway. One shoreline sampling for TOC was conducted during the winter season at Bailey's Harbor located on the Green Bay peninsula in the northern basin of the lake.

A detailed account of experimental techniques has been presented by Strand (1978), Strand et al. (1978), and Doskey-and Andren (1979). Briefly, TOC was collected using Delron model DC1-6 cascade impactors. Duplicate samplers were located on the bow of each ship, ahead of smoke stacks and ventilation systems, and from 2 to 7.6 meters above the water line. Impactor stage cutoffs for unit density spheres are reported to be 16, 8, 4, 2, 1 and 0.5 micrometers with an efficiency greater than 80%. TOC collected on precombusted glass fiber filters was determined by the method of Menzel and Vaccaro (1964).

PCBs were collected using a modified high volume sampler operating at a flow rate of 0.7 m^3/minute. Particulate associated PCBs and vapor phase PCBs were partitioned via a glass fiber filter. A cartridge filled with XAD-resin was placed behind the filter to collect vapor phase PCBs. The analytical methodology included soxhlet extraction of both filters and XAD-2 with petroleum ether and cleanup of the extracts with alumina. Cleaned extracts were injected into a Varian 1700 gas chromatograph equipped with electron capture detectors.

RESULTS AND DISCUSSION

TOC

Table 1 presents data on total particulate matter (TPM), total organic carbon (TOC), and their ratios for the Lake Michigan cruises. The 1976-77 data are represented by two concurrent sample collections for each cruise. The range of the TOC/TPM fraction extends from 7.5 percent to 54 percent. Results from previous studies in urban areas are

quite similar. Cukor et al. (1972) found that the TOC fraction ranged from 2 percent to 48 percent of the TPM in samples collected near a highway in New York City and Mueller et al. (1972) found a range of 18 percent to 44 percent in Pasadena, California. Thus, organic matter contributes a sizable portion to the TPM and the similarity of these ranges suggests that urban areas upwind of the Lake could be a partial source for the observed organic carbon levels.

TABLE 1. TOTAL ATMOSPHERIC PARTICULATE MATTER AND THE PERCENT RATIO OF TOTAL ORGANIC CARBON

Cruise Date	Total Particulate Matter $(\mu g/m^3)$	Total Organic Carbon $(\mu g/m^3)$	TOC/TPM (%)
6/20/75	N.D.[1]	2.3	--
6/20/75	"	2.4	--
6/21/75	"	4.3	--
6/21/75	"	4.6	--
6/22/75	"	4.1	--
6/23/75	"	5.9	--
6/23-24/75	"	15.5	--
2/26-28/76[2]	10.0	4.5	45
2/26-28/76	9.5	4.5	47
8/3-6/76	28.0	14.1	50
8/23-27/76	126.0	19.8	16
8/23-27/76	151.0	19.3	13
9/14-16/76	34.0	14.2	42
9/14-16/76	34.0	18.3	54
5/17-19/77	133	10.4	8
5/17-19/77	133	10.0	7.5
6/9-10/77	25.4	8.9	35
6/9-10/77	25.4	5.2	20

[1] Not determined.
[2] Duplicate samples.

Figures 1 and 2 are typical graphs of aerodynamic equivalent size versus TOC for several cruises, both in the central and southern basins of Lake Michigan. The important feature of these diagrams is the observation that the highest concentration of TOC is associated with the smallest particle sizes. Except for the collection made 23-26 August 1976, more than 70 percent of the TOC mass is found on stages 5, 6, and the backup filter (i.e., < 1 μm). Although the total amount of TOC varies, similar shapes of the particle size function have also been observed in samples collected in both the Atlantic and Pacific oceans (Barger and Garrett, 1970; Hoffman and Duce, 1977). The similarity of our inland lake values to the oceanic ones suggests a common mechanism of formation. This process has been hypothesized as a gas-to-particle

conversion (Ketserides et al., 1976; Hoffman and Duce, 1976; Duce and Hoffman, 1976). The relative source strengths of natural and anthropogenic organic carbon is, however, uncertain.
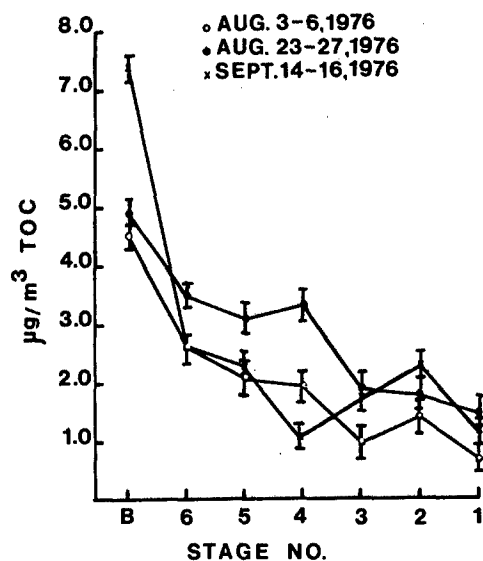
Figure 1. TOC versus stage for summer and fall 1976 cruises. Error bars are equal to the dimensions of the data point squares, circles, and triangles.
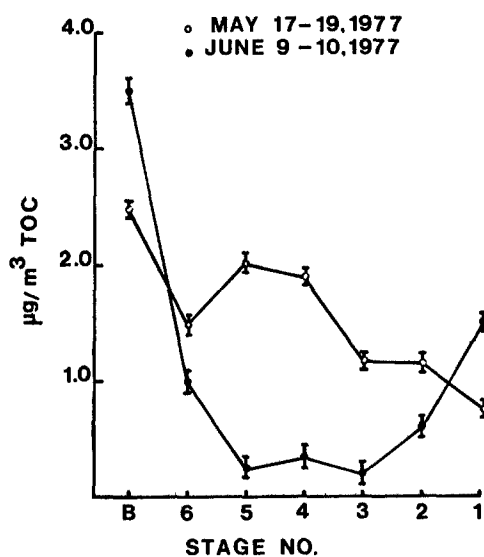
Figure 2. TOC versus stage for spring 1977 cruises.

It is also possible to make atmospheric deposition estimates to Lake Michigan using the size fractionated TOC data sets. Dry deposition calculations were made using a modified method (Clough, 1973) of Chamberlain's (Chamberlain, 1966) equation which relates the air particulate concentration to flux. Deposition velocities were obtained from Semmel and Suter (1974) and Moller and Schuman (1970). The calculations are only order of magnitude estimates because (1) more data sets representing seasonal trends should be included; (2) only one complete data set is available for the northern basin; (3) available deposition velocity data represent monodisperse particles whereas cascade impactors collect particle size ranges; (4) a more rigorous treatment of particulate deposition requires detailed meteorological data since deposition is strongly dependent on surface roughness; and (5) some, as yet unquantifiable, dry vapor deposition undoubtedly takes place. An extended account of computational details, combined with a critical analysis of the results can be found in Strand (1978).

The total dry TOC flux to Lake Michigan is calculated to range from 14 to 71 x 10^6 kg/yr. Wet deposition of TOC was estimated to range from 38 to 170 x 10^6 kg/yr for a total annual TOC deposition of 52 to 241 x 10^6 kg/yr. This estimate is not inconsistent with a total atmospheric particulate matter loading to Lake Michigan of about 1 x 10^9 kg/yr, calculated using independent data (Strand, 1978).

The overall TOC budget for Lake Michigan is presented in Table 2. This shows that net primary productivity predominates in the carbon flux of the lake. However, atmospheric and river inputs are quite similar.

TABLE 2. INPUTS, RESERVOIRS AND LOSSES OF ORGANIC CARBON IN LAKE MICHIGAN

Reservoirs	10^9 gC/yr
Dissolved Organic Carbon[1]	49,000
Particulate Organic Carbon[1]	6,700
Plankton [2]	2
Annual Inputs	
Net Primary Productivity [2]	5,800
Atmospheric [3]	146
Rivers [1]	160
Annual Outputs	
Sedimentation [4]	260
Outflow	30

[1]Andren et al., 1975.
[2]Kitchell, J., personal communication, 1979.
[3]This study (average).
[4]Klein (1975).

The data presented in Table 3 represent 6 cruises in 1977, lasting from 1-3 days each. The samples show an average concentration of 1.0 ng/m^3, with an average of 13 percent being captured by the glass fiber filter. Adsorbed vapor on the XAD-2 resin showed an average composition of 75 percent Aroclor 1242 and 25 percent Aroclor 1254, while material collected on the glass fiber filter was composed of 69 percent Aroclor 1242, 23 percent Aroclor 1254, and 8 percent Aroclor 1260.

TABLE 3. LAKE MICHIGAN SAMPLES - PCB CONCENTRATION IN TOTAL SAMPLES

Sample	Amount of Air Sampled (m^3)	Total PCB (ng)	1242 (%)	1254 (%)	1260 (%)	Total PCB Conc. (ng/m^3)	TSP* (μg/m^3)
2	813	1003	69.6	30.4	---	1.23	69.9
4	900	716	82.8	17.2	---	0.80	17.3
5	929	722	75.1	20.6	4.3	0.78	17.9
6	1102	601	78.2	19.6	2.2	0.55	31.0
8A	897	1301	65.8	32.8	1.4	1.45	28.4
9A	716	997	65.5	33.6	0.9	1.39	54.5
Average			72.8	25.7	1.5	1.03	36.5

*TSP = total suspended particulates.

While accurate measurements of PCBs in the atmosphere are hampered by technical and analytical problems (National Academy of Sciences, 1979), these data indicate that, at least operationally, PCBs exist mostly in the vapor phase. Whether these PCBs are associated with particles too small to be collected by glass fiber filters [having a particle diameter cutoff of 0.3 μm (Lockhart et al., 1964)] is presently uncertain. However, if the particle adsorbed PCBs are in equilibrium with the vapor in the atmosphere and if most of the filter retained particles are deposited on top of the filter where a minimum pressure drop exists, it appears that the observed vapor/particulate partitioning is real. Junge (1977) has constructed a theoretical adsorption model of atmospheric organic vapors based upon the number of particles per unit volume of air, the surface area of particles, and the saturation vapor pressures of the organic compounds. Calculations for rural air (Doskey, 1978) using this approach indicate that 10 to 45 percent of Aroclors 1254 and 1260 and less than 5 percent of Aroclors 1242 and 1248 should be associated with particulate matter.

Murphy and Rzeszutko (1977) found that the total annual PCB input via precipitation to Lake Michigan was about 5000 kg in 1975-76. It thus becomes instructive to investigate the extent of dry deposition. From the data presented, it is obvious that an atmospheric flux model for PCBs must include both particulate and vapor phase components. A model developed by Doskey (1978) includes wet, vapor, and particulate deposition modes of PCBs to Lake Michigan. The model also includes transport from the water surface to air by the processes of revolatilization and bubble ejection.

The following equation is most commonly used to calculate particulate fluxes (Chamberlain, 1966):

$$F = V_D \bar{C}_B$$

where F = flux, V_D = deposition velocity, and $\bar{C}_B$ = average concentration of the contaminant in air at a selected reference height.

The transfer of vapors between air and water is most often modelled using the Whitman two layer model (Whitman, 1923; Mackay and Leionen, 1975; Duce and Duursma, 1977; Bidleman et al., 1976; Doskey, 1978). This model divides the air/water interface into two physical regions, i.e., one which is close in composition to air and one which approximates the composition of water. The vapor transport across these two regions is accomplished via diffusion. The magnitude of the driving force is thus dependent on the concentration difference and the Henry's Law constant (Andren et al., 1975). The magnitude of H will determine whether a vapor is gas phase controlled (SO_2, for example) or liquid phase controlled (benzene, for example). The overall transport, expressed on a gas phase basis, is calculated by the expression:

$$F = K_{OG} \ (P-C/H)/RT$$

where F = flux, K_{OG} = overall gas phase mass transfer coefficient, C = the contaminant concentration in water, P = the partial pressure of the contaminant in air, R = the ideal gas law constant, and T = the absolute temperature. The overall gas phase mass transfer coefficient is defined by the following equation:

$$\frac{1}{K_{OG}} = \frac{1}{K_G} + \frac{HRT}{K_L}$$

where K_L and K_G are the individual liquid and gas phase mass transfer coefficients, respectively. Typical values for the individual mass transfer coefficients for CO_2 (K_G = 0.2 m/hr) and H_2O (K_L = 30 m/hr) have been reported by Liss and Slater (1974). Estimates of K_L and K_G for gases other than CO_2 and H_2O may be made by multiplying these values by the ratio of the square roots of the molecular weights of H_2O and CO_2 and the other gas.

Mackay and Leinonen (1975) have reported values for K_{OL}. It is, however, believed that these are in error because the Aroclor saturation vapor pressures are poorly known. Doskey (1978) has critically evaluated the available information on K_{OL} for PCBs and estimates that the overall gas phase mass transfer coefficient for Aroclors 1242 and 1254 are 7.9 m/hr and 7.0 m/hr, respectively. The data indicate that most PCBs are gas phased controlled, with the possible exception of micro- and di-chlorobiphenyls. This indicates that very small amounts of the deposited PCBs will revolatilize once deposited. Adsorption of PCBs by particulate matter in the surface organic microlayer should also retard any revolatilization.

These considerations were taken into account by Doskey (1978) who used the data presented in Table 3 and the precipitation data from Murphy and Rzeszutko (1977) to calculate the total annual atmospheric input of PCBs to Lake Michigan. Results are presented in Table 4. These calculations indicate that dry deposition may be equal in magnitude to wet deposition. Revolatilization seems to be negligible. Although most of the atmospheric PCBs seem to exist in the vapor form, a substantial fraction is deposited in association with particulate matter. Finally, a comparison of the PCB input inventory compiled by Murphy and Rzeszutko (1977) indicates that the atmospheric route is presently of major importance.

TABLE 4. TOTAL ATMOSPHERIC PCB FLUX TO LAKE MICHIGAN[1].

	kg/yr
Dry Deposition [2]	
Particulate	1200
Vapor	2800
Wet Deposition[3]	5000
Revolatilization	0
Net Atmospheric Input	9000
Streams and Wastewater Effluents[3]	750

[1]Area of Lake Michigan = 5.6 x 10^{10} m^2.
[2]From Doskey (1978).
[3]From Murphy and Rzeszukto (1977).

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METHODS FOR PROCESSING AND DISPOSAL
OF HAZARDOUS WASTES

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INTRODUCTION

During recent decades, the increased demands of our society for an ever-widening spectrum of consumer goods has intensified the problem of environmental degradation. Not only has rapid industrial expansion led to the generation of increased quantities of waste products, but also the diversity of chemicals appearing in these wastes has magnified. Many of the effluent streams discharged from industrial operations as well as from municipal water and wastewater facilities contain materials which are potentially hazardous to the health and welfare of man and the living world around us. Recent statistics published by the Environmental Protection Agency indicate that 10 to 15% of the 344 million metric tons of wet industrial waste produced annually can be considered hazardous (Murray, 1979).

DEFINITION OF HAZARDOUS WASTES

Hazardous wastes have been variously defined. The Resource Conservation and Recovery Act of 1976 (RCRA) defines a hazardous waste in Section 1004(5) as:

> A solid waste, or combination of solid wastes, which because of its quantity, concentration, or physical, chemical or infectious characteristics may –
>
> > Cause or significantly contribute to an increase in mortality or an increase in serious irreversible, or incapacitating reversible illness, or
> >
> > Pose a substantial present or potential hazard to human health or the environment when improperly treated, stored, transported, or disposed of, or otherwise managed.

More recently, EPA has attempted to identify hazardous wastes in terms of certain characteristics as (USEPA, 1974):

Ignitability,
Corrosivity,
Reactivity,
Toxicity.

THE PROBLEM IN NEW YORK STATE

New York State has some particular problems related to specific industries in certain areas of the state, e.g., leather tanning in the Johnstown-Gloversville area, photographic industry in Rochester, pulp and paper in the Adirondack area, and the chemical and steel industries in the Buffalo-Niagara Falls area. Iannotti et al. (1979) have recently completed an inventory of hazardous waste generation in New York State and have noted that over 1.2 million tons of hazardous wastes are produced in the state annually. This quantity is generated from some 4000 industrial sources, with more than 90% of the waste being generated by 100 firms. It is further reported that much of this waste is currently being disposed of by what may be considered to be nonsecure methods.

The magnitude and complexity of the hazardous waste problem in New York State has highlighted the need for assembling technical information on the processing and disposal of these materials in the best possible manner.

This paper has been based upon a recent study which has been directed towards the definition of the hazardous waste problem in New York State, and a review of the available technology most appropriate to the treatment and disposal of the toxic wastes occurring in the state. While this study focused on the problem in New York State, the approach used would find application in other parts of the country.

In this work, it was felt that the most meaningful approach would be:

To group the wastes found in New York State according to their principal characteristics and properties;

To list the principal unit operations and unit processes that appear to be suitable for waste treatment;

To match and cross reference individual and/or combinations of operations and processes most suitable for treating and disposing of each of the listed types of wastes.

Recommendations for best treatment technology have been presented accordingly with suitable reference citations whenever appropriate. In general, only techniques currently in use in full-scale or large pilot plant scale have been considered.

TYPES OF HAZARDOUS WASTES OCCURRING IN NEW YORK STATE

The waste streams from various industrial processes contain, in many cases, a wide variety of materials, many of which are considered to be hazardous. Since the identity and quantity of specific toxic materials in individual waste streams, originating from industrial processes, may vary over extremely wide ranges, even for the same or similar industries, it has been found feasible to classify the toxic components of wastes according to certain characteristics or properties, which because of their objectionable nature, require treatment or removal. Thus, the treatment process required for any given waste stream will be determined by the combination of operations required for the treatment of the individual components of the waste stream. In certain unique cases, reactions betweeen components prior to treatment may occur; this situation requires individual analysis beyond the scope of this study.

A classification of wastes based on the contained toxic components is shown in Table 1. A code number is listed for each waste type for subsequent identification.

As already noted, any given waste stream may contain several toxic or hazardous components. For instance, a waste stream from a chrome plating operation might contain appreciable quantities of chromium, sulfuric acid, floating oil and an acid sludge. Consequently, an integrated treatment process might include treatment steps appropriate for waste types 102, 104, and 202. It should be noted, however, that waste streams from chrome plating operations originating from a different location might have a very different composition and might contain other toxic materials whose treatment might necessitate other treatment steps. Hence, an overall treatment process must be tailored to a specific waste stream and may depend upon the approach used for the complete spectrum of wastes being generated within the plant.

TABLE 1. TYPES OF HAZARDOUS WASTES

INORGANICS

Acid Wastes
101 Acid solutions containing minor contaminants
102 Acid solutions containing metals
103 Acid solutions containing organics
104 Acid sludges
105 Acid gases

Alkaline Wastes
111 Alkaline solutions containing metals
112 Alkaline solutions containing organics
113 Cyanide solutions - nonmetallic
114 Cleaning solutions - miscellaneous
115 Alkaline sludges

Other Inorganic Wastes
121 Salt solutions
122 Solids
123 Metals
124 Nonmetals

ORGANICS

Concentrated Liquids
201 Lightly contaminated halogenated
202 Lightly contaminated nonhalogenated
203 Lightly contaminated solvent mixtures
204 Highly contaminated halogenated
205 Highly contaminated nonhalogenated
206 Highly contaminated solvent mixtures

Dilute Aqueous Solutions
211 Readily oxidized, halogenated
212 Readily oxidized, nonhalogenated
213 Difficult to oxidize, halogenated
214 Difficult to oxidize, nonhalogenated

Organic Solids
221 Salts
222 Tars and residues
223 Sludges

Organic Gases/Vapors
231 Combustible
232 Noncombustible

Special Wastes
311 Strong oxidizing agents
312 Explosives
313 Biological wastes

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TREATMENT METHODS

A treatment process is usually made up of a series of operations, the sequence of which is dependent upon the nature of the waste being treated, and the type, quantity and concentration of the individual components. Many other factors may influence the selection of operations that are chosen, such as economics, geographical considerations, and Federal, State, and local environmental regulations. Specific examples are presented when individual wastes are considered.

The principal unit operations that have application to hazardous waste treatment are listed in Table 2. A brief description of each of these is given in sections that follow. For details of specific methods and design principles, it is suggested that any of a number of standard texts on unit operations, unit processes, industrial waste treatment, or municipal waste treatment be referred to as cited (Rich, 1961, 1963; McCabe and Smith, 1976; Tchobanoglous et al., 1977; Metcalf & Eddy, Inc., 1979; Weber, 1972; Gurnham, 1965; Eckenfelder, 1966; Nemerow, 1978; USEPA, 1975, Ramalho, 1977; Besselievre, 1969; Gurnham, 1955; Crawford, 1976; Marchello, 1976; Wang and Pereira, 1979).

TABLE 2. UNIT OPERATIONS IN HAZARDOUS WASTE TREATMENT

Operation	Description
Sedimentation	Separation of Solids and Fluids by Gravity Settling
Flotation	Use of Air to Float Lighter Particles to Surface for Skimming
Filtration	Use of Beds of Sand or Coal, or Fabric for Removal of Particles
Dialysis	Separation of Solutes by Diffusion Through Thin Porous Membranes
Reverse Osmosis	Use of High Pressure to Force Solvents Through Membranes
Incineration	Combustion Under Controlled Conditions
Chemical Oxidation	Use of Chemical or Electrochemical Agents to Oxidize Waste Materials
Chemical Reduction	Use of Chemical or Electrochemical Agents to Reduce Waste Materials
Precipitation	Use of Chemical Additives to Cause Materials to Drop from Solution
Adsorption	Removal of Dissolved Material by Concentration at a Solid Interface
Coagulation/ Flocculation	Growth and Aggregation of Solid Particles in Solution
Heat Exchange	Transfer of Energy Between Fluids
Extraction	Separation of Components of Mixtures by Preferential Solvents
Evaporation	Separation of Solids & Liquids by Boiling Off Liquid from the Mixture
Distillation	Separation of Liquids Based on Differences in Boiling Points
Absorption	Removal of Components of Gas Mixtures by Dissolving in a Liquid Stream
Neutralization	Counteracting the Acidity or Alkalinity with an Alkali or Acid Respectively

It is recognized that other unit operations and unit processes may have application to waste treatment, but the emphasis here has been on current best technology. For instance, various biological processes such as trickling filters, activated sludge processes, lagoons, and composting have application in the treatment of various organic materials, but except in special isolated cases would not be considered to be best technology for toxic wastes.

For a more detailed description of treatment methods applicable to toxic wastes, reference may be made to the report prepared by Rensselaer Polytechnic Institute entitled "Technology for Managing Hazardous Wastes" for the New York State Environmental Facilities Corporation.

While various processes may be used to reduce the hazard potential or volume of a given waste, the ultimate disposal of residual material to the land must always occur. In all cases where residuals from treatment processes are to be disposed of, the use of an engineered secure landfill facility for the disposal of residuals is necessary. Only when residuals can be shown not to have any potential hazard would ultimate disposal to an ordinary landfill be recommended.

An important aspect of hazardous waste management is the possibility of incorporating recovery techniques as part of waste processing and disposal. Many waste streams contain materials of high potential value, which through proper and appropriate processing may be recovered for reuse. Hence, the possibility exists of specifying treatment methods that will both mitigate the hazardous properties of some wastes and at the same time result in the recovery and/or utilization of the stream. Such an approach has the potential of improving the economics of waste treatment. In general, four broad paths by which resource recovery might be accomplished are:

Direct recycle for primary use,

Use as a raw material in a second industry,

Recovery of energy from waste processing,

Utilization for pollution control purposes.

While an individual hazardous waste stream may be a potential candidate for resource recovery by more than one of the above strategies, the consideration of benefits which might be derived provides a reasonable guidepost to the type of pretreatment which must be carried out to achieve such benefit.

TREATMENT OF SPECIFIC WASTES

To demonstrate how treatment processes may be developed to treat specific wastes, four rather common waste streams have been chosen as examples. Two have been selected which involve treatment and disposal, and two which involve resource recovery as well as waste treatment.

TREATMENT OF ACID WASTES CONTAINING METALS

Frequently acid wastes may contain materials other than acids, whose presence may considerably complicate the treatment process. Many metal-finishing operations and electroplating processes generate both dilute and concentrated acid solutions containing dissolved metals whose presence magnifies the hazardous nature of the waste. Many of the metals can be removed by neutralizing with an alkali and raising the pH to a high enough value to precipitate the metal as the hydroxide which can usually be separated from the solution by sedimentation and/or filtration. While any of a number of alkaline materials may be used, lime probably has the widest use as a neutralizing agent. The sludge may either be dried and disposed of via secure landfill, or if the value of the metal is sufficient, recovery of the metal for reuse may be justified. Ion exchange may often be used for metal recovery. A possible process is illustrated in Figure 1.
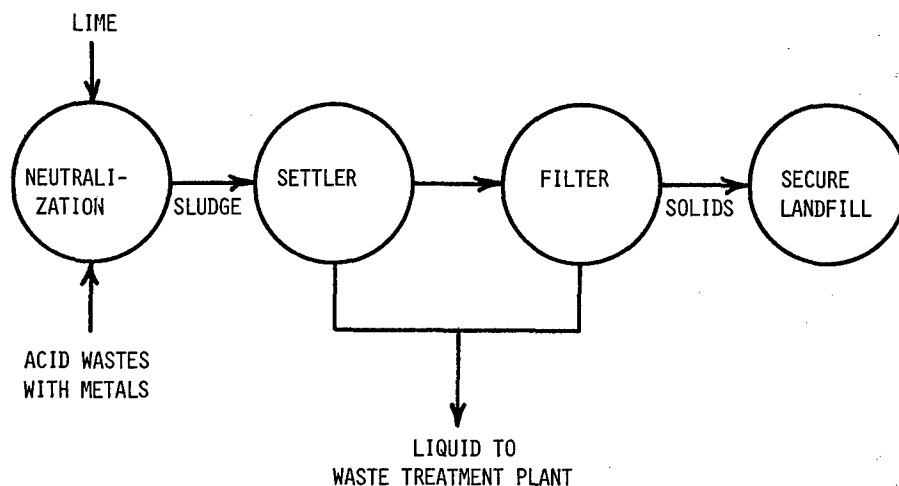
Figure 1. Possible treatment of acid wastes containing metals.

TREATMENT OF ORGANIC WASTES

For many broad classes of organic liquids, incineration is a viable disposal technique particularly for relatively high concentrations of organics. Care must be exercised in the design and operation of incinerators used for treating many toxic materials to ensure both high temperatures and sufficient contact time for complete destruction of the toxic agent. For instance, temperatures of the order of 1300°C and residence times of 2.0 seconds have been recommended for some wastes. In any case where incineration is used for the treatment of halogenated materials, particular attention to the control of acid components in the stack gases is required. A possible treatment process is shown in Figure 2. Recovery of heat energy may be feasible from an incineration process.
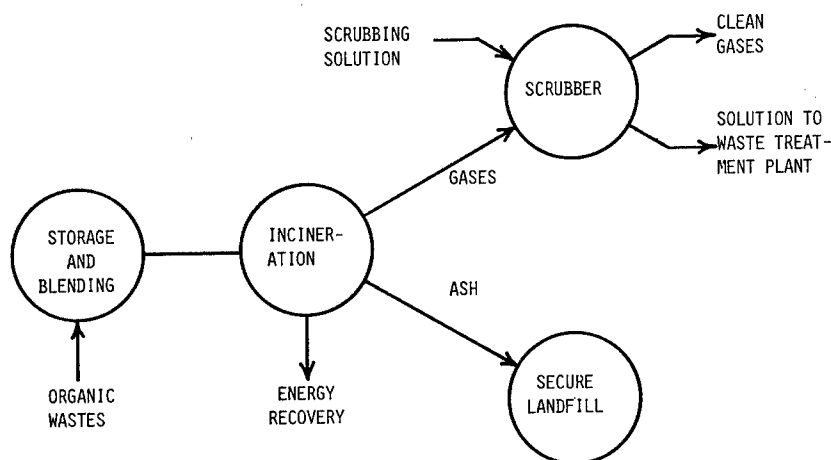
Figure 2.   Possible treatment of organic wastes.

RECOVERY OF METALS FROM SLUDGES

Sludges from many processes, such as metal plating operations, may contain metals which are hazardous, but in sufficient concentrations to make their recovery attractive. A possible treatment and recovery process is shown in Figure 3. The process entails leaching the metal from the sludge with an acid and separating the acid solution from the metal-free solids by filtration. The filtered solids could then be disposed of in a secure landfill. The acid solution would be neutralized with caustic to precipitate the metal hydroxides which would be separated from the neutral solution by filtration. The solution would be sent to a waste treatment plant if necessary. Caustic would be used in this case to minimize sludge formation. The flowsheet is shown in Figure 3.
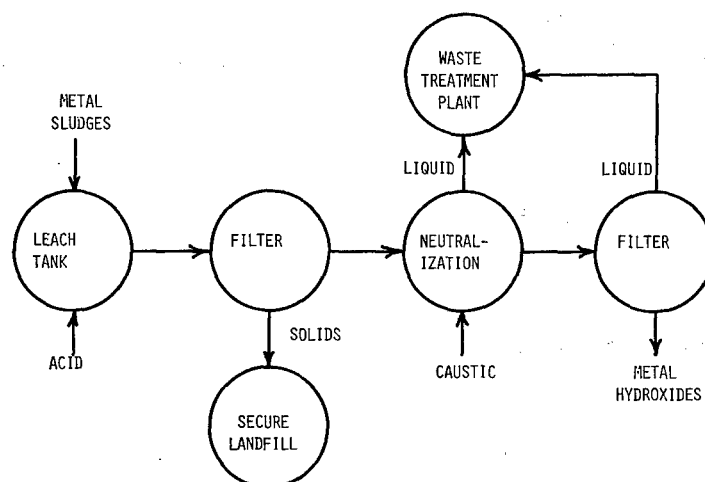
Figure 3. Recovery of metals from sludges.

RECOVERY OF MATERIAL AND ENERGY FROM WASTE SOLVENTS

In a wide variety of industrial operations, the recovery of solvents from waste streams is possible and feasible. Usually distillation is employed for the separation of the volatile components of the waste stream. If the residue from the distillation contains combustible materials, it may be disposed of by incineration with possible recovery of heat from the process. The solid residue can be disposed of in a secure landfill and the effluent gas scrubbed for removal of noxious components before discharge. Such a process is illustrated in Figure 4.
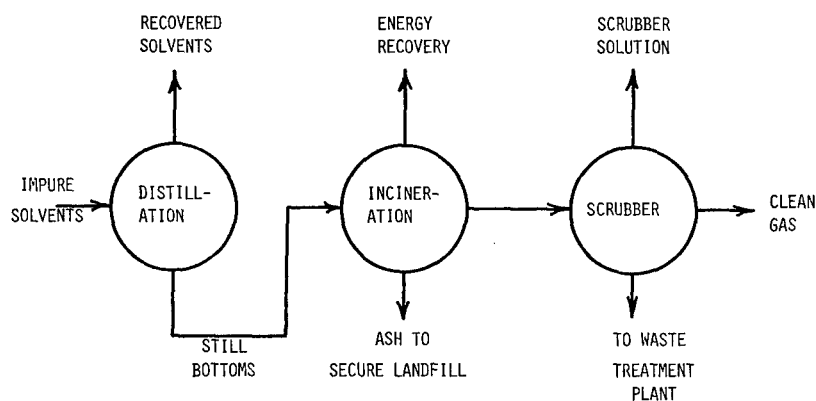
Figure 4. Recovery of material and energy from waste solvents.

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SUMMARY AND CONCLUSIONS

Based upon analysis of the processes available for treatment and ultimate disposal of hazardous wastes, the best current treatment technologies for various classes of materials generated in New York State have been summarized in Tables 3 and 4. This information can be used for the conceptual design of specific treatment systems, once the physical and chemical characteristics of a given waste are known. It should be noted that the recommended current best technology is based on the selection of the most economic treatment strategies consistent with minimal environmental health impacts, and has as a primary objective the reduction of the hazardous potential of the waste.

TABLE 3. SUMMARY OF CURRENT BEST TREATMENT TECHNOLOGY FOR HAZARDOUS WASTES IN NEW YORK STATE

Types of Waste	Potential for Resource Recovery	P-10 Sedimentation	P-20 Flotation	P-32 Filtration	P-70 Extraction	P-80 Distillation	P-90 Absorption	C-10 Neutralization	C-21 Incineration	C-23 Ozonation	C-24 Chlorination	C-40 Coag./Floc.	C-50 Precip.	C-60 Adsorption	C-70 Ion Exchange	B-12 Activ. Sludge	M-20 Detonation	M-41 San. Landfill	M-42 Secure Landfill	C-30 Reduction	Comments
101-Acid soln.-no contam.		x		x				x													
102-Acid soln.-with metals																					
Heavy metals (except Cr)	x	x		x				x				x	x						x		
Chromium	x	x		x				x				x	x						x	x	Possible recovery by ion exchange
Noble metals	x							x							x						
Pickle liquor	x	x		x				x				x	x						x		
103-Acid soln.-with org.																					
Emulsified org.	x	x	x	x				x	x			x									
Dissolved org.	x							x													See also waste types 201-214
104-Acid sludges																					
Inert solids		x		x	x			x										x			
Solids with metals	x	x		x	x			x											x		Possible recovery of metals
Organic solids					x			x	x										x		
105-Acid gases	x						x	x													
111-Alk. soln.-with metals	x							x											x		
112-Alk. soln.-with org.	x							x													See also waste types 201-214
113-Cyanide soln.								x			x										Ozonation also applicable
114-Cleaning soln.								x													See also waste types 201-214
115-Alkaline sludges																					
Inert solids		x		x	x			x										x			
Solids with metals	x	x		x	x			x				x							x		Possible recovery of metals
Organic solids		x		x				x	x										x		
121-Salt solutions	x														x				x		For exceptions see discussion
122-Solids	x																				Possible recovery or reuse
123-Metals																					
Heavy metals	x			x											x						
Alkali metals									x										x		Controlled combustion
Volatile metals	x							x	x												Recovery from flue gases
124-Nonmetals																					
Phos. sulf. compds.							x	x	x												Possible reduction of S Compounds
Asbestos																			x		Encapsulation recommended

(Left margin vertical label: Inorganics)

Types of Waste	Potential for Resource Recovery	P-10 Sedimentation	P-20 Flotation	P-32 Filtration	P-70 Extraction	P-80 Distillation	P-90 Absorption	C-10 Neutralization	C-21 Incineration	C-23 Ozonation	C-24 Chlorination	C-40 Coag./Floc.	C-50 Precip.	C-60 Adsorption	C-70 Ion Exchange	B-12 Activ. Sludge	M-20 Detonation	M-41 San. Landfill	M-42 Secure Landfill	Comments
Concentrated Liquids																				
201-Clean, halogenated	x					x	x		x										x	
202-Clean, nonhalogenated	x					x			x										x	
203-Clean, solvent mixtures	x					x			x										x	
204-Dirty, halogenated	x						x		x										x	
205-Dirty, nonhalogenated	x								x										x	
206-Dirty, solvent mixtures	x								x										x	
Dilute Aqueous Solutions																				
211-Readily oxidized, halogenated											x					x				
212-Readily oxidized, nonhalogenated											x					x				
213-Difficult to oxidize, halogenated									x					x						
214-Difficult to oxidize, nonhalogenated									x					x						
Organic Solids																				
221-Salts and other solids	x							x	x										x	Hydrolysis may be required
222-Tars and residues	x							x	x										x	
223-Sludges	x			x				x	x										x	
Organic Gases/Vapors																				
231-Combustible								x	x											
Special Wastes																				
311-Strong oxidizing agent											x									Controlled hydrolysis
312-Explosives																	x			Controlled incineration or detonation
313-Biological wastes								x	x											Sterilization prior to incineration

The most common applicable treatment methodologies as listed in Tables 3 and 4 are:

Incineration,
Neutralization,
Precipitation,
Various separation processes,
Secure landfill for treatment residuals.

These methodologies are used in the treatment of a large fraction of the total hazardous wastes generated. However, it must be emphasized that the hazardous waste streams encountered are so varied in composition, quantity, and toxicity, and occur in so many possible combinations that no single process or process train will be sufficient to treat all wastes.

Inspection of Tables 3 and 4 will show that, while various intermediate processes may be used to reduce the hazard potential or volume of a given waste, the ultimate disposal of residual material to the land must always occur. In all cases where residuals from treatment processes are to be disposed of, the use of an engineered secure landfill facility for the disposal of the residuals is necessary. Only when residuals can be shown not to have any potential hazard would ultimate disposal to an ordinary sanitary landfill be recommended.

Besides the current best technology for treatment discussed here, other developing techniques that may have application for New York State wastes should be noted. They include but are not limited to:

The use of rotary cement kilns,
The use of plasma techniques,
The use of biological treatment methods,
The use of fluid bed technology.

Many of these methods may well find prominence in waste treatment of the future. It further may be noted that some wastes are amenable to resource and energy recovery operations which may alter the configuration of treatment processes for some wastes.

It has not been the intent of this study to make a comprehensive and highly technical review of the field of hazardous waste disposal. Rather, the intent has been to give an overview of current best technology applicable to the handling of wastes generated in New York State and to provide guidance and direction to the New York State agencies responsible for the development, implementation, and management of hazardous waste programs.

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PUBLIC HEALTH PERSPECTIVES
OF THE LOVE CANAL

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Just as the threat of a national cholera epidemic in 1921 was the precursor to federal support of state health programs, so also, I suspect, the Love Canal will prove to be the seminal event in the development of a national strategy for dealing with hazardous wastes -- one hopes, a strategy in which the cool reason of science will overcome the heated passion of politics. As a battle-scarred veteran of the Love Canal -- indeed, some of the monosyllabic invective hurled in my direction I cannot recall being subjected to even in my salad days at Harvard -- I fervently hope no state or state health department will be abandoned by the scientific community as New York was, and still is, in the wake of Love Canal. Eighteen months after Love Canal, I feel a great kinship to the professional football quarterback whose team has just been upset: unhappy about my teammates' lack of support, but even more distraught at the Monday morning second-guessing of my peers.

But much like that quarterback, I do not intend to let the slings and arrows of outrageous fortune, in the shape of shrill and parochial polemics, deter me from my quest as a scientist to discover and analyze the true public health implications of the Love Canal.

Niagara Falls is a city on the western frontier of New York State. Its population at the last census was 85,615. According to data compiled by the New York State Department of Commerce, the principal enterprise of Niagara Falls is the manufacture of chemicals and allied products. As of 1970, located within Niagara County, there were nine major chemical producing companies employing some 5,300 people.

The principal chemical producing firm in Niagara County is the Hooker Chemical Company, employing some 1,500 people. A variety of organic pesticides are manufactured by Hooker in Niagara Falls.

The heavy concentration of chemical manufacturing in Niagara County has made the county a major national reservoir of hazardous chemical wastes. The Love Canal landfill is located in the southeast corner of the city of Niagara Falls, about one-quarter mile from the Niagara River. The site itself covers about 16 acres.

The Love Canal derives its name from William T. Love, a visionary entrepreneur who proposed to take advantage of the natural topography and build a navigable power canal, bypassing Niagara Falls, during the last decade of the nineteenth century. However, economic vicissitudes and the development of alternating current -- which permitted the transmission of electricity over long distances -- led to the abandonment of the site after only a small portion of the canal was dug. The partially dug, abandoned canal site was utilized as a municipal and chemical dumping ground for a period of at least 25 years, ending in 1953, when the site was sold by Hooker to the city of Niagara Falls and its Board of Education. The site was covered with earth, and soon thereafter homes were built directly adjacent to it and a public elementary school was built directly on top of a section of the landfill.

Beginning in the mid-1960s, residents of the landfill area began complaining about chemical odors present in the neighborhood and in the basements of their homes. Local officials hired an engineering consultant to investigate possible remedies, but did little to correct the situation. In 1976, almost serendipitously, the State Department of Environmental Conservation began to collect soil and other environmental samples in the neighborhood, in search of the source of Mirex contamination of Lake Ontario fish. Mirex, a pesticide used in southern states to control the fire ant, was manufactured at Hooker's Niagara Falls plant.

At the request of state officials, the U.S. Environmental Protection Agency lent assistance to an expanded study of air and groundwater pollution there. These air, soil, and water samples were sent to the State Health Department laboratories for analysis, and in the spring of 1978, the State Health Commissioner learned for the first time that these samples were from a chemical landfill in the heart of a residential neighborhood.

The first reaction of the State Health Commissioner was to declare the landfill a public health nuisance, and to order county health officials to cover or remove all visible chemicals and pesticides, and restrict public access to the site. The Commissioner's order, dated 25 April 1978, constituted the first official recognition that the Love Canal chemicals represented a potentially serious threat to the health and welfare of people living nearby.

As new environmental data flowed in and were analyzed, it became apparent that unacceptably high levels of toxic vapors were present in the basements of many homes in the first ring surrounding the landfill.

Sampling of ambient air in the basement of first-ring homes indicated the presence of some 80 different chemical compounds, including benzene, lindane, toluene, and trichlorethylene. Basement contamination was greater by far in the first ring of homes than in ring 2 homes, in more than half of which there was no measurable chemical contamination.

According to the residents, chemical odors became most noticeable during periods of heavy rain or snowfall, when water and chemicals rose to inundate the landfill. The outward migration of chemicals, some suggested, was through a network of low-lying swale areas. Based on historical archives and examination of aerial photographs, we were able to trace the location of former streambeds, ponds and other low-lying areas in the Love Canal neighborhood.

In July of 1978, Governor Carey signed legislation granting special emergency powers to the State Health Commissioner to deal with Love Canal health problems and appropriating $500,000 to conduct expanded health and epidemiologic testing, which eventually was to embrace some 4,000 people living in the vicinity of the landfill. An outside panel of nationally recognized experts in toxicology, epidemiology and industrial hygiene was convened to review the State's findings at Love Canal and to make recommendations for dealing with health and environmental problems encountered there.

In August of 1978, the State Health Commissioner, acting under his newly granted authority, declared that a state of emergency existed at the Love Canal landfill and ordered the closing of the elementary school there and the evacuation of pregnant women and children under the age of two, the two sub-groups considered most vulnerable to toxic chemical exposure.

The Governor followed up this move by announcing that, primarily as a safety measure, the State would purchase the homes of, and pay for the cost of relocating, the 239 families living in the first two rings of houses surrounding the landfill. The Governor created an interagency State Task Force to deal with relocation problems and to oversee remedial construction work at the landfill. Governor Carey also contacted the White House, requesting federal disaster assistance to deal with the Love Canal crisis.

Within two months, almost all of the families in the first two rings of homes had been relocated, and remedial construction, to prevent water penetration of the site and halt the outward flow of chemicals, began.

Today, remedial construction work is nearly completed. Epidemiological, environmental and toxicological testing continues in an ever-widening ring around the Canal site. And, with the Legislature's recent appropriation of $5 million to rehabilitate the Love Canal neighborhood and buy the equity of homeowners who wish to move from the area, New York State's public investment in resolution and abatement of the Love Canal catastrophe exceeds $30 million.

SUMMARY AND CONCLUSION

To date, more than 200 organic compounds have been identified in soil, sediment and water samples collected from the landfill and the area that surrounds it. We are continuing our efforts to identify the chemical agents responsible for the excess morbidity and feto-toxic effects found among Love Canal residents. What is impossible to estimate is the psychological harm that has attended the social upheaval of the Love Canal.

Life, according to the English novelist Samuel Butler, is the art of drawing sufficient conclusions from insufficient premises. This is the public health lesson of the Love Canal.

Throughout the past eighteen months, we have been faced with the paradigm of discerning the normality or abnormality of our epidemiologic evidence. Constantly, we are compelled to compare our data with population groups that, in social, educational, vocational and even geographical context, are jarringly inconsistent with the predominantly industrial households of the Love Canal. This is the single most important lesson of the Love Canal: we desperately need to build our reservoir of epidemiologic information concerning the impact of long-term, low-dose exposure to man-made chemicals, and we have to establish causality with precision in the face of such confounding health influences as cigarette smoking, dietary fat intake, work environment, and even genetic factors.

It is not inconceivable that significant deleterious health effects of the chemicals buried at Love Canal will not become manifest for 25 or 30 years, and we should be prepared to recognize this phenomenon, should it occur. This is precisely why we should undertake detailed epidemiologic studies now, involving population groups comparable in occupational background, economics and other characteristics of the people of the Love Canal.

Statistically speaking, even at the Love Canal, relatively few persons have been shown to have been seriously harmed by exposure to man-made chemicals. But the public perception of harm, encouraged by some in the scientific community who have consciously or unconsciously suffused their findings with a social ideology, has been very great indeed. The question has been raised, should government respond to the psychological implications of harm with the same concern as it does when the harm is all too real or evident, such as the injury and destruction that commonly accompany hurricanes, floods or other natural disasters?

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All the more reason, then, for the scientific community to identify and quantify risks and relate health effects to types and levels of exposure. For we need to rigorously establish what is normal just as we must be able to recognize and identify abnormality. To do less would be to make Love Canal a greater tragedy than it really is.

And if I may conclude on a sober note, I would like to quote Dr. Philip Handler's recent address on environmental carcinogenesis, in which he said the following:

"What seems lost on some who would participate in the debate on the place of technology in our society, particularly those concerned with possible environmental carcinogenesis by radiation or chemicals, is that the necessity for scientific rigor is even greater when scientific evidence is being offered as the basis for the formulation of public policy than when it is simply expected to find its way on the market place of accepted scientific understanding. Science itself can benefit by early publication of properly documented preliminary findings. But surely public policy should not rest on observations so preliminary that they could not find acceptance for publication in an edited scientific journal. And yet, that has happened repeatedly."

PROBLEMS ASSOCIATED WITH DISPOSAL OF
HIGH ENERGY FUELS AND EXPLOSIVES.

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No textual material was made available for this paper. The information included has been reproduced from the graphic aids used in the oral presentation.

DOD OPEN-BURNING DISPOSAL OF WASTE MUNITIONS

Definitions
Magnitude
Nature
Problem Resolution
Conclusions/Recommendations

WASTE MUNITIONS (PEP)

Waste PEP: Consists of propellants, explosives, or pyrotechnics (PEP) and other such energetic or hazardous materials which do not, or cannot be refined to meet required specifications.

Sources: Off-specification and scrap materials which are generated from primary production, loading, rework, demilitarization, resource recovery operations, and RDT & E.

PEP-Contaminated Wastes: Consists of various nonenergetic materials which have become contaminated with PEP and/or other such energetic or hazardous materials.

Sources: Cleaning rags, cardboard shipping boxes, dunnage, metal tanks, and wood or other building materials.

OPEN BURNING

The burning of materials, in the open air or in a receptacle, without significant control of the combustion and in such a manner that the products of combustion are emitted directly into the ambient air without passing through a device intended to control gaseous or particulate emissions.

MAGNITUDE OF DOD OPEN-BURNING DISPOSAL OF WASTE MUNITIONS

54 Installations
30 States and Territories
8K Tons/Year Waste PEP
26K Tons/Year PEP-Contaminated Wastes
Declining

COMPLEXITY OF DOD WASTE MUNITIONS DISPOSAL

Hazards
Diversity of Wastes
DOD Unique
Environmental Constraints
Legal Aspects
Economics
Sale/Recycle Problems
Unavailable/Unproven Technology
R&D/Operation Transition
Demil/Disposal Interface
Geographically Dispersed
Transportation Constraints

APPLICABLE LAWS AND REGULATIONS

Laws

1. Clean Air Act of 1963
2. Air Quality Act of 1967
3. Clean Air Amendment of 1970

Regulations

1. Part 76 of Title 40, Code of Federal Regulations (CFR) - Originally 42 CFR Part 76 (June 6, 1966)
2. Executive Order 11752 (December 17, 1973)
3. Revocation of Part 76, Title 40 CFR (March 25, 1975)
4. Compliance with Stationary Source Air Pollution Standards (May 12, 1975)
5. Summary of State Open Burning and Incinerator Regulations

Navy Instruction

1. OPNAV Instruction 6240.3E

PART 76 OF TITLE 40, CFR

Part 76 - Prevention, Control, and Abatement of Air Pollution from Federal Government Activities: Performance Standards and Techniques of Measurement.

#76.8.a.2. Deteriorated or Unused Explosives, Munitions, Rocket Propellants, and Certain Hazardous Wastes May be Burned in Open Fires, in Accordance with Recognized Procedures.

40 CFR Part 76, Originally 42 CFR Part 76, was Promulgated on June 6, 1966, by Secretary of HEW under Section 5 of Executive Order 11282 (May 26, 1966). It was Redesignated as 40 CFR Part 76 on November 25, 1971.

REVOCATION OF PART 76, TITLE 40 OF CFR

Part 76 - Prevention, Control, and Abatement of Air Pollution from Federal Government.

Activities: Performance Standards and Techniques of Measurement.

"The Administrator (EPA) Has Concluded That Part 76 is Substantially Inconsistent with Regulatory Scheme Mandated by the Clean Air Amendment of 1970 and Should be Revoked as Confusing and Unnecessary."

Effective Date: March 25, 1975
F.R., Vol. 40, No. 58, 13216

COMPLIANCE WITH STATIONARY SOURCE AIR POLLUTION STANDARDS

Action is Guideline to Provide Means of Determining Compliance Status of All Federal Stationary Sources of Air Pollutants Which are Subject to Federal, State, and Local Emission Limitations.

"Facilities Having One or More of the Following Points of Emission Should be Considered Major Emitters: ... Open Burning of Munition ..."

Effective Date: May 12, 1975
F.R., Vol. 40, No. 92, 20664

OPEN BURNING VS. OTHER DOD
WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
Safety	Air, Water, Soil & Noise Pollution
Economy	Aesthetic Impact
Energy Efficiency	Land Dedication
Versatility	Controversial
Reliability	Legality
Minimal Transportation	Major Emitter

RESOLUTION OF DOD WASTE - MUNITIONS DISPOSAL PROBLEM

Waste Reduction

Disposal Alternatives

    Destructive
        Nonincineration
        Incineration

    Nondestructive
        Sale
        Recycle
        Retention

    RDT & E Efforts
        DOD
        ERDA (Energy R&D Admn., was AEC)
        Others

DOD WASTE MUNITIONS DISPOSAL ALTERNATIVES

Open Burning
Biodegradation
Central Disposal Sites
Chemical Decomposition
Deep Land Burial (Salt Mines, etc.)
Deep Sea/Water Disposal/Dumping
Deep Well Slurry Injection
Detonation: Unconfined and Confined
Landfill (Shallow Burial)
Long-Term Storage/Retention
Incineration Approaches

BIODEGRADATION VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
Fertilizer End Product*	Water & Soil Pollution*
Mutual Sewage Sludge/PEP Disposal*	High Operating Costs
	Long Retention
	Incomplete Reaction*
	High Sludge/PEP Ratio
	Sludge Disposal
	Large Land Area
	Not Universal for PEP
	Cannot Handle Ordnance etc.
	Toxic Intermediates
	Odors
	Scale Up Problems*
	Unproven
	10-20 Years Away

*Possibly

CENTRAL DISPOSAL SITE VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
Handle Most Waste Munitions	High Cost
Handle Solid Wastes	Ash/Waste Water Disposal*
Energy Recovery*	Transportation
Reduce Air, Solid Waste & Noise Pollution	Varying Feed
	Safety Problems*

*Possibly

CHEMICAL DECOMPOSITION VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
Useful By-Products*	Requires Much RDT & E
Potentially Environmentally Acceptable	High Capital Costs
Conserves Resources*	Energy Inefficient
	Generally Unavailable
	Specific Feeds Usually Create Additional Wastes*
	Safety Problems*

DEEP LAND BURIAL VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
No Pollution Abatement Equipment	Not Ultimate Disposal
No Ash or Sludge	Higher Cost
	Long-Term Monitoring/Security
	Personnel & Public Hazards
	Land Acquisition*
	Land Restriction
	Long-Term Commitment
	Extensive Operations
	Controversial
	Transportation*
	Q-D Considerations
	EIS

*Possibly

DEEP WATER DISPOSAL VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
No Ash or Sludge	Ultimate Disposal?
High Applicability	Higher Costs
Environmental Impact Low*	Transportation
	Personnel & Public Hazards
	Extensive Operations
	Surplus Ships*
	Controversial
	Restricts Ocean Uses
	Justification
	CNO Approval
	EPA Permit
	EIS

*Possibly

DEEP WELL SLURRY INJECTIONS VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
No Ash or Sludge	Higher Costs
No Pollution Abatement Equipment	Land Acquisition*
	Solution or Slurry Only
	Transportation*
	Water Pollution*
	Monitoring Problems*
	Earthquakes
	Irreversible
	Controversial*
	Hazards*

DETONATION VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
UD Low Cost	UD Noise & Ground Shock
Energy Efficient*	UD Complaints
CD Only Envir. Accept. Appr.*	UD Air, Water, Soil Pollution*
CD Improves Public Relations*	Batch Process
Lower NO_x*	UD Weather Dependent*
	Not Universal

CD = Confined Detonation
UD = Unconfined Detonation
*Possibly

LANDFILL VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
No Pollution Abatement Equipment	Limited Versatility
Low Cost*	Water Pollution*
	Hazards*
	Restricts Land Use
	Finite Capacity*
	Site Specific
	Land Acquisition
	Variable Costs
	Transportation*
	Controversial*

*Possibly

LONG-TERM STORAGE/RETENTION VS. OTHER DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Advantages	Disadvantages
Defers Adverse Environmental Impacts*	Not Ultimate Disposal
Permits Technological Development	Higher Cost*
Enhances Public Relations*	Hazards*
Proven	Long-Term Monitoring/Security
Accumulates Large Lots	Transportation*
	Q-D Considerations
	Deterioration

*Possibly

INCINERATION APPROACHES FOR DOD WASTE-MUNITIONS DISPOSAL

Air Curtain (ACI)	Molten/Fused Salt
Closed Pit (CPI)	SITPA I & II
Batch/Popping Box	Vortex
Rotary Furnace	Vertical Induced Draft
Rotary Kiln	Multi Chamber
Fluidized Bed	Fuel Supplement
Wet Air Oxidation (WAO)	Miscellaneous

COMPARISON OF DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES[a]

Disposal Approach	Operational Mode	Developmental Status	Environmental Acceptability
Ideal Criteria	Continuous	Operational	High
Open Burning	Batch	Operational	Undesirable
Biodegradation	Batch	Experimental	Medium to High[b]
Central Disposal Site[d]	Batch to Continuous[d]	Planning	High
Chemical Decomp.[f]	Batch to Continuous	Various Devel. Stages	Medium to High
Deep Land Burial	Batch	Planning	High
Deep Water Disp.	Batch	Operational	Under Study
Deep Well Slurry Inj.	Continuous	Operation	High[b]
Detonation:			
Unconfined	Batch	Operational	Undesirable
Confined	Batch	Experimental	High
Landfill	Batch	Operational	High[b]
Long Term Stor/Ret	Batch	Operational	High
Incineration[f]	Batch to Continuous	All Developmental Stages	Low to High

(a) Based on waste-munitions categories known/believed suitable for disposal approach, (b) If no ground water pollution, (d) Primarily depot disposal system, (f) Evaluations dependent on process.

COMPARISON OF DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Disposal Approach	Public Acceptability	Equipment Complexity	Type of Solution
Ideal Criteria	High	Low	Permanent
Open Burning	Low to Medium	Low	Interim
Biodegradation	Low to Medium	Low[c]	Permanent
Central Disposal Site[d]	High[e]	High	Permanent
Chemical Decomp.[f]	High	Low to High[b]	Permanent
Deep Land Burial	Low[e]	Medium	Permanent
Deep Well Slurry Inj.	Low to Medium[e]	Medium	Permanent
Detonation: Unconfined	Low	Low	Interim
Confined	High	Low	Permanent
Landfill	High	Low	Interim
Long Term Stor/Ret	Medium to High	Medium	Interim
Incineration[f]	High	Low to High[c]	Interim/Permanent
Deep Water Disp.	Low[e]	High	Permanent

Disposal Approach	Adaptable Commercial Equipment	Energy Efficiency	Life-Cycle Costs
Ideal Criteria	Yes	High	Low
Open Burning	Not Applicable	High	Low
Biodegradation	Yes	Medium[h]	High
Central Disposal Site[d]	In Some Cases	Medium	High
Chemical Decomp.[f]	Yes	Low to High	Medium to High
Deep Land Burial	Yes	Low	High
Deep Water Disp.	Yes	Low to Medium	Low to Medium
Deep Well Slurry Inj.	Yes	Low	Medium to High
Detonation: Unconfined	Not Applicable	High	Low
Confined	No	Medium	Medium
Landfill	Yes	Medium to High	Low
Long Term Stor/Ret	Yes	Low to Medium	High
Incineration[f]	In Some Cases	Low to Medium	Low to High

(b) If no ground water pollution, (c) Process monitoring equipment may be complex, (d) Primarily depot disposal system, (e) Waste transportation may encounter public opposition, (f) Evaluations dependent on process, (h) Materials handling energy intensive.

COMPARISON OF DOD WASTE-MUNITIONS DISPOSAL ALTERNATIVES

Disposal Approach	Safety[g]	Additonal RDT&E Effort Required	Availability (Time Frame)
Ideal Criteria	High	Nil	Available
Open Burning	High	Nil	Available
Biodegradation	Medium to High	High	Long Range
Central Disp. Site[d]	Medium to High	Medium	Mid to Long Range
Chemical De-comp.[f]	Medium to High	Low to High	Mid to Long Range
Deep Land Burial	Low	Low	Long Range
Deep Water Disp.	High	Nil	Mid Range
Deep Well Slurry Inj.	Medium to High	Medium	Mid to Long Range
Detonation:			
Unconfined	High	Nil	Available
Confined	High	Low	Mid to Long Range
Landfill	Medium to High	Low	Mid Range
Long Term Stor/Ret	Low	Nil	Available
Incineration[f]	Medium to High	Low to High	Available to Long Range

(d) Primarily depot disposal system, (f) Evaluations dependent on process, (g) Decreases with waste quantity & AGE, (i) Approximate time frame: short term 0-1 year, mid range 1-5 years, long range 5-10 years.

SUITABILITY COMPARISONS OF DOD WASTE-MUNITIONS FOR DISPOSAL ALTERNATIVES

Disposal Approach	Propellants, Bulk	Explosives, Bulk	Pyrotechnics, Bulk
Ideal Criteria	Yes	Yes	Yes
Open Burning	Yes	Yes	Yes
Biodegradation	Possibly	Yes	No
Central Disp. Site	Yes	Yes	Yes
Chemical De-comp.	Yes	Yes	Yes
Deep Land Burial	Yes	Yes	Yes
Deep Water Disp.	Yes	Yes	Yes
Deep Well Slurry Inj.	Yes	Yes	Yes
Detonation[a]	Possibly	Yes	No
Landfill	No	No	No
Long Term Stor/Ret	Yes	Yes	Yes
Incineration[b]	Yes	Yes	Yes

(a) Unconfined and confined, (b) Suitability dependent on process.

SUITABILITY COMPARISONS OF DOD WASTE-MUNITIONS
FOR DISPOSAL ALTERNATIVES

Disposal Approach	PEP-Contaminated Dunnage	Selected Small Ordnance	PEP-Contaminated Equipment
Ideal Criteria	Yes	Yes	Yes
Open Burning	Yes	Yes	Yes
Biodegradation	No	No	No
Central Disp. Site	Yes	Yes	Yes
Chemical De-comp.	No	No	No
Deep Land Burial	Yes	Yes	Yes
Deep Water Disp.	No	Yes	Yes
Deep Well Slurry Inj.	No	No	No
Detonation[a]	No	Yes	No
Landfill	Possibly	No	No
Long Term Stor/Ret	No	Yes	Yes
Incineration[b]	Yes	Yes	Yes

(a) Unconfined and confined,   (b) Suitability dependent on process

OPERATIONAL AND ENVIRONMENTAL
CONCLUSIONS/RECOMMENDATIONS FOR DOD OPEN BURNING
DISPOSAL OF WASTE MUNITIONS

Variety Burned
SOPs Safety Orientated
Safe
Economical
Energy Efficient
Reliable
Environmental Impacts Minor/Vague
Termination Adverse for DOD
Implement Immediate/Mid Range Actions
Continue as Needed

PROBLEM RESOLUTION CONCLUSIONS/RECOMMENDATIONS
FOR DOD OPEN BURNING DISPOSAL OF WASTE MUNITIONS

8-10 Years for Elimination
Need Mid Range Measures
Pursue Both Reduction and Disposal
Coordinate Sale/Recycle
Quantify Disposal Comparisons
Proven Technologies Not Full Solution
Universal Solution Unlikely
Improve RDT&E/Operations Interface
Coordinate/Rank/Concentrate RDT&E
Solution Expensive

TECHNOLOGIES FOR CONTRIBUTING TO ELIMINATION OF DOD
OPEN BURNING DISPOSAL OF WASTE MUNITIONS

Proven	Promising	Unpromising
Rotary Kiln Incin.	Central Disposal Site	Biodegradation
Rotary Furnace	Chemical Decomposition	Deep Land Burial
Incin.	Unconfined Detonation	Deep Water Burial
	Confined Detonation	Deep Well Slurry Injection
	Air Curtain Incin.	Landfill
	Closed Pit Incin.	Long Term Storage/Retention
	Batch Box Incin.	Molten Salt Incin.
	Fluidized Bed Incin.	
	Wet Air Oxidation	
	SITPA II (Incin.)	

OPEN FORUM

DR. BENZ (University of California, Irvine): Dr. Shuster, in light of what the other speakers discussed, what would you call a secure landfill?

DR. SHUSTER (Rensselaer Polytechnic Institute): A secure landfill is intended, first of all, to accept only materials which in their own right would not cause any water or land pollution. In addition, there's been a lot of work done developing what are known as engineered or secured landfills which consist of landfills which are prepared with a lining that is highly resistant to diffusion of materials to surrounding strata. This might be done by plastic or clay liners. There are some people who argue that there is no such thing as a secure landfill, that any material placed in a landfill is going to diffuse out, and there is no barrier that can prevent diffusion. So one can argue that maybe there isn't such a thing as a secure landfill, but if you consider the fact that no materials can go into it which would cause pollution, then perhaps we can accept that as a definition.

DR. OLSEN (Ohio State University): You did not include radioisotopes in your list of hazardous materials. Was there a reason for that?

DR. SHUSTER: The disposal of radioisotopes was not included in our charge from the state. That is a problem in itself which we recognize, but it was not part of our study.

MR. VERNOT (University of California, Irvine): My question is for Mr. Sanchez. I don't know that you said it exactly, but did I understand correctly that a location for the central disposal site has been selected but the method of disposal has not yet been selected by the Navy?

MR. SANCHEZ (Naval Surface Weapons Center); There are two disposal sites. The Navy decided to dispose of high energy materials which are the propellants, pyrotechnics, and explosives of all types at the Hawthorn Ammunition Depot now called the Hawthorn Army Ammunition Plant because of the lake and the terrain there and the fact that the population nearby was very low. The Indian lands are adjacent to this area, and the closest civilization which has a population of 1000 is approximately 52 miles away. Normally, the prevailing winds are not in that direction. The back part of the arsenal land is adjacent to 78,000 acres of unused land that nobody wants, including the Indians. At the time this decision was made, this was an acceptable solution for disposal of high energy materials. The problem is that the equipment that was installed there is restrictive in nature. The amount of the explosive to be disposed of from all over the world is too great.

Currently, we are running into problems of transportability. Suppose the plant works right, and we do have materials at Hawthorn that we can take care of. The states of California and Nevada, for example, don't like to have explosive and toxic materials from other areas transported across or disposed of in their lands. The Army has responsibility for disposal of old war gases, defoliants, riot control agents and a large number of other agents. They have a very large plant, part of which is in operation. They use high temperature furnaces and, of course, controlled environments to scrub the air. It's a very expensive process and very limited. The problem right now is that the Army doesn't have permission, for example, to haul material across the mountains from the Rocky Mountain Arsenal for the purpose of being detoxified. The Navy also has that problem. There is no central facility yet.

DR. HODGE (University of California, San Francisco): Dr. Axelrod, do you have long-term plans for epidemiological studies on the people who lived in the Love Canal area?

DR. AXELROD (New York State Department of Health): Yes, we do. We're creating a registry of the residents who have lived there in the past in addition to which we are going back and creating a registry of the school which was located in the area. It was an elementary school, and presumably those children attending it would have had 7 years of exposure from residence in that area. I hope this information will provide a basis for some further studies and will be one of the few well documented areas of low level exposure to organic chemicals.

DR. HOUSEWRIGHT (National Academy of Sciences): Dr. Axelrod, you said that the psycho-social impact may be larger than the actual health effect. I wonder if you would care to expand on that a little bit.

DR. AXELROD: I think when we first started there were skeptics as to whether or not there were any health effects and that we were, perhaps, looking for things that weren't there. As we began to find things, people became very much concerned, and the psychological impact of the studies resulted in the appearance of many illnesses which could not be documented as having a physical basis. This does not mean that the illnesses were any less real. Also, we disrupted a community. We removed approximately 240 families initially. We created a spectre of a prison compound by erecting a fence to prevent access to the area. We created an area of desolation within a community and completely disrupted the community. The residents who had lived there for 25 and 30 years were now scattered to the winds. We destroyed friendships and relationships that had been made over a very long period of time. I think the final impact of all of these destructive forces that we brought into play by virtue of our findings have yet to be determined. They may well be as important as the long-term cancers associated with it.

DR. ANDREN (University of Wisconsin): Dr. Axelrod, what is being planned or being done about detoxification of the site at the Love Canal?

DR. AXELROD: There is a drain in place now, and the drains intersect the canal. There are lateral drains along the side. There are drains which intersect the canal, and the drainage runs to an on-site treatment facility which then discharges into the sewer system and then carries it to a fairly complex sewage treatment system located at Niagara Falls. Niagara Falls, because of its concentration of chemical industries, has constructed one of the largest carbon bed sewage treatment facilities in the country. After the local on-site treatment where the TOC has been reduced to acceptable levels for discharge into navigable waters based on current EPA standards, it still goes to an additional sewage treatment facility. We are presently examining the storm sewers with television cameras to determine the amount of sludge which may have been carried into the storm sewers and the contamination of streams which have drained the storm sewer system from the area. Our most recent findings are that several of the streams in the area have been contaminated with a variety of chemicals including dioxin from the canal.

DR. SMITH (National Institute of Occupational Safety and Health): Dr. Axelrod, what was the average residence time for those families along the canal?

DR. AXELROD: About 25 years.

DR. SMITH: Most of them had lived there since the homes were built?

DR. AXELROD: That's correct. There had been very few houses sold. It was a very stable, blue collar, lower income white neighborhood. A substantial number of the families had two members of the family working, the male member having worked in the chemical industry since its major development in the immediate post-war era when the major construction took place. Many of the individuals who lived there did so for 15 to 25 years.

DR. SMITH: So information from previous residents that would be in the immediate area that might be significant would probably be minimal then?

DR. AXELROD: We had a nationwide search for those individuals, and we have located a number of families who have moved out of the area. They will become part of our long-term study. We do have fairly good demographic data on the canal. Because of the fact that it's such a stable area, it's ideal for the kinds of studies we would like to carry out.

DR. SMITH: What was the group that was used for calculation of expected rates of miscarriage? Was that another group of residents near there?

DR. AXELROD: No, with respect to miscarriages, it was the Warberton data which was calculated for Montreal. We looked at several other kinds of statistical tabulations of expected miscarriages, and no matter which way it was examined or what kind of control group was selected, there was at least a 2 to 3-fold difference. If you use a case control method based on areas north of the Love Canal which are not directly impacted by the canal, you come up with approximately the same kind of difference. We are looking at a variety of different methods of determining the expected frequency of miscarriages. All of them seem to give about the same kinds of increase over the anticipated.

DR. BAETJER (The Johns Hopkins School of Hygiene and Public Health): If you pass this material from the canal through a filter bed, doesn't it contaminate the filter bed? Then what do you do about it?

DR. AXELROD: There are a variety of companies that provide the charcoal filter beds which also replace the charcoal which is then incinerated, and the charcoal is regenerated and returned. Most large treatment facilities using charcoal filters have ongoing contracts with companies that provide for both incineration disposal and regeneration and repletion of the carbon beds.

DR. VAN DER SCHALIE (U.S. Army Medical Bioengineering Research and Development Laboratory): I have a question for Dr. Andren. You indicated that the major means for concentration of PCBs in trout and salmon were eventual uptake from residues through food chain sources. I believe there are data for other chlorinated organics such as DDT that bioconcentration directly from the water may be a more important source. I was wondering if this might be a factor with PCBs although the water concentrations are very low.

DR. ANDREN: We have to remember that I directed those comments just to adult lake trout and salmon. When they are younger, the direct uptake from the water column is more important. We didn't look into those specifically in Lake Michigan. The way we did this is called bioenergetic modeling. What we did was ask a group of biologists to go out and determine exactly what made an adult lake trout or an adult salmon grow. How much did they eat to get to a certain weight? The biologists measured stomach content and constructed what they call a bioenergetic model which essentially is a growth model dependent upon temperature and the activity of a fish. Then we collected all the data from Michigan, Wisconsin, or wherever it was available and plugged that into this model. It was quite striking how well we could model some of the lake trout PCB levels. These levels fluctuate according to the seasonal fat content of fish, and we should be very careful when we look at trends.

LT. COL. MC GHEE (USAF, Office of the Surgeon): I was on a task force in Region 9 several years ago where we looked at matching the waste products from one industry with raw material from another industry to reduce pollution. We circulated a questionnaire to local industry, and we got a very poor response. Most industries were unwilling to identify their waste products for a variety of reasons including a concern about proprietary processes. I wonder if in the state of New York there are any plans to do anything with the inventory as far as matching on a statewide basis and what there might be on a nationwide basis for this type of problem?

DR. SHUSTER: There's some consideration being given to that idea of attempting to have a centralized information center regarding these wastes. You've identified the real problem which is that some industries feel that there may be proprietary information that might become available to other people. This is somewhat of a problem but it is being considered. We have also considered feeding information on all of the waste streams into a central place which would be available to other industries. For example, if I'm looking for an alkaline waste because I've got an acid waste, this information could be fed to them. Another difficulty recognized is that the composition of industrial waste tends to keep changing as processes develop and as industries change their manufacturing operations. It doesn't stay constant.

MR. WELCH (University of California, Irvine): I would like to ask Dr. Shuster is he has any specific recommendations in disposing of used motor oil.

DR. SHUSTER: There are some companies going into the business of recovering and purifying oil for reuse. There are a lot of problems there, though. The motor oil does tend to become contaminated with metal filings and with junk such as cigarette butts that people drop into it. I have a friend who runs a garage, and he operates a furnace with the waste oil that he takes out of cars. It's a special furnace that he uses to partly heat his own garage.

DR. NEWTON (University of California, Irvine): Dr. Andren, have you been able to determine where the majority of the PCB in Lake Michigan is coming from? Is it from Chicago or is it from Milwaukee? How do you put this in the context of the state of Illinois suing Milwaukee for polluting Lake Michigan?

DR. ANDREN: There doesn't appear to be any single source. The PCBs just permeate our society completely. We have them in this room in the capacitors and in fluorescent lights, for example. If we could immediately get rid of all of the PCBs, I think society would come to a screeching halt. There are a couple of hundred pounds in locomotives. All schools would have to close down. Hospitals would have to close down when they couldn't get fire insurance because of the capacitors which are fire insured.

All television sets would have to be thrown away because of the energy saving capacitors. Our whole environment is permeated with PCB-containing equipment. When we go to landfills, we can frequently find clouds of PCBs. When we get to population centers, and they don't necessarily have to be industrial in nature, we find elevated PCB levels. It's really very difficult to completely pinpoint all of the PCB sources.

INHALATION TOXICOLOGY

Chairman

Edmond H. Vernot
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SUBCHRONIC INHALATION TOXICITY OF DECALIN

C. L. Gaworski
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and

Major R. H. Bruner

Air Force Aerospace Medical Research Laboratory
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Decalin (decahydronaphthalene) is an alicyclic hydrocarbon commonly used as a solvent for oils, fats, and resins and has been substituted for turpentine in oil paints. Another important use of decalin is as a solvent and stabilizer for shoe creams and floor waxes, in which the mild terpene-like odor can be easily hidden. The military interest in decalin arises from its desirable characteristics for use as a high density fuel in the submarine launched cruise missile system.

There is presently no established threshold limit value for decalin, and the data available in the literature are insufficient for setting this limit. An acute oral LD_{50} of 4170 mg/kg of body weight for rats was established by Smyth et al. in 1951. Prior to this, Cardeni in 1942 reported that repeated daily 8-hour inhalation exposures of three guinea pigs to 319 ppm decalin resulted in one death on day one, a second death at 21 days, and the third death at 23 days. The microscopic examination of the tissues revealed lung congestion, kidney and liver injury. In a more recent inhalation study, Gage (1970) exposed eight rats to 200 ppm decalin for 20 days, 6 hours/day. No toxic symptoms were seen, and all tissues were grossly normal at necropsy. A single 4-hour inhalation exposure of eight rats to 1000 ppm caused mortality which was preceded by tremors and convulsions in three of the rats.

In a study reported by MacEwen and Vernot in 1978, Sprague-Dawley rats, CF-1 mice, and Hartley derived guinea pigs were exposed via inhalation to 50 ppm or 250 ppm decalin. The exposures were conducted on a 6 hour/day, 5 day/week basis for a total of 22 exposures. Rats exposed to decalin had respiratory tract irritation, hydropic change in the cytoplasm of hepatocytes, and hyalin droplet formation in the cytoplasm of the proximal tubular epithelial cells. Mice and guinea pigs exposed to decalin also exhibited signs of respiratory tract irritation.

The study reported here was designed to determine the toxic effects, as well as the oncogenic potential, of 90 days of continuous inhalation exposure of test animals to decalin vapors. The study was conducted for the U.S. Navy at the University of California's Toxic Hazards Research Unit facilities located at Wright-Patterson Air Force Base. A 90-day continuous exposure was chosen to simulate conditions on board submarines where decalin would be carried or transferred in enclosed areas and where crewmen might be exposed over the length of a cruise.

Decalin is a colorless liquid with a mild terpene odor. The materials used for these studies were typically 99.9% pure as determined by gas chromatographic analysis.

Decalin consists of two fused cyclohexane rings and exists in two isomeric configurations cis and trans. The physical properties of decalin along with the structure are shown in Figure 1.
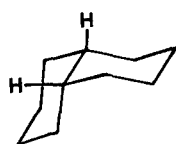
CIS – DECALIN
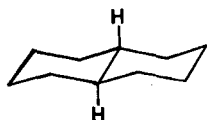
TRANS – DECALIN

Figure 1. Structure and physical properties of decalin.

MOLECULAR WEIGHT	138
BOILING POINT RANGE	188–195°C
SPECIFIC GRAVITY	0.885–0.890

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Decalin vapors were generated by passing the liquid down through two heated evaporator towers. Air passing up through the towers created a decalin vapor/air mixture. This mixture was split into the approximate volume ratio of the chamber concentrations before entering the respective chamber air streams. The chamber air stream flow was then used for maintaining finer control of the chamber contaminant concentration. Use of this type of contaminant vapor generation system resulted in total vaporization of decalin.

A Beckman 400 hydrocarbon analyzer was used for continuous mass analysis of the chamber atmosphere. A Varian 1200 gas chromatograph with a flame ionization detector was used for quality control checks of each drum of decalin prior to its use in the study. The GC was also used routinely to analyze the chamber atmosphere to verify that decalin vapor in the chamber was not appreciably altered by the generation system. The column used in the GC was a 12 ft. x 1/8 in. stainless steel tube packed with 10% SE 30 on Chromosorb W. The system was maintained isothermally at 40°C.

Groups consisting of purebred beagle dogs (3 male, 3 female), 150 Fischer 344 rats (75 male, 75 female), and 150 C57Bl/6 female mice were exposed to decalin vapor concentrations of 5 ppm or 50 ppm in Thomas Dome inhalation chambers (Thomas, 1968). A control group consisting of an equal number of animals was housed in laminar air flow rooms in a separate facility. All animals had food and water ad libitum.

Upon termination of the 90-day exposure period, all of the dogs and one-third of the rodents were killed for detection of any pathologic lesion resulting from exposure. The remaining rodents were held for 19 months of observation. An interim kill at that time should have allowed sufficient time for tumor formation to occur. It also will provide a statistically satisfactory sample of animal tissue for microscopic examination. The remaining rodents are being held for further observation for a period of 6 months (total of 24 months on study) at which time the final kill will occur.

TABLE 1. TISSUES TAKEN FOR HISTOPATHOLOGIC EXAMINATION

Gross lesions	Esophagus	Thymus
Tissue masses or	Stomach	Pancreas
suspect tumors and	Duodenum	Spleen
regional lymph nodes	Ileum	Kidneys
Skin	Colon	Adrenals
Mandibular lymph node	Anus	Bladder
Mammary gland	Mesenteric lymph	Seminal vesicles
Salivary gland	node	Prostate
Larynx	Liver	Testes
Trachea	Thigh muscle	Ovaries
Lungs and bronchi	Sciatic nerve	Uterus
Heart	Sternebrae, verte-	Nasal cavity
Thyroids	brae, or femur	Brain
Parathyroids	(plus marrow)	Pituitary

All animals were carefully observed throughout the exposure and postexposure periods for signs of altered physical condition. Rats and dogs were weighed individually at biweekly intervals during exposure and rats monthly during the postexposure period. Mice were weighed monthly throughout the study, and the group mean weights were monitored. All animals that died or were killed were necropsied and the tissues taken for histopathologic examination (Table 1). The brain, liver, spleen, and kidneys of individual dogs and rats were weighed during necropsy and the ratios of organ to body weight of exposed animals were compared to unexposed controls. Blood samples were drawn from dogs biweekly throughout the exposure and from rats at the time of scheduled necropsy for clinical determinations as shown in Table 2.

TABLE 2. HEMATOLOGY AND CLINICAL CHEMISTRY TESTS ON
DOGS AND RATS EXPOSED TO DECALIN VAPORS

Hematology	Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
Total RBC	Calcium
Total WBC	Albumin/Globulin
Differentials	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin	Bilirubin
Concentration (MCHC)	Creatinine
	BUN
	SGPT
	SGOT

Bromsulphalein (BSP) retention times were measured on all dogs at the conclusion of the exposure period to evaluate liver function.

Statistical analyses of body weights, hematology, clinical chemistry, BSP retention times, and organ weights were carried out using the Student's T method.

RESULTS

The results presented in this paper summarize those obtained through the 90-day exposure phase, and include histopathologic tissue examination of the animals necropsied at that time.

The mean body weights of dogs exposed to decalin were higher than unexposed controls through the exposure phase of the study (Figure 2). The unexposed control dogs went through an unexplained period of weight loss during the first 6 weeks of the study. This greatly contributed to the differences in body weight observed between

decalin-exposed and unexposed control dogs. Hematology and clinical chemistry tests performed biweekly on dogs during the 90-day exposure revealed no abnormalities attributable to decalin exposure.
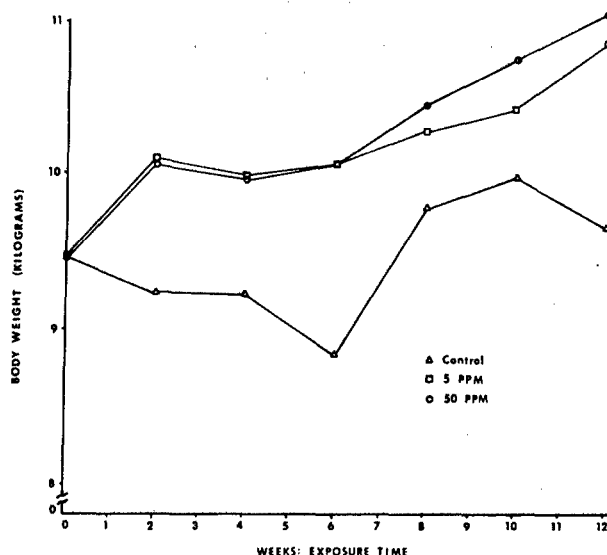
Figure 2. Effect of decalin on dog body weight.

Bromsulphalein retention time was measured on the dogs just prior to the sacrifice at the completion of the 90 days of exposure. The results of these tests are shown in Table 3.

TABLE 3. BROMSULPHALEIN RETENTION TIME IN DOGS AFTER 90 DAYS CONTINUOUS EXPOSURE TO DECALIN VAPORS

Concentration	% Retention at 10 Minutes*
Control	14.3 ± 7.1
5 ppm	11.3 ± 4.4
50 ppm	13.5 ± 8.2

*Mean $\pm$ S.D., N = 6

It can be seen from these results that liver function, evaluated by the BSP test, was not affected by decalin exposure.

Decalin-exposed dog brain, liver, kidney, and spleen weights were not significantly different from these organ weights from unexposed control dogs.

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Distinct dose-related pathological lesions were not observed either grossly or microscopically in dogs exposed to decalin vapors. Of moderate significance, however, was the finding of pulmonary inflammatory lesions in both control and exposed groups. These inflammatory changes ranged from mild, focal, chronic lesions in some dogs to diffuse, chronic active bronchopneumonia in others. In most cases, inflammation was attended by abundant eosinophil infiltrates. These findings suggest that many test subjects had mild verminous pneumonia caused by either migrating nematode larvae or adult <u>Filaroides</u> <u>sp.</u> lungworms.

The growth of male Fischer 344 rats was retarded by exposure to decalin vapors (Figure 3). Solid symbols in this figure represent mean values of the decalin exposed groups which were statistically different from control mean values at $p < 0.05$. Both groups of exposed males weighed significantly less than controls after 2 weeks of exposure. These differences in body weights continued through the 18th month of the study. Mean body weights of the male rats exposed to 50 ppm decalin vapors were generally less than the mean body weights of the male rats exposed to 5 ppm decalin vapors, indicating a dose response relationship.
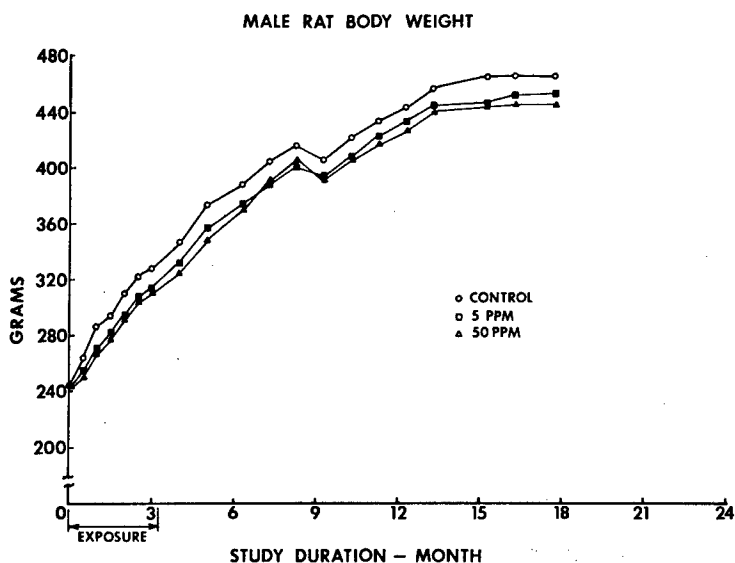
Figure 3. Effect of decalin on male rat body weight. Solid symbols denote values statistically different from controls at $p < 0.05$.

The body weights of female Fischer 344 rats are shown in Figure 4. Solid symbols represent mean body weights of test groups which were statistically different than the control group.

FEMALE RAT BODY WEIGHT

○ CONTROL
□ 5 PPM
△ 50 PPM

GRAMS

STUDY DURATION — MONTH
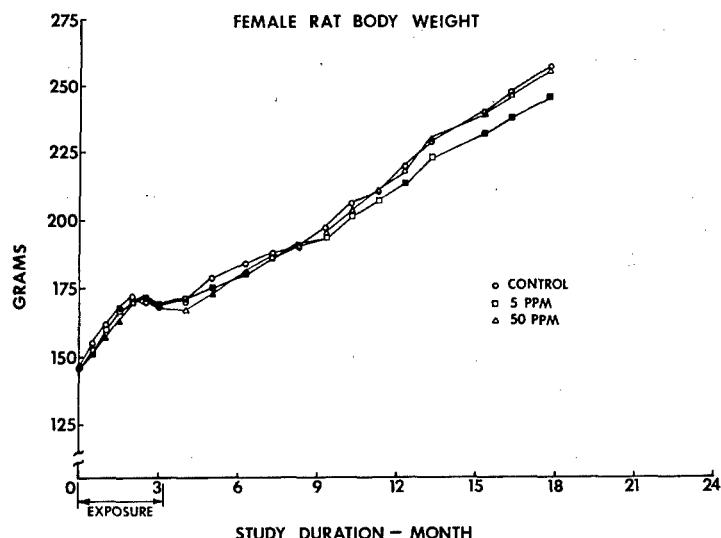
EXPOSURE

Figure 4. Effect of decalin on female rat body weight. Solid symbols denote values statistically different from controls at p < 0.05.

Decalin exposure had only a marginal effect on the weight gains of the female rats. The group mean weight of the females exposed to 50 ppm decalin was significantly less than controls after 6 weeks of exposure. However, at 8 weeks of exposure, the mean weight had returned to control values. All groups of females (control included) went through a period of weight loss toward the end of the exposure period. There was no obvious reason for this period of weight loss.

Blood samples were obtained via the portal vein from all of the rats that were necropsied at the conclusion of the 90-day decalin exposure period. Unfortunately, over 70% of the samples obtained were hemolyzed, leading to such uncertainties in the hematological and clinical chemistry results that they were rejected as nonvalid data.

Organ weights obtained from the rats at the conclusion of the 90-day exposure period are shown in Table 4 for males and females. Organ to body weight ratios of kidney, spleen, and brain of male rats exposed to 50 ppm decalin were greater than unexposed male control rats. The increase in kidney/body weight ratio in the male rats exposed to 50 ppm decalin was probably related to the tissue damage found in these rats. Increased kidney/body weight ratios were also evident in the male Sprague-Dawley rats exposed to 250 ppm decalin in the industrial type exposure described by MacEwen and Vernot (1978). All other changes in organ weights can be interpreted as reflections of the changes seen in body weight.

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TABLE 4. THE EFFECT OF 90-DAY CONTINUOUS INHALATION EXPOSURE TO DECALIN ON FISCHER 344 RAT ORGAN WEIGHTS[a]

Male

	Control	5 ppm	50 ppm
Body weight (gm)	327.4 ± 15.1	304.4 ± 13.6[b]	303.0 ± 14.7[b]
Liver weight (gm)	8.8 ± 0.5	7.5 ± 0.5[b]	8.2 ± 0.7[b]
Liver/100 gm body	2.69 ± 0.13	2.47 ± 0.11[b]	2.72 ± 0.14
Kidney weight (gm)	2.13 ± 0.33	1.92 ± 0.13[b]	2.32 ± 0.23[c]
Kidney/100 gm body	0.65 ± 0.09	0.63 ± 0.03	0.77 ± 0.05[b]
Spleen weight (gm)	0.56 ± 0.11	0.56 ± 0.06	0.60 ± 0.04
Spleen/100 gm body	0.17 ± 0.03	0.18 ± 0.02	0.19 ± 0.11[b]
Brain weight (gm)	1.88 ± 0.08	1.88 ± 0.07	1.90 ± 0.06
Brain/100 gm body	0.58 ± 0.03	0.62 ± 0.03[b]	0.63 ± 0.03[b]

Female

	Control	5 ppm	50 ppm
Body weight (gm)	165.6 ± 6.7	170.6 ± 7.6[c]	169.8 ± 5.9[c]
Liver weight (gm)	4.1 ± 0.3	4.1 ± 0.3	4.3 ± 0.4[b]
Liver/100 gm body	2.51 ± 0.13	2.40 ± 0.15[bc]	2.54 ± 0.18
Kidney weight (gm)	1.21 ± 0.07	1.22 ± 0.09	1.22 ± 0.08
Kidney/100 gm body	0.73 ± 0.05	0.71 ± 0.05	0.72 ± 0.05
Spleen weight (gm)	0.37 ± 0.05	0.38 ± 0.04	0.39 ± 0.04
Spleen/100 gm body	0.22 ± 0.03	0.22 ± 0.02	0.23 ± 0.03
Brain weight (gm)	1.73 ± 0.09	1.76 ± 0.08	1.77 ± 0.08
Brain/100 gm body	1.05 ± 0.07	1.03 ± 0.07	1.05 ± 0.07

a - Mean ± S.D., N = 25
b - Significant test vs. control, $p < 0.01$.
c - Significant test vs. control, $p < 0.05$.

Lesions in rats considered to be exposure related occurred only in male rats and were restricted to the kidneys where 100% of the 5 ppm decalin exposure group and 96% of the 50 ppm decalin exposure group exhibited changes compatible with a toxic tubular nephrosis. The lesions were dose related in severity. Lesions of this type were not observed in female rats or in the control animals. With light microscopy, the most striking lesions consisted of mild to moderate, focal

necrosis of tubular epithelial cells at the level of the corticomedullary junction with mild, cystic tubular dilatation and intraluminal casts of granular, amorphous cellular debris (Figure 5). Figure 6 shows a tubule at higher magnification. It is apparent that the tubule retained a lining of flattened, stretched-out epithelium where persisting cells attempted to resurface the denuded tubular basement membrane. These tubular changes were usually accompanied by the presence of moderately abundant cytoplasmic hyalin droplets in the proximal tubular epithelial cells (Figure 7). Hyalin droplets are regarded as microscopically visible aggregates of protein. Their presence in tubular epithelium indicates an inability to efficiently transport resorbed proteins from the glomerular filtrate to the capillary blood at the abluminal surface. Two pathogenic mechanisms, either alone or in combination, may be responsible for droplet formation. The first mechanism involves direct toxic injury to the tubular epithelial cells, causing obstruction of protein transport and increased cytoplasmic accumulations. The other process results from glomerular disease in which excessive proteins leak into the glomerular filtrate and subsequently overwhelm the transport capacity of the tubular cells. To differentiate between these two possible mechanisms, electron microscopic studies were conducted in an effort to demonstrate glomerular lesions which might promote excess proteinuria. The electron micrograph of a glomerular capillary shown in Figure 8 was obtained from a male rat exposed to 50 ppm decalin. All structures, including the basement membrane, endothelial lining, and epithelial cell foot processes are normal in appearance. Over 190 ultrastructural photomicrographs of renal tissue were examined. Although these photographs confirmed the presence of increased cytoplasmic protein droplets in the proximal tubular epithelium and focal necrosis of tubular epithelium at the corticomedullary junction, there were no distinct morphologic changes observed in glomeruli.

The occurrence of renal lesions is quite high in the Fischer 344 rat strain. Coleman et al. (1977) detailed the incidence of pathological changes during aging in Fischer 344 male rats. In all but one of 144 rats studied, some sort of renal pathology was observed. There was a high correlation between increasing age and increasing severity of chronic nephropathy centered mainly on changes in the glomeruli. In the present study of decalin, there was an absence of glomerular involvement in the renal lesions observed in the male rats exposed to decalin, and the lesions appear to be distinctly different from those seen in cases of chronic nephropathy. This finding suggests that hyalin droplet formation and tubular epithelial cell necrosis observed at the conclusion of the 90-day exposure period were probably the result of the direct toxic effect of decalin or one of its metabolites.
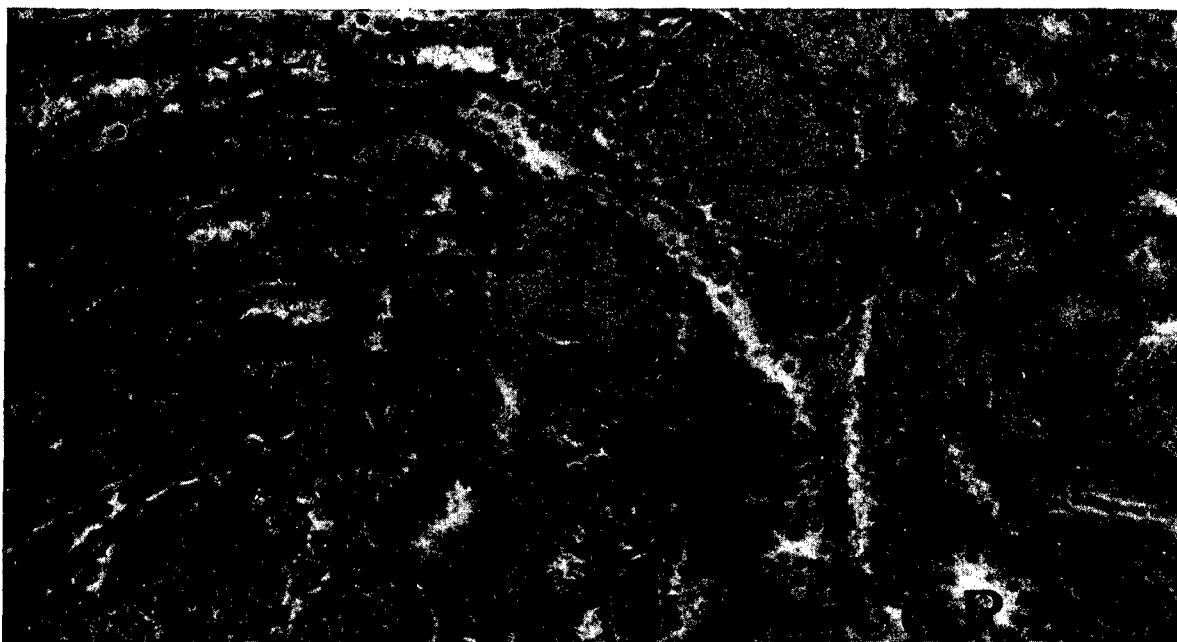
Figure 5. Dilated tubules at the cortico-medullary junction (arrows) filled with amorphous, cellular debris from a male rat exposed to 50 ppm decalin (high dose group). (X 50)
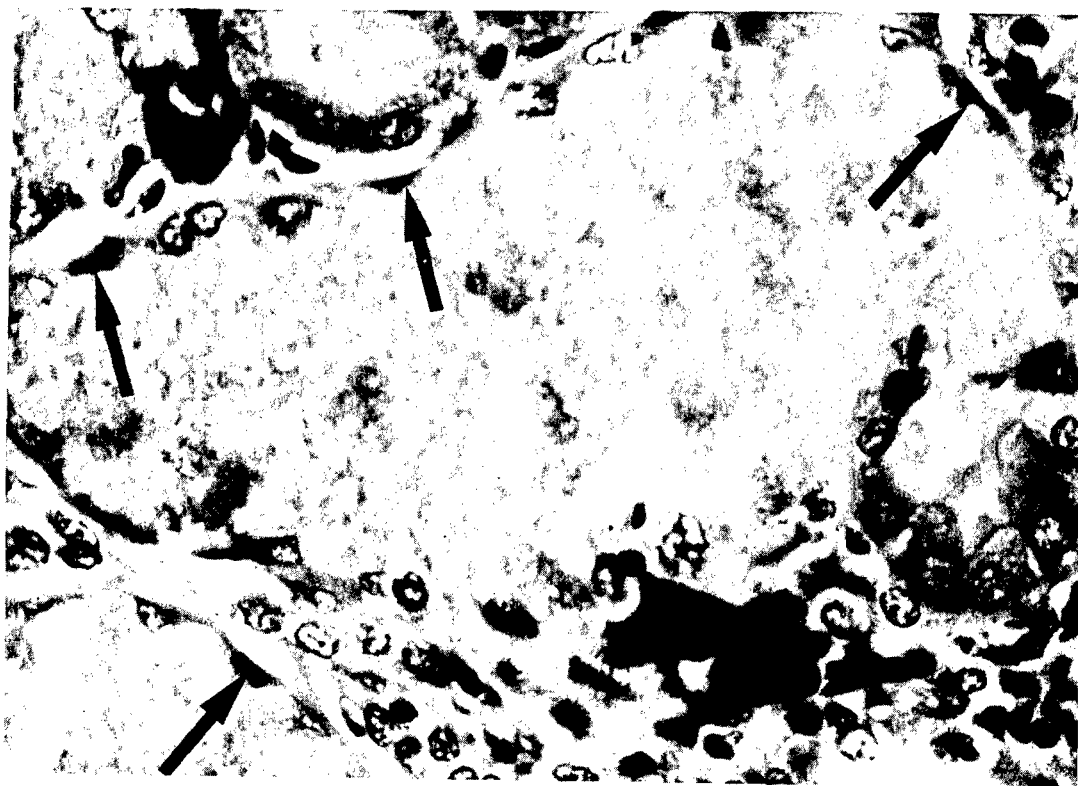
Figure 6. Higher magnification of tubular lesions illustrating attempts by attenuated tubular epithelial cells (arrows) to maintain surface integrity. (X 100)
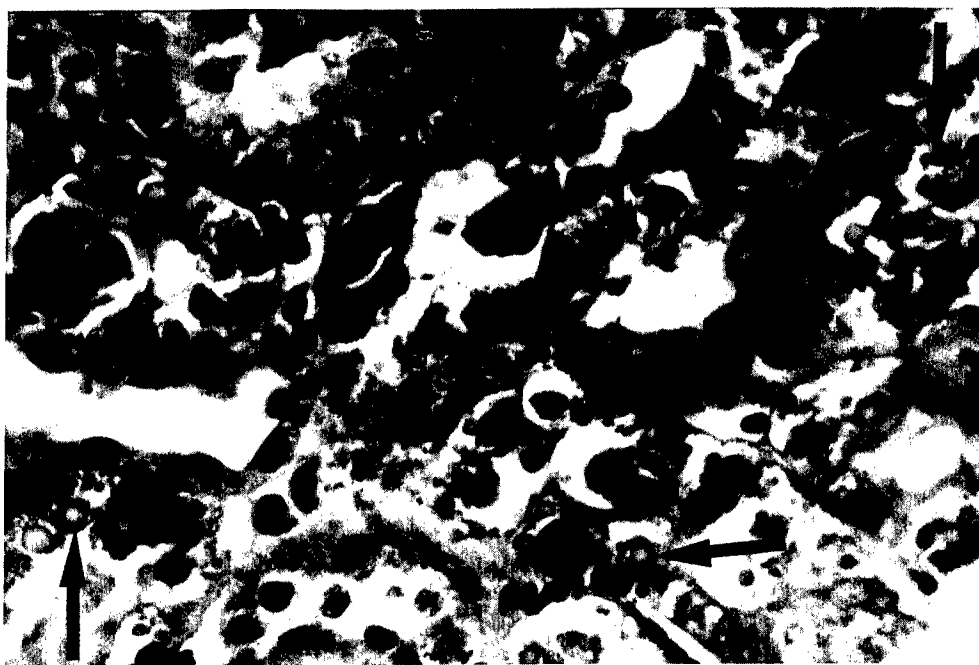
Figure 7. Proximal convoluted tubules exhibiting abundant cytoplasmic hyalin droplets (arrows). (X 100)

Figure 8. Ultrastructure of male rat renal glomerulus from 50 ppm decalin exposure group exhibiting normal capillary tuft structures including epithelial cell foot processes (fp), basement membrane (bm), and endothelial cell (EN). (X 4000)

In mice, lesions that were considered to be dose dependent were limited to the liver where 87% of the 5 ppm decalin exposure group and 94% of the 50 ppm decalin exposure group exhibited varying degrees of hepatocellular cytoplasmic vacuolization (fatty change). This lesion was present in only 6% of the unexposed control animals. Electron microscopic examination of the hepatocytes further indicated that increased cytoplasmic lipids were present in exposed animals and that these changes were accompanied by slight increases in smooth endoplasmic reticulum. Interpretation of ultrastructural findings, however, was largely subjective due to the relatively small sample size (3 exposed and 3 control mice) and the limited quantity of photomicrographs (33). It should be emphasized that fatty change and increases in smooth endoplasmic reticulum are hepatocellular cytoplasmic alterations which may result from a variety of toxic or metabolic insults.

SUMMARY

A 90-day inhalation study was conducted to determine the toxic effects as well as the oncogenic potential of decalin. Dogs, rats, and mice were exposed to vapor concentrations of 5 ppm or 50 ppm decalin for the 90-day period. Pathological lesions were observed in the livers of decalin exposed mice and in the kidneys of decalin exposed male rats.

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EMERGENCY EXPOSURE LIMITS FOR JP-10 SYNTHETIC JET FUEL

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Two studies were conducted in our laboratory during the past year to develop information necessary for setting Emergency Exposure Limits. The first of these was done on methylcyclohexane and was reported in our last conference in March (Kinkead et al., 1979). The second study, reported here, was done on JP-10, a constituent of JP-9 jet fuel.

JP-10 is a single chemical entity identified as tricyclo(5.2.1.0^{2,6})-decane. Gas chromatographic analysis of the material indicated that it was 98% pure with 2% miscellaneous unidentified impurities. The quantities of JP-10 needed for the experiment were supplied by the Air Force.

The known physical properties of JP-10 are as follows:

Molecular Weight - 136
Boiling Point - 360°F
Density, 70°F - 0.940
Viscosity, 70°F - 3.5
Flash Point - 135°F

Since no information was available in the literature concerning the acute toxicologic properties of JP-10, a series of acute studies was done to characterize them and provide background information.

A single-dose oral LD$_{50}$ for JP-10 could not be determined in either rats or hamsters using our standard oral dosing technique. The oral LD$_{50}$ for both species is greater than 20 ml/kg and would not be considered toxic by accepted toxicity standards.

Mice proved to be less resistant to the toxic effects of JP-10 administered by this route with a resultant LD$_{50}$ of 3.9 ml/kg. Deaths occurred within 48 hours of dosing with convulsions immediately preceding death.

Single injection IP LD$_{50}$'s were determined for all species. Although mice still appear to be the most susceptible species, the differences between the species are slight.

The results of the irritation tests were negative. JP-10 caused no irritation to the eyes or to either abraded or intact skin of rabbits after 24, 48, or 72 hours.

Eight of twenty guinea pigs showed a sensitization response with a mean score of 43. The number responding and the mean reaction score indicate that JP-10 has a moderate potential for sensitization and produces a mild response.

Acute inhalation studies with female mice, male and female rats, and male hamsters again showed mice to be the most susceptible species. Exposure to essentially saturated vapors for two hours resulted in death of all exposed mice and female rats. Five of six male rats died after two hours of exposure while no hamsters succumbed following four hours of exposure.

Four-hour LC$_{50}$ exposures were performed on male and female rats giving the following results:

Male rats   = 1221 (1174-1259) ppm
Female rats = 1194 (1104-1287) ppm.

An exact LC$_{50}$ was not obtained for mice because the difference between zero and 100% mortality was so slight that an attempt to achieve partial mortalities was not made. A concentration of 900 ppm resulted in no deaths while 955 ppm caused complete mortality in groups of mice.

Symptoms in these exposures were eye irritation, fine tremors, prostration, followed by convulsions and death. Survivors of high level exposures showed hind quarter paralysis which lasted throughout the 14-day observaton period or to time of death, whichever occurred first.

The purpose of this experiment was to establish Emergency Exposure Limits for time periods of 60, 30, and 10 minutes. An Emergency Exposure Limit is defined as that concentration which will not cause chronic or irreversible tissue damage or produce CNS effects which could impair coordination and prevent a man from self rescue.

Groups consisting of 20 male Sprague-Dawley rats and 20 female ICR mice were exposed to JP-10 vapors for one-hour periods. A control group of each species was maintained for comparison to the test group. Ten animals of each species, including controls, were killed immediately following exposure for examination while the remaining animals were observed for 28 days postexposure.

Once the concentration causing no observable effect in rodents was achieved, four dogs were also exposed at that level. A group of four control dogs was maintained for comparative purposes.

Rodent exposures were conducted in a one cubic meter Rochester chamber while the dogs were exposed in a larger, two cubic meter chamber. The rats and mice were group-housed in large cages (24" x 24") during exposure to allow for greater freedom of movement and thus provide more opportunity for visual observations. Dogs were not confined within the exposure chamber for the same purposes.

The animals used in this experiment were male, Sprague-Dawley rats and female ICR mice, both supplied by Harlan Industries of Indianapolis, Indiana. The purebred beagle dogs were provided by the Air Force. The rodents were fed ad libitum and were cage changed twice per week. The dogs were fed once per day and had their cages cleaned daily.

JP-10 vapors were produced by generating a known liquid flow through a spiral glass evaporator. A measured airflow through the evaporator carried the vapors into the exposure chamber. An airflow of a minimum of 10 cfm was maintained in the chamber.

The chamber concentration was measured using a hydrocarbon analyzer which was calibrated using 100 liter Mylar® bags containing known standard concentrations of the contaminant.

All animals were carefully observed for signs of toxic stress during and after the exposure period. The rats were weighed immediately prior to exposure, on the first, second, and third days postexposure, and weekly thereafter.

Prior to exposure, the beagle dogs were trained to perform four basic tasks: to fetch; to come; to stay; and to lead. Considerable time was spent in acclimating the dogs to the laboratory environment. Approximately six weeks of training were required. The dogs were tested weekly prior to the experimental exposure and twice immediately after exposure.

Besides the field trial evaluation, the dogs were examined neurologically after exposure using the following tests: flexor reflex; extensor thrust reflex; tonic neck reflex; tonic eye reflex; righting reflex; and placing reflex. Each reflex was tested by the method described by Hoerlein (1971).

The dogs were weighed before exposure and at 2 and 4 weeks postexposure. On the same schedule, blood samples were taken on each dog for the following determinations:

HCT	SGPT	Sodium
HGB	SGOT	Potassium
RBC	Alkaline Phosphatase	Glucose
WBC	Bilirubin	BUN

Gross and histopathologic examinations were done on all experimental and control animals from this study. All major organs were examined and sampled with special emphasis on the liver and kidney.

The first one-hour concentration tested, 150 ppm to both rats and mice, caused immediate hyperactivity in both species. Coordination in both species remained normal throughout the exposure.

The second one-hour exposure, to a mean concentration of 254 ppm, also caused hyperactivity in both animal species. After 51 minutes of exposure, one mouse had a tonoclonic spasm which lasted for 20 seconds, after which it appeared relatively normal. The convulsion of this animal would negate this concentration for consideration as an emergency exposure level.

No signs of stress were noted during the subsequent 28-day observation period in either species, and mean body weight gains of the test animals compared favorably with their respective controls. Gross and histopathologic examinations of the animals that were sacrificed immediately following exposure and those sacrificed after 28 days revealed no lesions which could be attributed to the JP-10 exposure.

From the rodent data, a concentration of approximately 150 ppm appeared to be a safe one-hour exposure limit for rats and mice. The dog exposure was then designed for a nominal concentration of 150 ppm wherein the dogs would be carefully observed during exposure and tested postexposure for neurological effects.

The four test dogs were exposed to a mean measured concentration of 151 ppm for one hour. The dogs behaved normally throughout the exposure showing no signs of irritation or CNS effects. Immediately following the exposure, each dog performed the four trained tasks with its assigned animal trainer. All dogs performed this exercise adequately

to the standard established during the training program. The subsequent neurological testing of each dog revealed no exposure related effects. During the 28-day postexposure observation period, all dogs appeared normal. Blood samples examined at 14 and 28 days postexposure showed all normal values. Gross and histopathology at necropsy revealed no exposure related lesions.

In a similar manner, two concentrations were selected for testing at the 30-minute time period. The first exposure was to a mean concentration of 823 ppm. This resulted in the usual increase of activity in both species, as well as tonoclonic spasms in several mice starting at 16 minutes and continuing to the conclusion of the 30-minute exposure. Two mice died after being removed from the exposure chamber. The rats showed no adverse symptoms during or following exposure.

Following the 28-day observation period, mean body weight gains of the test rats and surviving mice did not differ significantly from their respective control groups. Gross and histopathologic examination revealed no lesions related to exposure.

The second rodent exposure for this time period was done at 723 ppm. The only toxic sign noted was increased activity of both species for the duration of the exposure. The 28-day observation period for these animals was uneventful, with mean weight gains and gross pathology within normal parameters.

To complete this phase of the study, four dogs were exposed to a mean concentration of 718 ppm JP-10 for 30 minutes. The vapors caused slight lacrimation in the dogs after six minutes of exposure. The lacrimation did not increase in severity during the remaining 24 minutes of exposure. One dog had a violent coughing spell after 25 minutes of exposure which continued, off and on, for approximately three minutes. During the final two minutes of exposure, the dog appeared normal.

Because of an unavoidable delay in receiving additional rats, mice alone were exposed to the following 10-minute exposures. The first exposure, to a mean concentration of 1218 ppm, caused an increase in activity as seen in the previous exposures. No other observable symptoms were noted throughout the remaining exposure time or during the subsequent 28-day observation period.

The second exposure was to a mean concentration of 1311 ppm. During the 10-minute exposure period, the only sign noted was the usual increased activity of the mice. However, upon removal, one mouse had fine tremors and poor coordination while several other mice showed eye irritation and slight loss of coordination.

Gross and histopathology of the mice sacrificed immediately following exposure and after 28 days did not show any lesions which could be attributed to the chemical insult. Mean weight gains of the test mice after 28 days compare favorably with the mean weight gains of the control mice.

Because coordination effects precluded the higher concentration, a single rat exposure was performed at 1015 ppm. The only sign of stress noted was hyperactivity similar to that seen in all previous rodent exposures. Mean body weight gains and pathological examinations failed to show any differences when compared to the respective control groups.

The trained dogs were exposed to a mean concentration of 1000 ppm for 10 minutes. By two minutes, all dogs showed signs of eye irritation although only one dog experienced lacrimation. Two dogs displayed fine tremors at the conclusion of exposure. In general, all dogs appeared less active during exposure compared to the two previous dog exposures.

Following exposure, all dogs performed the learned tasks with their trainers. Reflexes, tested by the Air Force veterinarians, appeared to be normal. Blood parameters examined at 14 and 28 days were all within normal limits. Gross and histopathology at necropsy revealed no exposure related lesions.

The lack of serious toxic signs as well as the fact that no significant histopathology was found at the lower concentrations tested results in the following recommendation for short-term exposure limits. The concentrations and times are 150 ppm for 60 minutes, 600 ppm for 30 minutes, and 1000 ppm for 10 minutes. The selection of 600 ppm for 30 minutes is based on the slight lacrimation of the dogs' eyes and mild cough experienced at 700 ppm. We believe that a concentration of 600 ppm would eliminate this problem.

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A PROFILE APPROACH TO THE EVALUATION
OF COMBUSTION TOXICITY

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INTRODUCTION

Interest in combustion toxicity originated from findings that the majority of fire fatalities are due to inhalation of smoke and toxic gases, not to physical factors such as burns. Consequently, a great deal of research was conducted in the hope of reducing fire hazard. Yet, after years of research to develop tests to measure the combustion toxicity of materials, no small scale laboratory method has been proven singularly relevant to fire safety issues.

Many factors contribute to overall fire hazard including ignition and flammability properties, smoke and toxic gas evolution, heat stress, oxygen depletion, building design, and numerous others. Fire safety issues, therefore, are very complex and will not be resolved by the singular input of any of these factors. Combustion toxicity data are only one input for and should not be construed as an evaluation of overall fire hazard.

Combustion toxicity research, by itself, has proven to be extremely challenging. Combustion atmospheres are complex mixtures of physical and chemical components each exerting its own toxicological or physiological effect(s). Thus, numerous toxic effects (sensory irritation, hypoxia, incapacitation, etc., to death) are elicited by combustion atmospheres. Further, the conditions within an individual fire are everchanging along the course of that fire's development. Thus, the relative composition of the chemical and physical components of combustion atmospheres is everchanging.

There are an infinite number of fire scenarios ranging from low temperature thermodegradation (representative of the workplace environment) through smoldering combustion (representative of a developing fire), to flaming combustion (representative of a fully developed fire or incineration process). Indeed, combustion toxicity, as it pertains to building fires, is only a small subset of the broader classification of thermodegradation toxicity.

In brief, there is no "ideal" fire which can be used in the laboratory to model all real-life fire or thermodegradation hazards. Further, there is no single toxic effect which reflects the entire nature of a material's combustion toxicity. The nature and severity of combustion toxicity (and fire hazard) are everchanging functions of combustion conditions. The challenge of combustion toxicity research is to elucidate this everchanging, condition-dependent, nature of combustion toxicity.

In this study several contemporary biological indices were used to study the toxicity of Douglas fir combustion atmospheres as a function of combustion temperature. Combustion temperature was chosen because it is a principal variable in real fires. Alternatively, combustion toxicity could be studied as a function of some other condition such as radiant flux intensity.

From these data, combustion product toxicity versus combustion temperature profiles were constructed. Profiles such as these may eventually provide the perspective necessary for real-world application of combustion toxicity data.

EQUIPMENT AND METHODS

Most of the equipment and methodologies used in this study have been described in detail in the literature.

EXPOSURE SYSTEM

The rectangular exposure chamber is constructed of 1/2" sheets of polymethylmethacrylate, is 48" long x 18" tall x 14" deep, and has an approximate internal volume of 175 liters. Animal exposure ports were located midway from top to bottom on the front face of the chamber.

COMBUSTION SYSTEM

The cup furnace was built by and is identical to that used by Potts and Lederer (1977) of Dow Chemical Company. This was positioned under one end of the exposure chamber.

A Theall Engineering Company (Oxford, Pennsylvania) Model 1000 continuously proportional controller was used to regulate furnace temperature.

EXPOSURE PROTOCOL

Combustion toxicity was studied at three nonflaming temperatures (300, 375, and 450°C) and one flaming temperature (500°C).

For each exposure, eight male Sprague-Dawley rats, weighing from 250 to 300 grams, were placed in whole body restrainers and loaded "head-only" into the exposure chamber. These rats were obtained from Charles River Breeding Laboratories in North Wilmington, Massachusetts.

With the furnace equilibrated at the desired furnace temperature ($\pm$ 10°C), exposures were started by dropping the weighed sample into the cup furnace and quickly sealing the chamber door. Combustion products evolved directly into and accumulated in the exposure chamber.

Test groups were exposed to the resulting atmosphere for thirty minutes. Surviving rats were held for a fourteen-day recovery and observation period.

A nominal smoke concentration (sample weight divided by chamber volume) was calculated for each exposure. During all exposures, chamber atmospheres were monitored for temperature, carbon monoxide (CO), carbon dioxide (CO_2), and oxygen (O_2). Biological indices were:

1. Respiratory rate depression as an index of sensory irritation. This has been used extensively by Barrow and co-workers (1979) at the University of Pittsburgh.

2. Loss of shock avoidance as a measure of incapacitation. The hind-leg flexure model used was developed at the Flammability Research Center in Salt Lake City, Utah (Packham et al., 1973; Einhorn and Hartzell, 1977).

3. Arterial blood chemistry (Hb, COHb, O_2Hb, pH, PCO_2, and PO_2) studied via samples from a femoral artery cannula (Packham et al., 1973).

4. Lethality.

At each temperature an LC_{50}, EC_{50} (for loss of shock-avoidance), and a RD_{25} (for respiratory rate depression) were calculated. Arterial blood chemistry was also studied at each temperature.

RESULTS AND DISCUSSION

Only the most significant aspects of these results are summarized. The classical aspects of toxicology, dose-response and time-to-effect, are discussed as they pertain to these data. Then the unique aspect of combustion toxicity, the temperature or condition dependency of these data, is reviewed. Chamber analytical and temperature data were unremarkable and are not presented.

Respiratory rate depression is a graded response; percent response refers to the average decrease in breathing rate for a test group. Dose-response curves for respiratory rate depression (Figure 1) are bimodal. The initial, low-concentration (0.08-0.8 mg/liter) response resulted from exposure to sensory irritants in these combustion atmospheres. This was followed by an accommodated breathing rate over a wide range of nominal smoke concentrations, and the second, high-concentration, response which was due to near-lethal hypoxia.
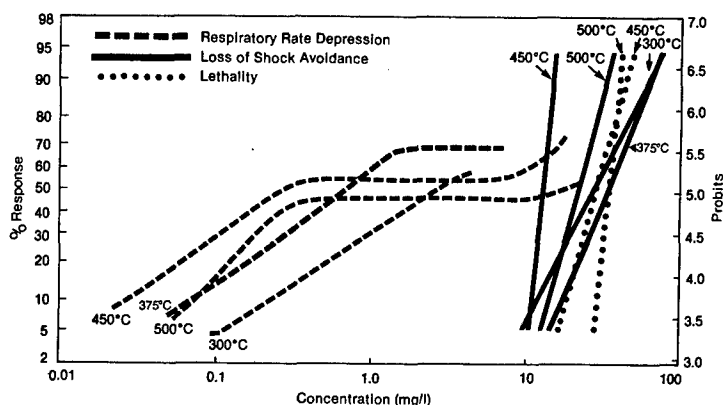
Figure 1. Dose-response curves (Douglas Fir).

The RD_{25}, that concentration causing a 25% decrease in the average respiratory rate of the test group, was chosen as the endpoint for the sensory irritant response.

Lethality and loss-of-shock avoidance are all-or-none responses; percent response refers to the fraction of the test group exhibiting the response. The dose-response curves for lethality and loss of shock avoidance (Figure 1) are quite similar. These effects resulted only after exposure to relatively high smoke concentrations (10-40 mg/liter) and have very steep slopes. The reason for this similarity is that these indices reflect different stages of the same toxic action. Loss of shock avoidance (as will be discussed later) resulted from hypoxia or near lethal carbon monoxide intoxication, and lethality for the most part was due to carbon monoxide intoxication. As such, these two indices provided essentially the same information regarding these atmospheres.

Another classical aspect of toxicity is time-to-onset of toxic action. In these exposures, time-to-respiratory rate depression (Figure 2) was rapid (2-3 minutes). Initially there was a sharp decrease in respiratory rate, followed by accommodation, and finally the animals settled into a steady compensated rate for the duration of the exposure.

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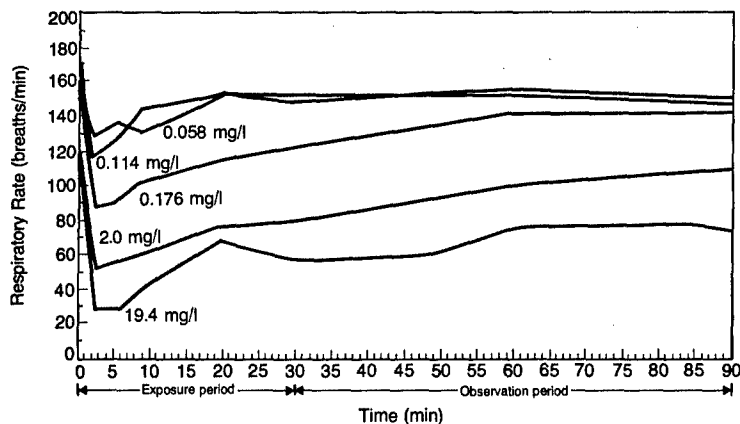
Figure 2. Respiratory rate data (450°C combustion atmospheres of Douglas Fir).

Respiratory rate depression is manifested in the blood by changes in pH, PO_2, and PCO_2. Figure 3 shows PCO_2 changes for several exposures conducted at 450°C. These changes paralleled changes in blood pH and PO_2. Note that PCO_2 changes occurred early in the exposure (3-7 minutes), just subsequent to respiratory rate depression.
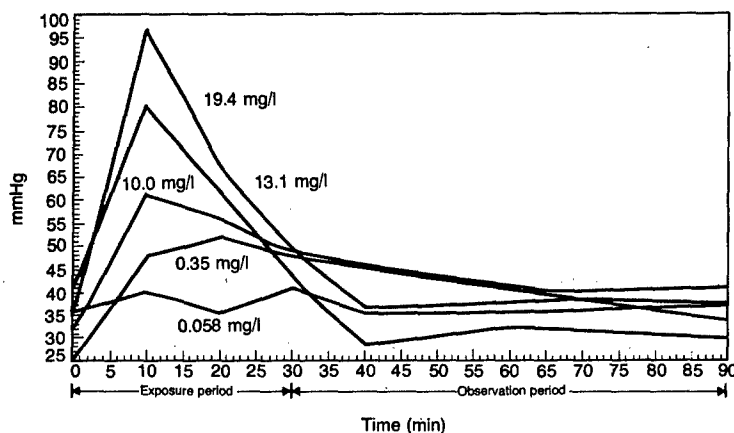
Figure 3. Blood PCO_2 data. (450°C combustion atmospheres of Douglas Fir).

Time-to-death, although not specifically measured in this study, occurred late in the exposure period (20 to 30 minutes) or shortly thereafter. The reason is illustrated by carboxyhemoglobin (COHb) loading curves (Figure 4). In the first 10 minutes loading was slow, with rapid loading from 10 to 20 minutes, and maximum concentrations expressed from 20 to 30 minutes. Assuming carbon monoxide as the cause of death, this late loading explains why death occurred late in these exposures.

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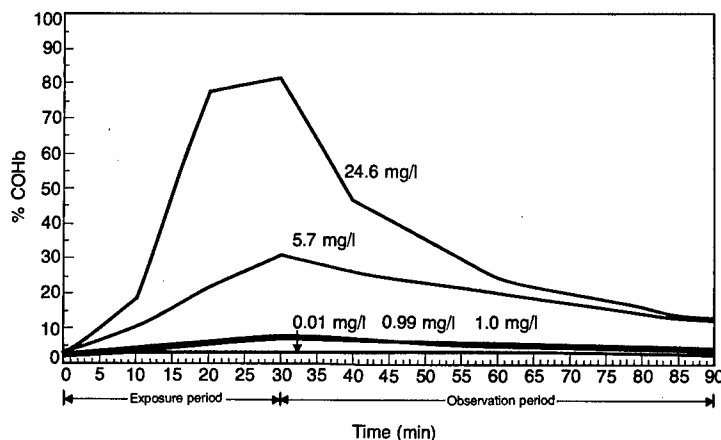
Figure 4. Carboxyhemo-globin data: 500°-535°C Douglas Fir combustion atmospheres.

Unlike lethality or respiratory rate depression, loss of shock avoidance did not exhibit a clear-cut trend in time-to-onset, occurring early as well as late in the exposure period. Loss of shock avoidance paralleled hypoxia or depressed oxygen availability (O_2Hb, Figure 5). Initially, O_2Hb depression parallels respiratory rate depression and subsequently, COHb loading. Thus, loss of shock avoidance seems due to hypoxia caused either by respiratory rate depression, COHb loading, or possibly by other factors.
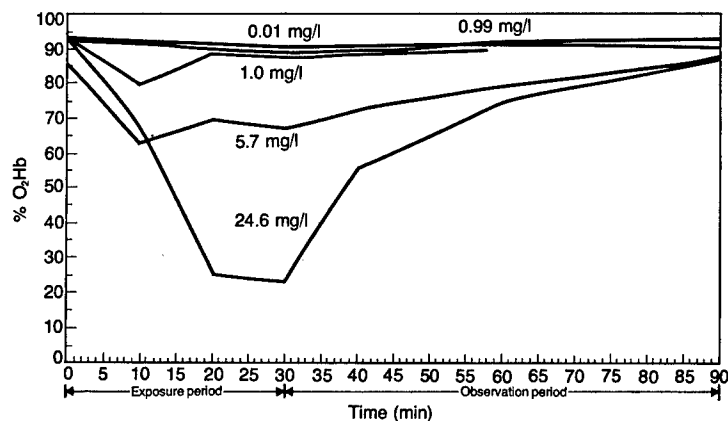
Figure 5. Oxyhemoglobin data: 500°-535°C Douglas Fir combustion atmos-pheres.

Finally, the aspect of combustion toxicity which makes it more complicated than general inhalation toxicity is condition dependency. Table 1 summarizes respiratory rate depression (RD_{25}), loss of shock avoidance (EC_{50}), and lethality (LC_{50}) data. It can be seen from the table that the most toxic atmospheres of Douglas fir resulted from combustion at 450°C. Further, respiratory rate depression and loss of shock avoidance are the predominant toxic effects at low temperatures (300-375°C where little or no lethality was observed).

TABLE 1. SUMMARY OF RESULTS

Combustion Temperature (°C)	RD_{25} (mg/liter)	EC_{50} (mg/liter)	LC_{50} (mg/liter)
300	0.8	27	--
375	0.2	35	40 (ALC)
450	0.08	13	28
500	0.15	20	32

If these LC_{50}'s, EC_{50}'s, and RD_{25}'s are plotted as a function of combustion temperature, Combustion Temperature versus Combustion Toxicity Profiles are generated (Figure 6). These graphically:

a. compare the relative sensitivities of these toxicity indices,

b. illustrate the condition dependency of combustion toxicity, i.e., the everchanging and wide ranging nature of combustion toxicity; and thus

c. portray combustion toxicity data in a perspective amenable to real-world application.
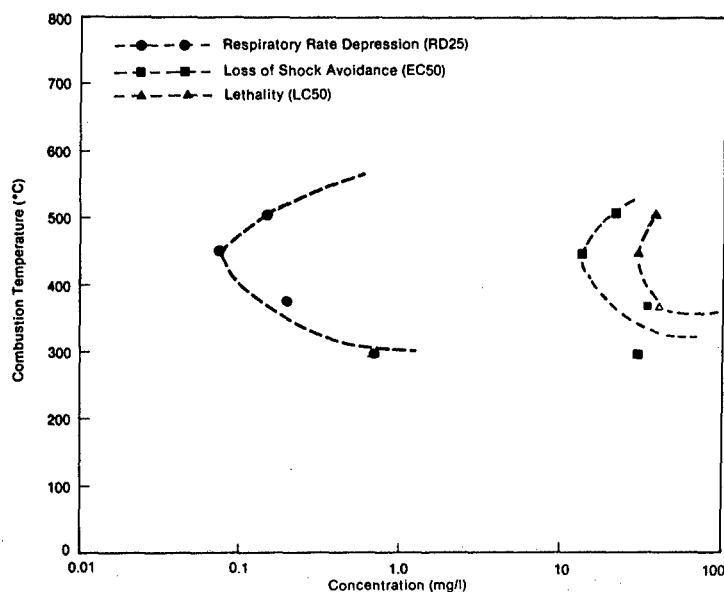
Figure 6. Toxicity – temperature profiles (Douglas Fir).

The nature of combustion toxicity is characterized by condition dependency and multiplicity of toxic effects. A profile approach for the evaluation of combustion toxicity is necessary if we are to use this information as an input in evaluating real-world uses of materials. Further, such judgments will have to weigh the relative significance of toxic effects such as respiratory rate depression (which occurred at low temperatures, low dose, and had fast time-to-effect) against those of lethality (which occurred at higher temperatures, higher dose, and had longer time-to-effect).

Eventually the real question to be answered is "what contribution does combustion toxicity make to real-world fire hazard?" Fire hazard reflects the total of individual hazards stemming from flammability, heat release, toxic gases, etc. These factors are strongly interrelated in real-life situations, and tests that identify or qualify individual hazardous properties of materials (such as toxicity) do not provide an acceptable basis for predicting fire safety performance (or hazard potential) of materials in a fire.

Douglas fir (or wood in general) is by universal use a material of accepted practical fire hazard. However, a wide range of toxic conditions and effects have been illustrated by these toxicity-temperature profiles. These results, in concert with the relative acceptance of the hazards associated with Douglas fir, point out that even though a material possesses measurable toxicity, there can be many justifiable uses for that material.

The relative importance of toxicity to fire hazard is yet to be assessed. A profile approach, however, is essential if judicious decisions are to be made regarding the contribution of combustion toxicity to real-world fire hazard.

SUMMARY

Combustion Toxicity versus Combustion Temperature Profiles were established for Douglas fir. Biological indices of sensory irritation, incapacitation, blood chemistry and lethality were examined. A profile approach to evaluation of combustion toxicity provides a general understanding of the everchanging nature and severity of combustion toxicity. Further, combustion toxicity data serve only as an input to hazard analysis and should not be construed as evaluating fire hazard.

ACKNOWLEDGEMENTS

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ELEVATED CARBOXYHEMOGLOBINS IN FIRE FIGHTERS*

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INTRODUCTION

In a survey of 946 Milwaukee fire fighters conducted in 1975, an average carboxyhemoglobin (COHb) of 3% was found in 442 nonsmokers (unpublished data). This elevated COHb level was 2.5 times greater than that found in the blood of 2,745 nonsmoking Milwaukee blood donors (1.2%) (Stewart et al., 1974). Elevated COHbs in nonsmoking fire fighters, 5.0% (n = 27), 2.5% (n = 32), and 3.2% (n = 32), have also been reported by other laboratories (Sammons and Coleman, 1974; Levy et al., 1976; Lawther, 1971). Presumably, the elevated COHb levels were a result of exposure to carbon monoxide (CO) while extinguishing fires inside burning buildings where CO levels as high as 27,000 ppm have been measured (Burgess et al., 1977), or during the clean up operation when pockets of fumes still remain in the building.

The potential for a fire fighter to have an elevated COHb is further increased due to possible CO exposure on multiple occasions during the workday. These exposures may not be significant individually, but together they could accumulate to produce a large, even lethal, level of COHb. The effect of the resultant CO-induced hypoxic stress

on the cardiovascular system may be compounded further because the fire fighter often will be exposed simultaneously to a high work load, heat stress, emotional stress and/or pulmonary insult from other combustion products; and in addition, he may have underlying heart disease.

In order to monitor COHb levels and to provide appropriate treatment when CO overexposure occurred, the Emergency Medical Technicians (EMTs) from two Milwaukee fire department rescue squads were equipped and trained to monitor COHb levels of fire fighters after "working fires" using an alveolar breath sample technique. Measurements made during this program revealed, however, that the fire fighters were also being exposed to high CO levels from exhaust fumes originating within their own quarters. Subsequently, a survey of atmospheric CO concentrations in fire houses was conducted.

The results of monitoring COHb levels of 781 fire fighters after 64 working fires and the results of a survey of the atmospheric CO concentration in 32 fire houses over a 48-hour period are presented.

METHODS

COHb LEVEL ESTIMATION

Two fire department rescue squads were equipped with Energetics Science CO Analyzers (Ecolyzer, Model 2100), equipped with an external activated charcoal filter and powered from the squad's own 12V battery. After each working fire, alveolar breath samples were obtained from the fire fighters for COHb estimation by the method previously described (Stewart et al., 1976). Symptoms of red eyes and/or coughing were recorded at the same time the breath samples were obtained. When it was determined that a nonsmoking fire fighter had a COHb in excess of 9% (50 ppm CO in alveolar air), 100% oxygen was administered. When his COHb was in excess of 13% (75 ppm CO in alveolar air), he was transported to a hospital for medical evaluation. Similarly, for a fire fighter who smoked and whose body had already compensated for an elevated COHb (Stewart et al., 1974; Seevers and Deneau, 1964), the level for oxygen administration was 13% (75 ppm CO) and for medical evaluation was 17% (100 ppm CO). Those fire fighters placed on oxygen were rechecked at 30-minute intervals until their COHb dropped below the above criteria. A 5-minute fresh-air breathing period was required prior to rechecking to allow blood equilibrium to be reestablished.

CALIBRATION

The CO analyzers were calibrated with 50 ppm standards prepared by adding 0.625 ml of pure CO to a Saran® bag containing 12.5 liters of CO-free purified air. Weekly calibrations showed the sensitivity decreased only 2-4 ppm/week.

FIRE HOUSE ATMOSPHERIC CO LEVEL

The atmospheric concentration of CO was continuously measured and recorded in 32 fire houses for a minimum of 46 hours in each using two CO analyzers and a dual channel recorder. The CO level was recorded from both the apparatus floor and the dormitory or living quarters. The average CO exposure was determined by weighing the area beneath the curve on the chart paper. Each CO analyzer was zeroed and calibrated every 48 hours and the zero was checked every 24 hours. Loss of sensitivity to a 50 ppm standard over a 48-hour period was never greater than 2 ppm and the baseline was stable.

In addition to the atmospheric CO concentrations, COHb levels were estimated using alveolar breath samples while the fire fighters were on duty in their quarters and again at completion of their 24-hour shifts. This allowed a comparison of the COHb levels with the fire house atmospheric CO exposure level.

RESULTS

COHb MONITORING

The mean COHb for fire fighters checked after 64 working fires was $5.0 \pm 3.0\%$ (29.5 ± 13.6 ppm CO in alveolar air; n = 463) for nonsmokers and $8.0 \pm 3.0\%$ (43.3 ± 17.0 ppm CO in alveolar air; n = 318) for smokers. As a result of monitoring these 781 fire fighters, 48 (6.1%) were placed on oxygen as determined by the above criteria, and 6 (0.7%) were taken out of service and transported to a hospital for medical evaluation. The incidence of overexposed fire fighters per fire ranged from none or the one motor pump operator who stood too close to the exhaust to keep warm, to entire engine companies of men requiring oxygen.

A grouping of symptomatology (red eyes and/or coughing) with the incidence of elevated COHb levels which required treatment is shown in Table 1. The presence of these symptoms was not a reliable indicator of an elevated COHb.

TABLE 1. INCIDENCE OF ELEVATED COHb COMPARED WITH OTHER SYMPTOMS

Other Symptoms	Nonelevated COHb Returned to Duty[a]	Elevated COHb Placed on Oxygen[b]	Elevated COHb Transported to Hospital[c]
None	487	19	1
Red Eyes Only	149	12	2
Coughing Only	9	0	0
Red Eyes and Coughing	82	17	3
Total	727	48	6

a - COHb < 10% for nonsmokers; < 13% for smokers
b - COHb > 10% for nonsmokers; > 13% for smokers
c - COHb > 13% for nonsmokers; > 17% for smokers

FIRE HOUSE ATMOSPHERIC CO LEVEL

The Threshold Limit Value (TLV) is defined as "the maximum average atmospheric concentration of contaminants to which workers may be exposed for an 8-hour day without injury to health" by the American Conference of Government Industrial Hygienists (ACGIH) (Patty, 1958). The current TLV for CO is 50 ppm.

During a total of 68 days of monitoring atmospheric CO concentrations for at least 46 hours in 32 fire houses, the 24-hour average CO concentration to which each shift of fire fighters was exposed exceeded the current 8-hour TLV on 9 days (13%), and a NIOSH proposed lower TLV of 25 ppm was exceeded on 30 days (44%). Even though the 24-hour average in some cases was below 50 ppm, there were 8-hour intervals in which 50 ppm was exceeded due to a large number of false alarms in the early evening hours. In addition, due to the large difference in the number of responses per day between fire houses, whereas on the average the TLV was exceeded 13% of the time, some houses rarely exceeded it, and some busy houses exceeded it every day.

Figure 1 is a partial record of Engine 31's quarters where the 24-hour CO level on the apparatus floor was 86 ppm. Since the half-life for the COHb level in the body is 5 hours (Peterson and Stewart, 1970), and since these fire fighters had not had any major exposure for 5 hours prior to going off duty when their COHb was measured at 9%, one can estimate their peak COHb was at least 18%. The Coburn-Forster-Kane equation for CO absorption, with appropriate assumptions for variable values, has previously been shown quite accurate for predicting COHb levels during fluctuating CO exposures (Stewart et al., 1974; Peterson and Stewart, 1975). Using this equation and the apparatus CO concentration, the maximum predicted COHb level was 17.2%, which agrees with the 18% estimated above. This level was achieved during a shift with six fire responses, of which five were false alarms and the sixth was a fire which was quickly extinguished. Figure 2 is a partial record of Engine 13's quarters where the average apparatus floor CO level was over 100 ppm for seven hours. The 24-hour average for that particular day was 56 ppm with the main exposure occurring in the first 12 hours; the nonsmoking fire fighters went off duty with a mean COHb of 3.5%. Engine 29's quarters, a frame house which was airtight relative to the other fire houses, had a 24-hour level of 27 ppm where the only source of CO was from tobacco smoke (3 cigarette smokers). An increase in CO was also seen when a gas stove was used to fry liver for supper. The nonsmoking fire fighters in this house went off duty with a mean COHb of 4%.
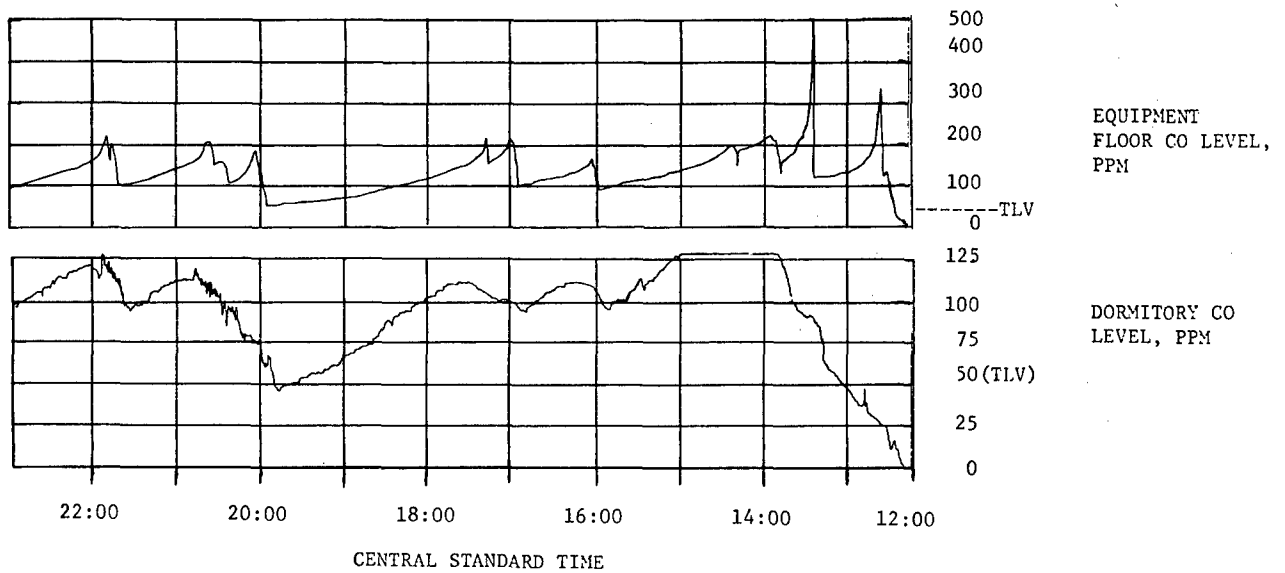
Figure 1. Carbon monoxide exposure level in fire houses. Engine 31 - 21 February 1977.
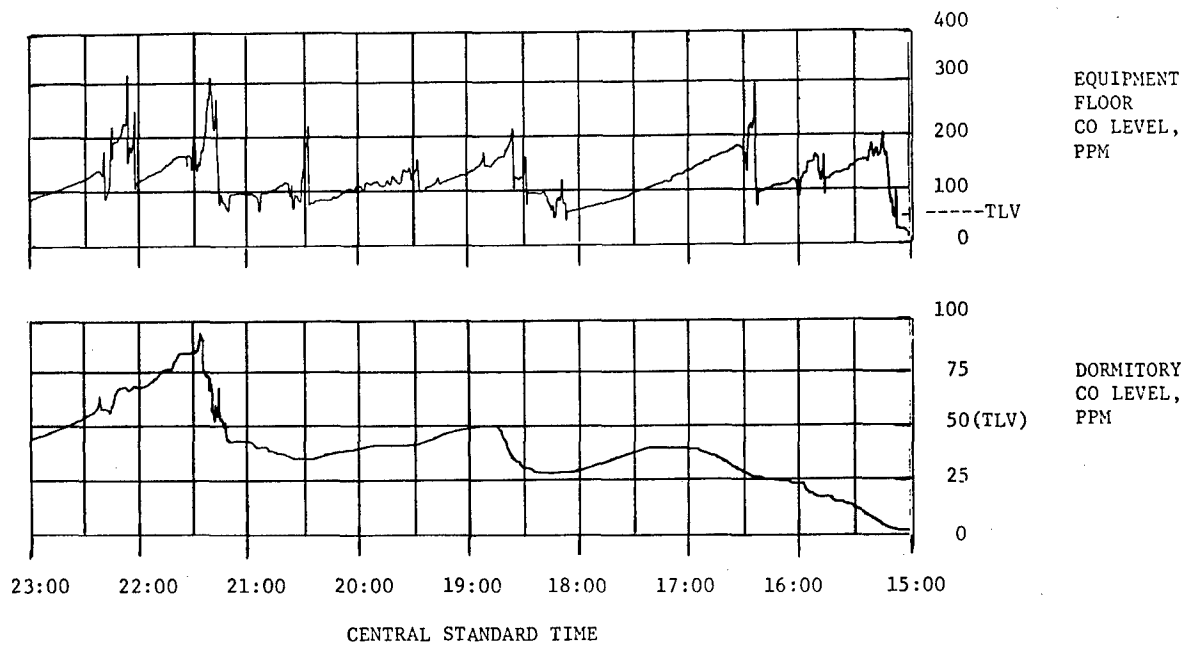
Figure 2. Carbon monoxide exposure level in fire houses. Engine 13 - 13 December 1976.

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The CO level in these fire houses is dependent on several factors: (1) The number of responses. (There were 10,000 false alarms in Milwaukee in 1976.) (2) The number of pieces of apparatus responding from each fire house. (3) The amount of CO pollution in the apparatus' exhaust. (The increase in the CO level in these fire houses produced by a single response ranged from 20 to 120 ppm with the diesel engines producing less CO.) (4) The length of time the apparatus engines run inside the fire house. (5) The internal volume of a fire house. (6) The air exchange rate of a fire house. (The half-life for atmospheric CO in these fire houses ranged from 15 to 260 minutes.)

A key factor controlling an individual fire fighter's exposure to CO within the fire house is his location within the fire house. In older fire houses, the fire fighters spend most of the day in areas just off the apparatus floor. In the new houses being constructed, where the living quarters are isolated, the exposure is less. The CO level found in the living quarters compared to that found on the apparatus floor ranged from 7% in the new house to 74% in the older houses. However, the danger of overexposure in a new house still exists when the fire fighters are working in the apparatus floor area.

The COHb level data presented in Table 2 shows that for non-smoking fire fighters who have had no prior "fires" on a given day, approximately 50% of their COHb level after a fire is due to the fire exposures and 50% is from the prior exposure while in the fire houses. In some cases, however, as seen with the above data from Figure 1, probably 100% of the 18% COHb saturation calculated there was produced from CO exposure within the fire house.

TABLE 2. CARBOXYHEMOGLOBIN LEVELS IN MILWAUKEE BLOOD DONORS AND FIRE FIGHTERS

| | | Fire Fighter** | |
	Milwaukee Blood Donor*	On Duty (No Fires)	After A Working Fire
Nonsmoker	1.2 ± 0.5% N = 2745	3.0 ± 1.0% N = 442	5.5 ± 3.2% N = 1347
Smoker	4.2 ± 2.8% N = 1592	7.0 ± 1.5% N = 478	8.7 ± 3.4% N = 1001

*X ± S.D. as determined directly from blood samples.
**X ± S.D. as estimated from alveolar breath samples.

DISCUSSION

Milwaukee and other fire fighters are being repeatedly overexposed to CO. Other investigators have shown a relationship between elevated COHb levels, as found in this study, and angina pectoris, atherosclerosis, and both electrocardiographic and electroencephlographic changes (Aronow et al., 1972; Aranow and Isabell, 1973; Aronow, 1973; Astrup, 1969; Zanda, et al., 1972; Zanda et al., 1973). These COHb levels are, therefore, sufficiently great to pose a serious health hazard.

The overexposure at fires occurred even with the routine use of respirators. This is probably indicative of improper use, such as poorly fitting masks, or more likely, failure to use the respirator during the entire operation, including the post-fire clean up where pockets of toxic fumes and CO-producing smoldering coals exist. Use of the alveolar breath sample technique, however, provides an accurate and precise method for detecting the elevated COHb in the field.

In the busy fire houses, overexposure occurs routinely but could be eliminated with the installation of additional ventilation systems which would automatically purge the apparatus floor area after a piece of apparatus leaves or returns to quarters. Additional use of this system could also eliminate the pollution produced from such practices as moving the apparatus to wash the floor or running the engine to build up air pressure. Since an average fire fighter does not work at a "working fire" daily, the chronic overexposure to CO in fire fighters is occurring within the fire house.

It should be noted that the 50 ppm TLV is for 8 hours of exposure, and there is no recommended limit for a 24-hour day. Such a limit would probably be lower, and if the proposed lower 8-hour TLV of 25 ppm were enacted, a 24-hour TLV could be even lower still. This would only intensify the problem disclosed by these data.

ACKNOWLEDGEMENT

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CHRONIC EFFECTS OF INHALATION EXPOSURE TO HYDRAZINE

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Hydrazine (N_2H_4) is a highly reactive reducing agent which is widely used as an intermediate in organic synthesis and either singly or in combination with other hydrazines as a missile propellant. An important and increasing use of hydrazine is that of a boiler feed water additive as an oxygen scavenger. It is a colorless polar liquid, weakly basic and fumes in air. It has a slightly ammoniacal odor.

Clark (1968) provided a detailed review of the toxicology and pharmacology of propellant hydrazines. Hydrazine is a strong convulsant at high doses but may cause central nervous system depression at lower doses. Animals may die acutely of convulsions, respiratory arrest, or cardiovascular collapse within a few hours of an acute exposure by any route of administration, or may die 2 to 4 days later of liver and kidney toxicity (Weir et al. 1964; Witkin, 1956). Jacobson et al. (1955) reported the 4-hour LC_{50} value as 252 ppm (330 mg/m^3) for the mouse and 570 ppm (750 mg/m^3) for the rat. House (1964) exposed monkeys, rats, and mice to a hydrazine concentration of 1.0 ppm continuously for 90 days. Though mortality was very high, some animals survived. Ninety-six percent of the rats and 98% of the mice died during the exposure while monkeys proved to be the most resistant species with only a 20% mortality. Comstock et al. (1954) exposed dogs, in separate experiments, to 5 and 14 ppm. Two dogs survived the repeated 6-hour exposures to 5 ppm hydrazine for 6 months, and 2 of 4 dogs lived after 194 6-hour exposures to 14 ppm. Two of four dogs died during the third and fifteenth weeks in a debilitated condition. The dog that died during the fifteenth week had a severe convulsive seizure prior to death. Prior to death, both dogs showed signs of anorexia and general fatigue. Changing diets and forced feedings resulted in the survival of the remaining two dogs.

A 6-month chronic inhalation study of hydrazine was reported by Haun and Kinkead (1973) which employed four exposure groups and an unexposed control group. Each group was comprised of 8 male beagle dogs, 4 female rhesus monkeys, 50 male Sprague-Dawley rats, and 40 female ICR mice. The experimental groups were exposed to vapors of hydrazine either at concentrations of 1.0 or 0.2 ppm continuously, or at 5.0 and 1.0 ppm intermittently. The continuous exposures were designed to approximate the same weekly doses of hydrazine received by the intermittent exposure groups with continuously exposed animals receiving 168 and 33.6 ppm-hours of hydrazine/week and intermittently exposed animals 150 and 30 ppm-hours/week. Dogs exposed at the higher dose levels, either intermittently or continuously, exhibited 10-20% reductions in erythrocyte, hematocrit and hemoglobin values which continued throughout the 6-month exposure but returned to control values within 2 weeks after the exposure ended. Hematology values for dogs exposed to lower doses remained within the normal limits of the control group.

Rats showed a dose-related growth rate depression and a sustained difference in group average weights of up to 35 grams throughout the exposure. Weight loss in dogs which occurred only in the high dose group was recovered within 2 weeks postexposure, suggesting that the loss was due to appetite suppression.

Gross and microscopic examination of tissues from these animals taken at termination of the exposure showed fatty liver changes in mice and dogs at the high exposure dose levels but no exposure-related changes in the livers of monkeys and rats.

Ten mice and 10 rats from each of the exposure groups were held for a year postexposure period. Most of the rats in the two high dose groups died within 6-8 weeks postexposure from chronic pulmonary disease. This infection spread to the other groups housed in the same animal room. Consequently, none of the rats survived long enough to evaluate the carcinogenic potential of inhaled hydrazine for this species.

Approximately half of the mice in each group were alive one year postexposure. Tumorigenic changes in these mice were reported by MacEwen et al. in 1974. Mice exposed to the high doses (continuous exposure to 1 ppm hydrazine or intermittent exposure to 5.0 ppm) had increased incidences of alveolargenic carcinomas, lymphosarcomas, and hepatomas. Both lower dose groups had an increased incidence of alveolargenic carcinomas when compared with unexposed controls. The total tumor incidence appeared to be dose-related: approximately 87% tumor incidence occurred at the high dose level; 33% at the low dose level; and 12% in the unexposed control group. Although the group sizes were very small, the findings were important in that they demonstrated tumorigenic response at the current Threshold Limit Value.

The findings of MacEwen et al. (1974) were consistent with other reports of tumor induction following repeated oral administration or injections of hydrazine. The induction of lung tumors in mice by hydrazine sulfate was reported by Biancifiori et al. (1962a, 1962b, 1963a, 1963b, 1966), Biancifiori (1969 and 1971), Roe et al. (1967), and Toth (1969, 1971, 1972).

Since hydrazine inhalation at the Threshold Limit Value increased the incidence of pulmonary tumors in mice, a more comprehensive oncogenic study of hydrazine effects on multiple species was undertaken and the results reported herewith.

The objectives of this study were to evaluate (a) the chronic effects of inhaled hydrazine on rats, mice, hamsters, and dogs, and (b) the oncogenic potential of hydrazine in rodents observed for a maximum period of 1½ years after one-year of industrial-type inhalation exposure. The animals used in this study were C57Bl/6 mice obtained from the Jackson Laboratories, CDF (Fischer 344 derived) albino rats from Charles River, Engle Golden Syrian hamsters, and beagle dogs. The number of animals of each species and sex are listed in Table 1 which also shows the chambers used and exposure concentrations.

TABLE 1. EXPERIMENTAL DESIGN FOR HYDRAZINE INHALATION EXPOSURE CONCENTRATIONS

Hydrazine Concentration, ppm	Animal Numbers, Sex, and Species	Chamber Number
0.05	100♂, 100♀ rats; 400♀ mice	7
0.25	200♂ hamsters; 400♀ mice	5
0.25	100♂, 100♀ rats; 4♂, 4♀ dogs	6
1.0	200♂ hamsters; 400♀ mice	1
1.0	100♂, 100♀ rats, 4♂, 4♀ dogs	4
5.0	100♂, 100♀ rats; 200♂ hamsters	8
Control	150♂, 150♀ rats, 800♀ mice; 200♂ hamsters; 4♂, 4♀ dogs	Vivarium

The exposure concentrations were selected to span the range from a certainly toxic level to the current OSHA Threshold Limit Value for exposure to hydrazine (1 ppm) and the proposed ACGIH Threshold Limit Value of 0.1 ppm. The 5 ppm exposure concentration was selected as a maximum tolerable exposure dose which would produce some biological response without causing death in hamsters and rats. Mice and dogs were not exposed at this concentration because prior studies (Haun and Kinkead, 1973) had shown that repeated daily exposures to 5 ppm hydrazine caused death in these species.

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The inhalation exposures were conducted on a 6 hour/day, 5 day/week schedule for a one-year period without exposures on weekends and holidays.

The animals were exposed in Thomas Dome exposure chambers (Thomas, 1968) at a slightly negative pressure (725 mm Hg) to insure a complete seal and to prevent contamination of the surrounding laboratories and personnel. All animals were observed hourly during the 12-month hydrazine exposure phase of the study and daily during the post-exposure phase. Rats, dogs, and hamsters were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in cage groups and group means followed on a monthly schedule throughout the entire study.

Blood samples were drawn from dogs at biweekly intervals during the exposure phase and clinical determinations made for the following battery of tests:

RBC	Glucose
WBC	Total Protein
HCT	Albumin
HGB	Globulin
Sodium	A/G Ratio
Potassium	SGPT
Calcium	Alkaline Phosphatase.

Animals that died or were killed during the study were necropsied following the National Cancer Institute protocol. The necropsy consisted of an external examination, including all body orifices, and the examination and fixation of portions of approximately 44 tissues.

The average body weights of the groups of male and female rats are shown in Figures 1 and 2, respectively, for the entire study. Although not dose dependent, growth was reduced in all hydrazine-exposed rats during exposure but the effect was most significant in the male rats exposed to the 5 ppm concentration. The differences between exposed and control animals were maintained at relatively constant levels during the first 12 months postexposure but became less significant during months 25 to 30 of the study as the weight decline of the aging animals was observed.
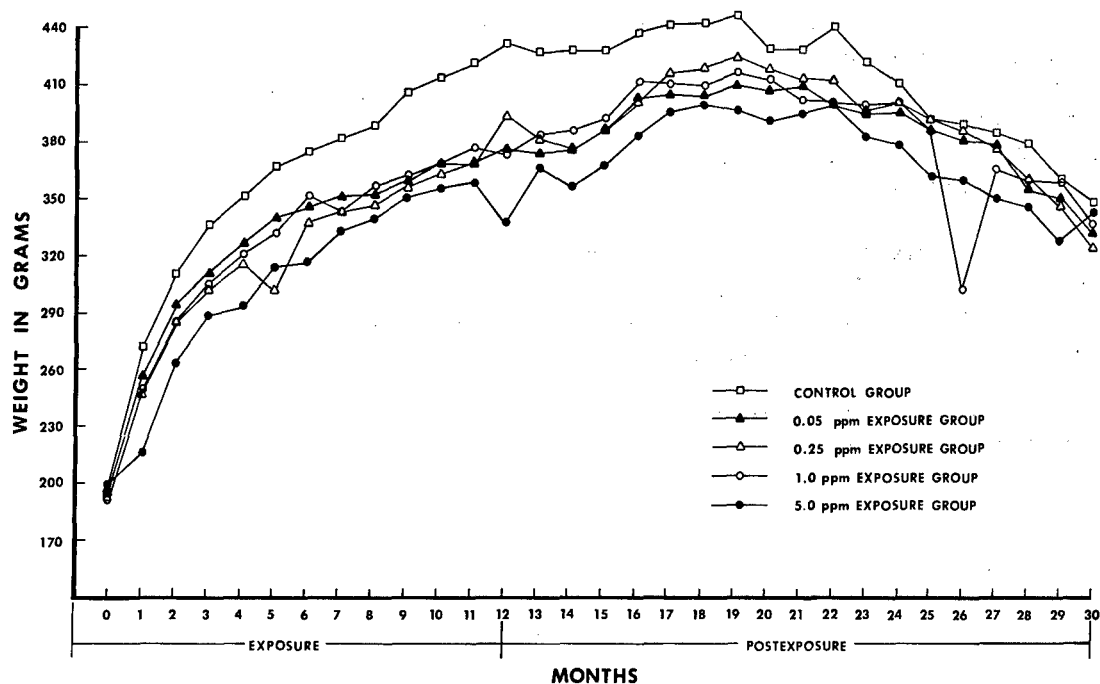
Figure 1. The effect of chronic inhalation exposure to hydrazine on the growth of male Fischer 344 rats.

The effect of depressed growth in female rats was not as pronounced as in males during the exposure phase but was significant and became more noticeable during the postexposure observation period.
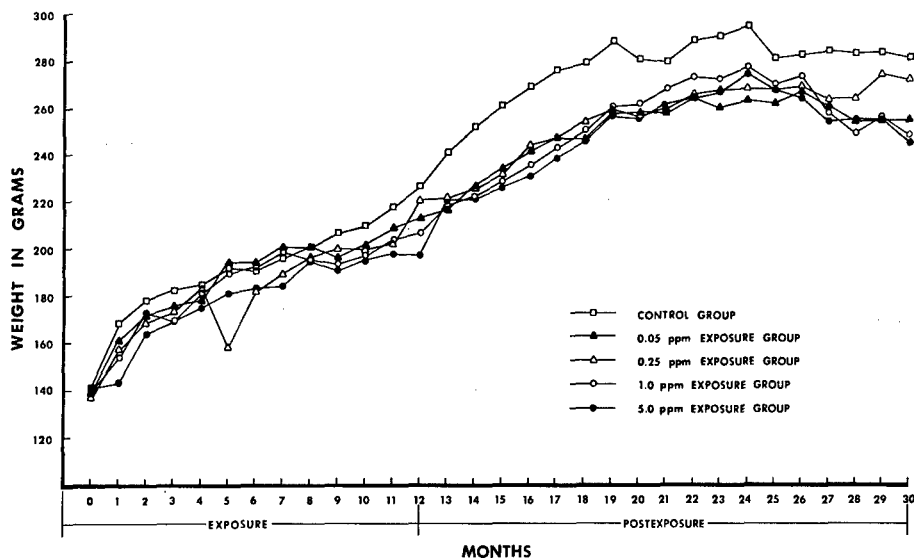
Figure 2. The effect of chronic inhalation exposure to hydrazine on growth of female Fischer 344 rats.

Hamster body weights shown in Figure 3 were depressed for all exposure groups but also exhibited an inexplicable cyclic phenomenon common to all groups exposed as well as the unexposed that was relatively severe in all groups. In the final months, only the 5 ppm hydrazine exposed group continued to show a significant weight difference from controls.

Mean body weights of mice are shown in Table 2 along with cumulative mortality. Mice were not exposed to the 5 ppm hydrazine atmosphere. Body weights of mice were unaffected by chronic exposure to inhaled hydrazine at 1 ppm or less.

There was no significant increase in the mortality experience of the hydrazine exposed mice, rats, hamsters, or dogs.
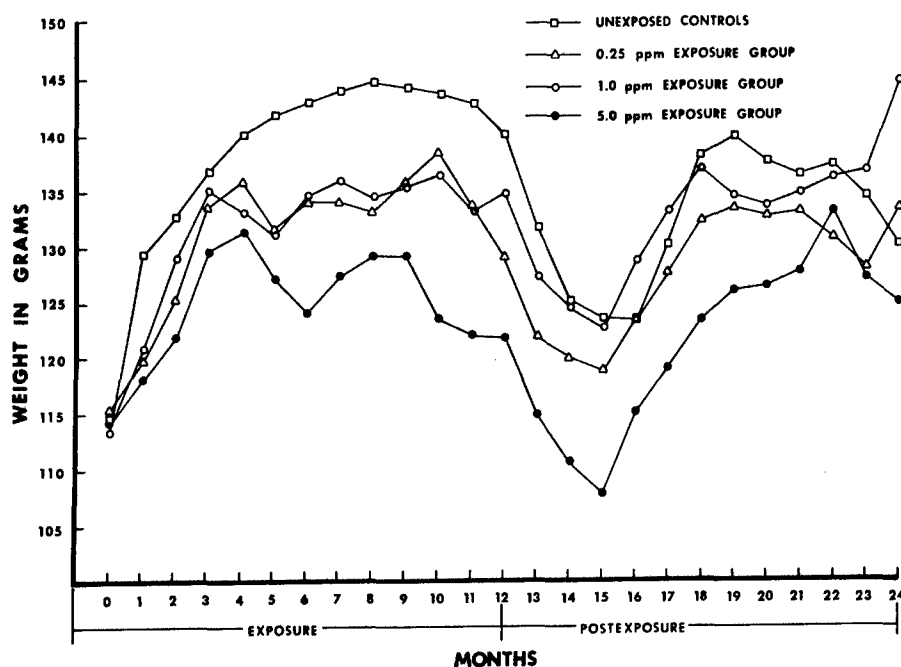

Figure 3. The effect of chronic inhalation exposure to hydrazine on the growth of male Golden Syrian hamsters.

TABLE 2. MEAN BODY WEIGHTS AND CUMULATIVE MORTALITY FOR MICE EXPOSED TO HYDRAZINE FOR ONE YEAR

Exposure Time (Months)	Set 1 Unexposed Controls Percent Mortality	Weight, g	Set 1 Exposed, 0.05 ppm Percent Mortality	Weight, g	Set 1 Exposed, 0.25 ppm Percent Mortality	Weight, g	Set 2 Unexposed Controls Percent Mortality	Weight, g	Set 2 Exposed, 1.0 ppm Percent Mortality	Weight, g
Pre	0	16.2	0	18.0	0	16.4	0	17.0	0	16.9
1	1	20.0	1	21.5	0	20.5	0	19.3	1	20.2
2	1	21.6	1	22.2	1	22.0	0	20.7	2	22.0
3	1	22.3	1	23.2	2	22.6	0	21.1	2	22.7
4	1	22.5	1	23.6	2	23.4	0	22.0	2	23.4
5	1	22.5	1	24.1	3	24.0	1	22.3	2	24.1
6	1	23.1	1	24.2	4	24.5	1	22.5	3	24.5
7	1	23.6	2	24.2	4	25.1	1	23.6	5	25.4
8	1	24.0	2	25.3	4	25.5	2	25.6	5	26.0
9	2	23.8	2	25.3	4	26.5	2	25.3	5	26.1
10	2	24.1	3	25.6	4	25.9	2	24.9	6	25.9
11	3	24.6	5	26.9	6	27.0	3	25.2	7	27.2
12	3	25.1	6	27.0	7	27.6	Not Weighed		7	26.8
Months Post										
1	3	26.5	7	26.4	7	26.5	4	25.2	8	26.0
2	5	27.5	8	26.9	8	27.5	6	25.5	13	26.6
3	6	27.4	9	27.0	8	27.2	7	26.6	13	27.4
4	7	27.2	14	26.6	9	26.8	8	26.4	14	26.8
5	8	27.0	16	26.2	11	27.0	11	27.5	15	27.8
6	13	27.6	20	26.0	12	26.1	16	27.6	20	27.3
7	14	28.7	22	26.7	15	27.1	22	28.1	23	27.7
8	16	28.4	26	27.0	18	27.2	27	29.1	23	27.7
9	19	29.1	29	27.8	23	27.7	29	29.5	32	28.3
10	25	28.5	38	27.4	32	27.7	34	30.0	38	28.3
11	32	29.0	42	27.3	37	27.7	40	27.6	43	27.3
12	38	28.9	50	28.0	42	27.8	46	28.7	50	27.5
13	43	28.8	54	27.8	48	27.7	54	27.3	57	26.7
14	48	29.4	62	28.1	57	27.5	62	27.1	67	26.3
15	75	26.2	81	27.0	83	26.1	71	26.1	79	25.1
16	75	27.0	88	27.5	86	27.3	79	27.3	86	25.9

Approximately 4 months postexposure, rectal bleeding was observed in one dog in the 0.25 ppm hydrazine exposure group. A biopsy was taken from a 3 x 2 cm cauliflower-like growth on the ventral surface of the rectum. Histologic examination of the biopsied material led to a diagnosis of low grade adenocarcinoma. The tumor was removed three years postexposure, and the original diagnosis was reconfirmed. The tumor mass was confined to the mucosa, and there was no evidence of invasion of normal tissue.

An unexposed control dog died 32 months postexposure due to respiratory failure following extensive hemorrhage into the thoracic cavity. The hemorrhage resulted from rupture of numerous small capillaries formed in response to pyogranulomatous reaction involving the lung, pericardium, and diaphragm. Bacterial cultures made from material in this lesion isolated a Corynebacterium organism. At the time this paper was prepared, the dogs were still being maintained.

Gross histopathologic examinations were performed on all rodents that died during the course of the study or were sacrificed at completion of the postexposure period. Histopathologic examinations were conducted in accordance with the National Cancer Institute protocols on approximately 33 tissues from all animals with the exception of a few in which postmortem changes were extensive or cannibalism prevented examinations.

Surviving hamsters were sacrificed one-year postexposure, and their tissues were examined by pathologists of the Veterinary Science Division at Brooks Air Force Base. Tumor and nontumor nomenclature was developed by this group for automated data processing of the results from hamsters. Tumor incidence tables were compiled, and statistical analyses, using the Fisher Exact Test, were performed by the UCI staff.

Since rat mortality was very low after one-year postexposure, 10% of the survivors were sacrificed and tissues collected as previously described. The study was terminated after 30 months (18 months postexposure), and all surviving rats were necropsied.

Mouse mortality approached 90% in the 18th postexposure month for the first set of animals including the 0.05 ppm and 0.25 ppm hydrazine exposed mice and their controls. The second set of mice including the 1 ppm hydrazine exposure group and their controls was terminated at 132 weeks which was 3 weeks longer than the first set.

Tissues from both rats and mice were sent to the Huntingdon Research Centre in Huntingdon, England for histopathologic examination. Rats were examined by Dr. C. P. Cherry and mice by Dr. J. M. Offer under the supervision of Dr. D. E. Prentice.

Table 3 shows the tumor incidence in the various groups of exposed and control hamsters. The outstanding finding in hamsters is a statistically significant increase in benign nasal polyps. These tumors were seen in 16/160 of the 5 ppm exposed animals, only one in the control group. The only other tumor types of possible importance are those of the colon in the 5 ppm exposure group. There were 3 primary adenocarcinomas, one benign leiomyoma and one benign papilloma. When these tumor types were separately subjected to the Fisher Exact Test, none showed statistical significance. There was a rather large incidence of cortical adenomas in the adrenals of all groups of exposed hamsters but with incidence rates lower than that in the control group. This type of tumor is commonly seen in aged hamsters. Incidence of other tumors in the various organs was low. No biological significance is attached to the increase in benign thyroid adenomas limited to the 0.25 ppm hydrazine exposure group. The reduced incidence of adrenal cortical adenomas may indicate some antineoplastic activity as will also be seen with leukemia incidence in rats.

TABLE 3. TUMOR INCIDENCE IN CONTROL AND HYDRAZINE EXPOSED MALE GOLDEN SYRIAN HAMSTERS†

TUMOR TYPE	Unexposed Controls	0.25 ppm Exposed	1.0 ppm Exposed	5.0 ppm Exposed
Nares, Trachea, Bronchi				
Polyp (B)	1/181	0/154	1/148	16/160**
Basal Cell (P)	0/181	0/154	1/148	0/160
Basal Cell (B)	0/181	0/154	0/148	1/160
Adenoma (P)	0/181	1/154	0/148	0/160
Adenoma (B)	0/181	0/154	0/148	2/160
Lung				
Bronchogenic Adenoma (P)	1/179	0/154	1/146	0/155
Bronchogenic Adenoma (B)	0/179	0/154	0/146	2/155
Liver				
Reticulo-endotheliomas (B)	1/180	0/160	0/148	0/159
Spleen				
Hemangioma (P)	1/160	1/129	0/130	2/138
Reticulo-endotheliomas (P)	1/160	2/129	0/129	0/138
Reticulo-endotheliomas (B)	1/160	0/129	0/129	0/138
Bone Marrow, Blood				
Myelogenous (P)	0/157	0/134	1/136	0/135
Bone				
Osteoma (P)	0/177	0/152	0/148	1/156
Lymph Nodes				
Reticulo-endotheliomas (P)	5/167	4/143	5/140	6/146
Reticulo-endotheliomas (B)	0/167	1/143	0/140	0/146
Kidney				
Renal Adenoma (P)	1/179	2/164	0/145	0/160
Reticulo-endotheliomas (B)	1/179	0/164	0/145	0/160
Thyroid				
Adenoma (P)	1/145	1/117	0/127	0/137
Adenoma (B)	0/145	4/117*	1/127	0/137
"C" Cell Adenoma (P)	0/145	1/117	0/127	0/137
"C" Cell Adenoma (B)	0/145	0/117	0/127	4/137
Parathyroid				
Adenoma (B)	3/111	3/88	2/82	2/100

TABLE 3. (CONTINUED)

TUMOR TYPE	Unexposed Controls	0.25 ppm Exposed	1.0 ppm Exposed	5.0 ppm Exposed
Adrenal				
Cortical Adenoma (B)	40/177	18/155	19/141	23/153
Cortical Adenoma (P)	6/177	5/155	3/141	4/153
Stomach				
Papilloma (B)	0/169	1/149	0/140	0/145
Basal Cell (P)	0/169	0/149	2/140	1/145
Pleura, Peritoneum Mesenteries				
Fibroma (P)	0/161	2/152	0/139	0/147
Pancreas				
Islet Cell Adenoma (B)	0/114	0/98	0/99	0/107
Small Intestine				
Adenocarcinoma (P)	1/148	1/140	0/132	0/141
Colon				
Adenocarcinoma (P)	0/158	0/146	2/129	3/139
Leiomyoma (B)	0/158	0/146	0/129	1/139
Papilloma (B)	0/158	0/146	0/129	1/139
Total Tumors	0/158	0/146	2/129	5/139**
Skin				
Leiomyoma (B)	0/170	1/161	0/146	0/147
Squamous Cell Carcinoma (P)	0/170	1/161	0/146	0/147
Trichopithelioma (B)	0/170	1/161	0/146	0/147
Hemangioma (B)	0/170	0/161	1/146	0/147
Fibroma (B)	0/170	0/161	0/146	1/147
Pituitary				
Adenoma (B)	0/163	1/133	0/129	1/138

†   – Metastatic tumors in various organs were not counted.
(P) – Primary malignant tumors.
(B) – Benign tumors.
*   – Significant at the 0.05 level as determined using the Fisher Exact Test.
**   – Significant at the 0.01 level as determined using the Fisher Exact Test.

The nonneoplastic histopathology finding for exposed hamsters included descriptions and discussion of many lesions which occasionally occurred more frequently than in control animals. These probably reflected the aging process or the existence of chronic disease states to which hamsters are susceptible. Analysis of the incidence of such lesions would not elucidate the effect of hydrazine exposure on target organs. Therefore, the data were examined to select specific organ lesions which might have been related to exposure. This examination revealed that lesions in the nares, trachea, and bronchi (considered as one organ in the accounting), lung, liver, spleen, lymph nodes, kidney, thyroid, adrenal, colon, and testes occurred more frequently in exposed animals and could be possible sites of toxic action by hydrazine.

A compilation for each exposure group of the numbers of each type of lesion in some selected organs (Table 4) permitted the detection of significantly elevated incidence rates.

TABLE 4.   SOME SELECTED HISTOLOGIC CHANGES IN MALE
HAMSTERS AFTER REPEATED EXPOSURE TO INHALED HYDRAZINE
(% INCIDENCE)

Lesion	Unexposed Controls	Exposed 0.25 ppm	Exposed 1.0 ppm	Exposed 5.0 ppm
Liver				
Amyloidosis	23	42**	46**	50**
Hemosiderosis	23	39**	52	59
Bile Duct Hyperplasia	8	19**	19	28
Spleen				
Amyloidosis	24	30	44**	44**
Kidney				
Interstitial Amyloidosis	8	12	15	18**
Glomerular Amyloidosis	22	32*	46**	48**
Adrenal				
Amyloidosis	22	32*	37**	50**
Testis				
Senile Atrophy	18	26	27*	35**

*Statistically significant at the 0.05 level.
**Statistically significant at the 0.01 level.

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Two important observations emerged:

1.   Degenerative disease, characterized by amyloidosis in the livers, spleens, kidneys, thyroids, adrenals; and liver hemosiderosis, kidney mineralization, general degeneration of the adrenals; and senile atrophy, aspermatogenesis, and hypospermatogenesis, is a common finding in all groups of hamsters.

2.   The important fact is that these lesions occur with statistically significantly higher frequency in the exposed group; and in most cases, a dose response relationship can be seen.   The implication is that the stress of 12 months of hydrazine exposure at the various dose levels tended to increase the degenerative process in a dose dependent manner.

Nasal epithelial tumors were observed only in hydrazine exposed rats.   The majority of the epithelial neoplasms were benign and were mainly classified as adenomatous nasal polyps.   Small numbers of villous nasal polyps, muco-epidermoid papillomas, and squamous cell papillomas were also noted.   The incidence of these benign and several malignant epithelial tumors (shown in Tables 5 and 6) was elevated significantly in the 5 ppm hydrazine-exposed rats of both sexes.   An apparent dose-response was noted in that the incidence and degree of significance of the benign tumors were less in the 1 ppm hydrazine exposure groups (only one malignancy was found in both sexes).   No tumors of this type were seen in either control group of rats, and only one malignancy of the six tumors seen in about 400 rats exposed to 0.05 and 0.25 ppm.   Most of these tumors were seen after 2 years with the earliest occurring in a male rat at 88 weeks (36 weeks postexposure) and in a female rat at 98 weeks.

Varying degrees of acute inflammation were observed in the nasal cavity, larynx and/or trachea in some rats from the control and all treated groups.   The incidence and severity of the inflammatory changes were greatest in male and female rats from the group receiving 5.0 ppm, and in some of these affected animals, they were associated with focal hyperplasia and/or squamous metaplasia of the epithelium of the nasal cavity, larynx, and trachea.   These histopathologic changes were observed in rats dying during the study as well as in the animals killed at the 2-year interim sacrifice and at the 2½-year terminal sacrifice.

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TABLE 5. SELECTED TUMORS FOUND IN FEMALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TUMOR TYPE	UNEXPOSED CONTROLS (N = 147)	EXPOSED 0.05 ppm (N = 99)	EXPOSED 0.25 ppm (N = 100)	EXPOSED 1.0 ppm (N = 97)	EXPOSED 5.0 ppm (N = 98)
Nasal cavity:					
Epithelial (Benign)	0 (0)	1 (1)	0 (0)	4 (4)*	31 (32)**
Epithelial (Malignant)	0 (0)	0 (0)	0 (0)	0 (0)	5 (5)**
Pituitary:					
Adenoma	59 (40)	28 (28)	35 (35)	33 (34)	40 (41)
Adenocarcinoma	9 (6)	6 (6)	2 (2)	6 (6)	6 (6)
Thyroid:					
Adenoma	9 (6)	2 (2)	4 (4)	7 (7)	7 (7)
Carcinoma	17 (12)	1 (1)	8 (8)	15 (15)	5 (5)
Adrenals:					
Phaeochromocytoma	10 (7)	3 (3)	6 (6)	9 (9)	12 (12)
Uterus:					
Adenoma	1 (0)	0 (0)	0 (0)	2 (2)	3 (3)
Adenocarcinoma	10 (7)	4 (4)	5 (5)	7 (7)	6 (6)
Endometrial stromal sarcoma	0 (0)	2 (2)	1 (1)	1 (1)	3 (3)
Lymphoreticular Tissue:					
Leukemias	41 (28)**	18 (18)	21 (21)	13 (13)	13 (13)
Sarcomas	4 (3)	4 (4)	4 (4)	2 (2)	6 (6)
Mammary gland:					
Adenoma	4 (3)	4 (4)	6 (6)	8 (8)	8 (8)
Fibroadenoma	28 (19)	20 (20)	11 (11)	18 (19)	19 (19)
Adenocarcinoma	2 (1)	1 (1)	2 (2)	2 (2)	3 (3)
Liver:					
Liver cell tumor	3 (2)	0 (0)	0 (0)	6 (6)	3 (3)
Lung:					
Bronchial adenoma	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)

**Significant at the 0.01 level, control vs. test.
*Significant at the 0.05 level, control vs. test.
( ) = % incidence.

TABLE 6. SELECTED TUMORS FOUND IN MALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TUMOR TYPE	UNEXPOSED CONTROLS (N = 149)	EXPOSED 0.05 ppm (N = 99)	EXPOSED 0.25 ppm (N = 99)	EXPOSED 1.0 ppm (N = 98)	EXPOSED 5.0 ppm (N = 99)
Nasal Cavity:					
Epithelial (Benign)	0 (0)	2 (2)	2 (2)	10 (10)**	66 (67)**
Epithelial (Malignant)	0 (0)	1 (1)	0 (0)	1 (1)	6 (6)**
Pituitary:					
Adenoma	62 (42)	31 (31)	29 (29)	27 (28	26 (26)
Adenocarcinoma	4 (3)	0 (0)	5 (5)	4 (4)	5 (5)
Thyroid:					
Adenoma	15 (10)	5 (5)	7 (7)	9 (9)	2 (2)
Adenocarcinoma	7 (5)	6 (6)	5 (5)	9 (9)	13 (13)*
Adrenals:					
Phaeochromocytoma	16 (11)	14 (14)	13 (13)	18 (18)	11 (11)
Testes:					
Interstitial cell tumor	104 (70)	80 (81)	73 (74)	83 (85)	74 (75)
Prostate:					
Squamous carcinoma	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Liver:					
Liver cell tumors	9 (6)	11 (11)	8 (8)	6 (6)	4 (4)
Lung:					
Bronchial adenoma	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)
Lymphoreticular Tissue:					
Leukemias	36 (24)	20 (20)	28 (28)	22 (22)	10 (10)*
Sarcomas	8 (5)	9 (9)	3 (3)	6 (6)	3 (3)

**Significant at the 0.01 level, control vs. test.
*Significant at the 0.05 level, control vs. test.
( ) = % incidence.

The more severe grades of chronic respiratory disease were observed in lungs of some rats exposed to 5.0 ppm hydrazine and to a lesser degree in males exposed at 0.05 ppm. None of the males or the females exposed to 0.25 and 1.0 ppm showed epithelial hyperplasia. The morphological changes included peribronchial/peribronchiolar lymphoid hyperplasia, pneumonia, bronchopneumonia, and bronchiectatic abscesses. The affected animals usually showed the more severe grades of acute inflammation in the nasal cavity, larynx and/or trachea but with a higher prevalence as shown in Tables 7 and 8.

TABLE 7. PATHOLOGIC CHANGES SEEN IN FEMALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TYPE OF LESION	UNEXPOSED CONTROLS	EXPOSED 0.05 ppm	EXPOSED 0.25 ppm	EXPOSED 1.0 ppm	EXPOSED 5.0 ppm
Nasal:					
Squamous metaplasia	28/145(19)	18/97(19)	23/98(23)	24/94(26)	28/95(29)*
Epithelial hyperplasia	3/145(2)	2/97(2)	4/98(4)	5/94(5)	9/95(9)*
Larynx:					
Squamous metaplasia	6/138(4)	2/91(2)	2/91(2)	4/91(4)	14/91(15)**
Inflammation	22/38(16)	11/91(12)	4/91(4)	10/91(11)	48/91(53)**
Trachea:					
Squamous metaplasia	0/147(0)	0/96(0)	0/97(0)	0/95(0)	6/98(6)**
Inflammation	0/147(0)	3/96(3)	1/97(1)	4/95(4)*	29/98(30)**
Lung:					
Epithelial hyperplasia	0/147(0)	0/97(0)	0/100(0)	1/97(1)	3/98(3)
Adenomatosis	7/147(5)	3/97(3)	3/100(3)	4/97(4)	3/98(3)
Heart:					
Myocardial degeneration	125/147(85)	82/97(85)	91/100(91)	83/97(86)	89/98(91)
Myocardial fibrosis	49/147(33)	24/97(25)	24/100(24)	26/97(27)	23/98(23)
Thymus:					
Regression	85/147(58)	55/97(57)	59/100(59)	46/97(47)	50/98(51)
Lymph Nodes:					
Hyperplasia	3/147(2)	2/97(2)	4/100(4)	3/97(3)	11/98(11)**
Liver:					
Hepatocyte degeneration	18/147(12)	15/97(15)	14/100(14)	13/97(13)	15/98(15)
Hepatic hyperplasia	57/147(39)	42/97(43)	36/100(36)	58/97(60)**	64/98(65)**
Kidney:					
Progressive glomerulonephrosis	82/147(56)	34/97(35)	52/100(52)	54/97(56)	79/98(81)*
Uterus:					
Polyps	26/147(18)	23/97(24)	21/100(21)	19/97(20)	19/98(19)
Cystic endometrial hyperplasia	2/147(1)	1/97(1)	4/100(4)	1/97(1)	7/98(7)*
Endometritis	8/147(5)	5/97(5)	0/100(0)	6/97(6)	21/98(21)**
Squamous metaplasia	3/147(2)	1/97(1)	1/100(1)	0/97(0)	2/98(2)
Ovary:					
Atrophy	15/149(10)	13/97(13)	3/100(3)	15/97(15)	22/98(22)*
Oviduct:					
Salpingitis	0/147(0)	0/97(0)	0/100(0)	1/97(1)	20/98(20)**

**Significant at 0.01 level, control vs. test.
*Significant at 0.05 level, control vs. test.
( ) = % incidence.

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TABLE 8. PATHOLOGIC CHANGES SEEN IN MALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO HYDRAZINE

TYPE OF LESION	UNEXPOSED CONTROLS	EXPOSED 0.05 ppm	EXPOSED 0.25 ppm	EXPOSED 1.0 ppm	EXPOSED 5.0 ppm
Nasal:					
Squamous metaplasia	24/146(16)	19/96(20)	24/94(26)	25/97(26)	47/99(47)**
Epithelial hyperplasia	4/146(3)	9/96(9)*	3/94(3)	4/97(4)	21/99(21)**
Larynx:					
Squamous metaplasia	2/141(1)	2/95(2)	2/91(2)	3/91(3)	18/92(20)**
Inflammation	14/141(9)	42/95(44)**	7/91(8)	14/91(15)	72/92(78)**
Trachea:					
Squamous metaplasia	0/145(0)	0/97(0)	0/98(0)	0/95(0)	10/97(10)**
Inflammation	5/145(3)	17/97(18)**	2/98(2)	2/95(2)	52/97(54)**
Lung:					
Epithelial hyperplasia	0/149(0)	6/99(6)**	0/99(0)	0/98(0)	6/99(6)**
Adenomatosis	6/149(4)	9/99(9)	7/99(7)	9/98(9)	4/99(4)
Heart:					
Myocardial degeneration	98/149(66)	71/99(72)	73/99(74)	76/98(78)*	82/99(83)**
Myocardial fibrosis	104/149(70)	67/99(68)	68/99(69)	73/98(74)	52/99(53)
Thymus:					
Regression	67/149(45)	43/99(43)	57/99(58)*	48/98(49)	44/99(44)
Lymph Nodes:					
Hyperplasia	4/149(3)	5/99(5)	3/99(3)	5/98(5)	5/99(5)
Liver:					
Hepatocyte degeneration	18/149(12)	20/99(20)	16/99(20)	12/98(12)	7/99(7)
Hepatic hyperplasia	58/149(39)	39/99(39)	40/99(40)	41/98(42)	42/99(42)
Kidney:					
Progressive glomerulonephrosis	137/149(92)	90/99(90)	93/99(94)	90/98(92)	90/99(91)
Testes:					
Atrophy	119/149(80)	85/99(86)	77/99(78)	85/98(87)	84/99(85)
Interstitial hyperplasia	29/149(19)	12/99(12)	18/99(18)	11/98(11)	13/99(13)*
Prostate:					
Hyperplasia	8/149(5)	1/99(1)	11/99(11)	9/98(9)	13/99(13)

**Significant at 0.01 level, control vs. test.
*Significant at 0.05 level, control vs. test.
( ) = % incidence.

The incidence of focal liver cell hyperplasia tended to be greater in treated as compared to control female rats only at the exposure levels of 1.0 ppm and 5.0 ppm. This effect was seen in female rats dying during the study and in those killed at the 2-year interim sacrifice, but it was not noted in female rats killed at the 2½-year terminal sacrifice. There was no difference in the incidence of liver cell hyperplasia in treated as compared to control male rats.

There was no evidence that treatment with hydrazine increased the incidence of hepatic neoplasia. It was considered, therefore, that the slightly greater incidence of liver cell hyperplasia in treated as compared to control female rats arose fortuitously and that it was not related to treatment.

Acute endometritis was noted more frequently in female rats from the group receiving 5.0 ppm than in the controls or in rats from the groups receiving 0.05 ppm, 0.25 ppm, or 1.0 ppm. Acute salpingitis was present only in rats from the highest dosage group with the exception of one female from the 1.0 ppm dosage level and killed at termination.

Many microscopic variations from normal were seen in the aging mice, both control and hydrazine exposed groups. The only lesion of significance, an increased incidence of pulmonary adenomas in the 1.0 ppm hydrazine exposed mice, is shown in Table 9. This small increase in tumor incidence over unexposed control mice is similar to that previously reported in Swiss mice (MacEwen et al., 1974). An increased incidence of ovarian tubular adenomas was also noted in the group of mice exposed to 1.0 ppm hydrazine. This increase was not significant at the 0.05 confidence level, and its biological significance is uncertain since there was no suggestion of malignancy in this type of tumor in any of the exposed or control mice.

The occurrence of nonneoplastic lesions in the C57Bl/6 mice used in this study was similar in all groups with no apparent treatment effects.

TABLE 9. NEOPLASTIC PATHOLOGY IN CONTROL AND HYDRAZINE EXPOSED FEMALE C57Bl/6 MICE

TUMOR TYPE	Set No. 1			Set No. 2	
	Unexposed Controls (N=385)	Exposed 0.05 ppm (N=364)	Exposed 0.25 ppm (N=382)	Unexposed Controls (N=378)	Exposed 1.0 ppm (N=379)
Pituitary					
Adenoma	152 (39)	94 (26)	101 (26)	109 (29)	64 (17)
Carcinoma	7 (2)	10 (3)	3 (1)	8 (2)	2 (1)
Thyroid					
Adenoma	17 (4)	25 (7)	19 (5)	34 (9)	22 (6)
Carcinoma	2 (1)	1 (0)	1 (0)	2 (1)	1 (0)
Uterus					
Adenocarcinoma	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)
Lymphoreticular Tissue					
Leukemias	4 (1)	5 (2)	11 (3)	5 (1)	0 (0)
Sarcomas	145 (38)	154 (42)	150 (39)	154 (41)	139 (37)
Mammary Gland					
All tumors	1 (0)	1 (0)	0 (0)	1 (0)	0 (0)
Liver					
Liver cell tumor	4 (1)	9 (2)	6 (2)	6 (2)	11 (3)
Lung					
Adenoma	8 (2)	3 (1)	5 (1)	4 (1)	12 (3)*
Adenocarcinoma	2 (1)	1 (0)	2 (1)	3 (1)	3 (1)
Ovary					
Tubular adenoma	12/369(3)	10/340(3)	11/365(3)	13/365(4)	23/361(6)

*Significant at 0.05 level, control vs. test.
( ) = % incidence.

Three rodent species that inhaled hydrazine concentrations of 1.0 ppm or greater for a year developed oncogenic changes in the respiratory system. These changes appeared to be dose related in the rat in which the significant effects were epithelial tumors of the nasal turbinate. In the female rat, the tumor incidence was 4% and 37%, respectively, in animals exposed to 1.0 and 5.0 ppm hydrazine. In the male rats, the incidence was 11% at 1.0 ppm and 73% at 5.0 ppm. Nasal polyps were significant only in the 5.0 ppm hydrazine exposed hamsters. These tumors were not seen in any unexposed control rats and in only 1 of 181 unexposed control hamsters.

A previous report of hydrazine exposures (MacEwen et al., 1974) indicated a dose related increase in alveolargenic carcinomas in female ICR mice (a strain that normally has a high incidence) exposed to 1.0 and 5.0 ppm hydrazine. C57Bl/6 mice used in this study and exposed to 1.0 ppm hydrazine exhibited a significant increase in pulmonary adenomas. This concentration was the highest level tested in mice during the present study since the prior study had shown 5.0 ppm killed half of the mice during exposure.

A number of chronic nontumorous pathologic changes were seen in rats and hamsters exposed to hydrazine concentrations of 1.0 or 5.0 ppm. Significant differences between unexposed control male rats and the high level exposure groups occurred after one month of exposure and continued even after cessation of hydrazine treatment. Both male and female rats in the 5 ppm exposure group had a much higher incidence of upper respiratory inflammation and squamous metaplasia.

Male hamsters exposed to 5 ppm hydrazine had significantly lower body weights than control animals during their exposure and the 12-month postexposure holding period. Amyloidosis, a disease frequently seen in aged hamsters, was much more prevalent in the exposed groups and the incidence appeared to be dose related.

Although the mortality rates were comparable between the test and exposure groups of hamsters throughout the study period, there were greater numbers of changes in the hydrazine exposed animals than their unexposed controls that are usually associated with aging such as amyloidosis and senile atrophy of the testes.

Analysis of the oncogenic changes and other toxic effects of exposure to hydrazine indicates that the nononcogenic sequelae were more severe in producing debilitation and lethal effects. The oncogenic changes were mostly benign and observable only at the microscopic level producing little or no impairment of respiratory function and had no effect on life expectancy.

The respiratory system appears to be the primary site of hydrazine induced oncogenic changes regardless of route of administration. In studies conducted by Roe et al. (1967) and others, lung tumors were induced in Swiss mice after oral administration of hydrazine in water. Lung tumors were induced in C57B1/6 mice by Mirvish et al. (1969) after intraperitoneal injection. Rats given hydrazine sulfate by stomach tube by Severi and Biancifiori (1968) exhibited some lung tumors.

No statistically significant tumor induction was seen in rodents exposed to hydrazine concentrations of 0.25 or 0.05 ppm.

We conclude from these studies that hydrazine is a relatively weak tumorigen which exhibits a dose response related tumor induction at inhaled concentrations of 1.0 ppm and 5.0 ppm. Repeated exposures to hydrazine concentrations above 5.0 ppm result in early death of rodents and dogs, usually associated with malnutrition after chronic exposure.

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The incidence of benign and malignant tumors was highest in nasal turbinates of rats. This rat tissue has demonstrated extreme sensitivity to the action of respiratory carcinogens (HMPA and formaldehyde) and may not be directly extrapolatable to exposure of humans who are not obligate nose-breathers. Nevertheless, the toxic and oncogenic effects seen in this study indicate that the current OSHA Threshold Limit Value of 1.0 ppm for hydrazine is unsatisfactory and is too near concentrations which cause death in chronically exposed animals. More realistically, the ACGIH recommended TLV of 0.1 ppm would be expected to provide adequate protection.

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OPEN FORUM

DR. NEWTON (University of California, Irvine): I think the time frame of the frequency change, the acidosis change, and the oxygenation change are very interesting. I think that the ventilation would be very interesting to look at. Mr. Burgess, did you look at tidal volumes and inspiratory flows?

MR. BURGESS (E. I. duPont de Nemours and Company, Inc.): No, we didn't. Most of those procedures require some sort of anesthesia or surgical procedure. They would be interesting to know but were not a part of the protocol we were testing for evaluation of construction materials.

DR. NEWTON: In talking to firefighters, they say the worst fire to work with is a mattress fire. Have you looked at what the constituents are in a mattress fire other than what you would expect from burning polyurethane and incorporate those compounds into your protocol?

MR. BURGESS: Mattress fires are very common. Maybe that's one of the reasons it comes to mind. Most mattresses are polyurethane in construction, and its combustion products are irritant in nature. I suppose that's what you're talking about, the irritating aspects of the fire?

DR. NEWTON: Right. Firemen say the worst fires that they have, and the ones that require the use of self-contained breathing apparatus are mattress fires. It doesn't have to be a big fire.

MR. BURGESS: I really don't know, but I suspect that those are the most common types of fires. But I really don't know that they are any more irritating than any other materials.

DR. CAVENDER (ToxiGenics, Inc.): I have three questions for Mr. Burgess. What temperatures are attained in the static exposure system that you are dealing with? Secondly, how do you treat control animals to be able to compare them with the exposed animals that are clamped down in these nose-only holders? And finally, in considering a dynamic system versus this, how would you relate this to the normal situation? The Alarie respiratory rate measurement is really considered to be a defense mechanism rather than a toxic response. You certainly can compare compounds within the same classes as to relative toxicity perhaps, but to denote a toxic response from a defense mechanism doesn't seem to be really appropriate. I wondered if you would comment on that.

MR. BURGESS: Temperature in the chamber varied with the combustion temperature of the material, and in a nonflaming mode, temperatures in the chamber usually are below 30 degrees centigrade for the majority of tests. In a flaming mode, you can get higher temperatures generated in the chamber. They can be up to as high as 50 degrees

centigrade. We don't use control animals for determining an LC_{50} value. I assume if we don't expose an animal, they don't die. In terms of respiratory rate and the loss or shock avoidance techniques, we do use controls. We test them independently of these exposures, but they are placed in the same holders in the same way, for the same time period, and handled in the same fashion. The furnace is on, nothing is burned, but the effect of the heat produced from the furnace has been studied in control animals. We've looked at, and have started looking at more intensively, the effect of chamber temperature on controls. In terms of whether or not a change in respiratory rate is a toxic response or just an accommodation mechanism, it is a response, a physiological or toxicological response. You can argue the two. I'm thinking in terms of toxic hazard. Certainly the effects of carbon monoxide intoxication are considered toxic responses. I look at the respiratory rate response as being a very important physiological response in these atmospheres and one which shows a dose response.

DR. HENDERSON (Olin Corporation): A comment in terms of a real life situation. The avoidance response to shock really doesn't duplicate the real life situation. I think that the real life situation is the fire alarm going off on the second floor of this motel at 2:00 A.M. after a very hospitable party, and then whether you can awaken upon hearing that fire alarm and go through the avoidance of getting out from the fire. Which means then that rather than testing single responses, what we need to do is test a conditioned response of an animal, not from the smoke exposure but from enough smoke to ring a smoke alarm. Then I think we would be looking at a real life situation. Can we go up a flight of stairs, push a button, get through an opening, and get out when the smoke alarm is being triggered by a real fire? This is very nice toxicology you are doing, but I don't think it's going to help us save many lives in a real life situation.

MR. BURGESS: It's very difficult to train animals to make a specific response, especially rats. There's a long training period for higher level function. I don't know if loss of shock avoidance relates to incapacitation at all. I think that the two important effects that we've seen are lethality and respiratory rate depression or irritation. I would like to develop a test which would really reflect the ability of people to think in a fire which is what you are talking about but no suitable model has been developed yet for animals. These techniques here simply represent techniques which can be handled in the laboratory, within a reasonable time span, on a large enough group of animals to look at and to draw conclusions for screening super toxic materials. Toxicity is only one aspect, and these are only individual aspects of toxicity. What we really need to address is the fire hazard. There are many factors that we haven't found a way to address yet.

CDR JENKINS (Naval Medical Research Institute): I have one or two questions, then I would like to make a comment. Did you measure the rate of CO evolution in your chamber and correlate that with rate of appearance of carboxyhemoglobin?

MR. BURGESS: Yes, I didn't present that data this morning due to time limitations. If you look at CO generation and carboxyhemoglobin formation, they essentially parallel each other. I think that what you are getting at is perhaps the late climb in carboxyhemoglobin due to late generation.

CDR JENKINS: I was wondering if that relates to the respiratory rate decrease?

MR. BURGESS: Well, there were two breaks in the dose response curve, if you remember. There was an early break which I attributed to sensory irritation and a later break which we didn't define which appears to occur right at those points where CO concentrations increase. The second break in the dose response curve does appear to be due to effects of asphyxia or hypoxia.

CDR JENKINS: The comment that I have partially relates to Dr. Henderson's comment. In toxicology, we almost never do things that relate to real life. It's a laboratory science. Hazard evaluation is a judgment. We go to great pains to make sure that we have animals that are genetically homogenous, young, healthy adults, which in no way resemble the real life population. I think the key factor is that no two fire situations are really similar, and it depends on what the response is that the victim of the fire has to be able to perform. That is to say that if you have smoke in your bedroom in the motel, your biggest concern is to get out of the motel, and the paradigm that you would have to develop in the laboratory animal is probably the ability to go through a maze. On the other hand, if you see smoke while you are trying to land a jet aircraft on an aircraft carrier at sea, a lot more complex behavioral paradigms would have to be modeled. Again, if you are trying to model a situation of escape, you would not be able to compare that particular situation to that of a firefighter where the firefighter is not supposed to escape. The firefighter is supposed to be able to stay and put out the fire, especially with a shipboard fire at sea, because when the ship is on fire, the fire department is on fire. Sometimes, somebody keeps shooting at the fire department and trying to keep it on fire. You can have a great many real life situations. I think one of the things that Mr. Burgess' protocol can do as well as a number of others is to rank the toxicity of materials. Theoretically, if you have a choice, for example in this room of tiles for the ceiling and if you rank them in some way by the toxicity of their pyrolysis products and pick the safest tile, and the safest wall covering, and the safest upholstery, then the end result might be that you would optimize the situation if the room did catch on fire and perhaps increase the time for safe escape from a couple of minutes to perhaps five minutes. Fires and fire behavior are very complex and difficult to model in the laboratory.

MR. BURGESS: One reason that respiratory rate depression may be a good index for ranking materials is that one of the first things that firemen say they experience is the sensory irritation. You notice that the

exit signs are no longer visible. You really can't see well because your eyes are starting to tear, and your breathing becomes difficult because you're starting to experience real respiratory irritation. These types of effects are very important, and we can measure these effects in the laboratory.

DR. REISCHL (University of California, Irvine): I have a question regarding the respiratory rate. I assume your animals were hyperventilating when you loaded them into the chamber. Your observed depression in respiratory rate may be due to the animals settling down into the chambers. How much do you think this settling could account for the depression? Personally, I'm very much surprised your rats are not hyperventilating rather than hypoventilating.

MR. BURGESS: We let the animals settle down in these holders for at least 15 or 20 minutes before the exposure to see that they have achieved a steady constant breathing rate. There is variability between one exposed group and another. I'm assuming that they aren't hyperventilating and that this effect is due to the toxic components. I don't know if that answers your question, but I'm assuming that they aren't hyperventilating because we sit and watch them for quite a while to ascertain that they are breathing at a constant rate. What we are looking for is a relative change and not an absolute change in the breathing rate.

DR. REISCHL: Do you have control data for these animals being in the chamber for the 90-minute duration?

MR. BURGESS: We do. There wasn't anything on the slide but essentially those graph lines for control animals look like straight lines ± 10% throughout the entire 90-minute or 2-hour period. It is a gross thing to do. We put an animal in a whole body restrainer and put a little pressure transducer in there. We expect it to have some sort of stress associated with it, but we are looking at relative changes.

DR. BACK (Air Force Aerospace Medical Research Laboratory): The question is for Dr. Newton. Your first slide indicated that carboxyhemoglobin levels for the average nonsmoker is 1.2%, and then you said that the great bulk of the people in the United States, 30% to 50% or better, have blood levels above that amount, about 1.5% I believe you said. Now, how was that taken? The range of carboxyhemoglobin is 0.8 to 2.1 on that slide. Does that mean that every individual that went to 1.51 was included as an overexposure or did you use the upper range or what?

DR. NEWTON: I said that 45% of the nonsmoking blood donors had carboxyhemoglobin levels above 1.5% which is the EPA air quality standard which is supposed to be the upper limit.

DR. BACK: That's a peculiar way to do it. I would think that a national standard should be set at the upper edge of the normal range for nonsmokers. That would give you a falsely high indication of carbon monoxide overexposure if you consider anyone over 1.51% overexposed when your normal unexposed range is 0.8 to 2.1%. It seems to me that the upper end of that range ought to be the proper way of looking at it rather than 1.5% which is above your average. You also showed that nonsmoking firefighters reach an average carboxyhemoglobin of about 3% and that smoking firefighters had about 7% carboxyhemoglobin in their blood. After a fire, your nonsmoking group went to 5% COHb, and your smokers went to 8%. That's not surprising depending upon how long they were in there because nonsmokers are going to have relatively more carboxyhemoglobin than smokers under most fire conditions. The amount that a smoker is going to increase is the difference in the partial pressure of carbon monoxide that he's exposed to. As a matter of fact, in certain instances, a smoker in a room that doesn't contain any carbon monoxide is adding to that room, not subtracting from it, and he's not picking up any more from another smoker because he may already be at equilibrium with his environment. So going from 7% to 8% is not surprising at all. As a matter of fact, if he's not exposed to very much, he wouldn't pick up any.

DR. NEWTON: They must have been exposed to some carbon monoxide because their COHb levels did increase.

DR. BACK: Yes, to something, to some portion of it, but probably not back at the firehouse.

DR. NEWTON: No, at the scene of the fire because they took their breathing apparatus off too soon.

DR. BACK: Yes, they were exposed to something. I guess what I'm trying to get at is that the Air Quality Standards were set to protect the smoker from extraneous CO which would produce more carboxy-hemoglobin. What I am getting at is that an air quality standard should protect the nonsmoker less than the smoker.

DR. CULVER (University of California, Irvine): I'm not sure whether this is a question so much as an opportunity for me to climb on one of my favorite soap boxes. Mr. Gaworski's paper presents an illustration of something that I consider to be important. He is planning to hold his animals and look for cancer at a later date. This, of course, has become very popular today. I asked Dr. MacEwen whether he was also going to be looking for changes in pathology that were found follow-ing exposure and see whether or not those pathological changes had progressed or reversed at a later date of sampling. He said indeed, he was. It is my observation that this is not done frequently and that following chronic exposure studies, animals are not examined for chronic

change or noncancer pathology at periods remote from the time they were exposed. We need to know what to expect in humans in terms of progressive or regressive pathological changes resulting from toxic exposure once the individual has been removed from his exposure. That information can best come from the experimental laboratory. The other thing that we need is information on whether the pathology created by the exposure makes the organism or the individual more susceptible to subsequent exposures.

DR. HODGE (University of California, San Francisco): Dr. Culver's comment on the holding period is something I agree with entirely. I also feel uncomfortable when I hear of strict adherence to a practice which was exemplified in Mr. Kinkead's statement that the mouse still had hind quarter paralysis at the end of the 14-day observation period. Now, we don't have any rules and regulations about that, but I would feel much better if the standard practice were to hold animals until they return to a healthy status rather than follow the USDA or Food and Drug Administration or National Cancer Institute practice of killing the animals at 14 days. I would like to see us hold our animals for another 2 weeks, or 3 weeks, or whatever to find out if this is an irreversible change.

DR. MAC EWEN (University of California, Irvine): Dr. Hodge, in the proposed EPA protocols for testing under the Toxic Substances Control Act, that provision has been made, that if the animals still exhibit any symptoms or the weight losses have not returned to control levels or preexposure levels, then the animals are to be held indefinitely until they do return, and any subsequent deaths are to be included in that LD_{50} calculation or LC_{50} calculation. That "proposed" protocol will probably become a regulation in time.

I would like to add one comment about the hydrazine study. I forgot to mention in my presentation that the reason I didn't give any dog data was that the dogs were still alive when I was preparing this presentation. The dogs were terminated in September of this year. Dr. Hall is present, but I don't know whether he has looked at the tissue sections yet or not. Dr. Hall, have you?

DR. HALL (Air Force Aerospace Medical Research Laboratory): I finished them yesterday.

DR. MAC EWEN: Very good. Perhaps you can tell us something about what was seen in those tissues.

DR. HALL: The slides from these dogs were examined thus far only to determine whether there were neoplasms present. Nonneoplastic lesions will be catalogued later. We had one dog in the low dose group which had a mass at the mucocutaneous border of the anus biopsied during the second postexposure year. It was a papillary adenocarcinoma low grade tumor. A very unusual tumor in a dog. That same mass has

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since been biopsied and also noted at necropsy several months ago. The nature of that particular neoplasm hasn't changed. Neither has it gone away. There were no other tumors seen in these three groups of dogs on histopathologic examination of the tissue.

DR. REISCHL (University of California, Irvine): I have a question regarding the obedience training tests on the JP-10 exposed dogs. Did you use a graded evaluation scale to detect small decrements in their behavioral response?

MR. KINKEAD: Yes, we had a subjective grading system, ranging from 1 to 10. A veterinarian and I did the grading each time. Throughout the entire training period, these animals were graded, and we kept a record of their responses. It was a subjective scoring system, and most of the dogs improved with time until they were able to perform at a 9 or 10 rating repeatedly prior to exposure.

NOTICES

TECHNICAL REVIEW AND APPROVAL

AFAMRL 79-121

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

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