JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE
VOLUME 54, NUMBER 2, APRIL, 1979

ENVIRONMENTAL TOXINS*

GENERAL SESSION
TENNESSEE ACADEMY OF SCIENCE
88TH ANNUAL MEETING

INTRODUCTION

JAMES D. CAPONETTI

The University of Tennessee
Knoxville, Tennessee

Environmental toxins can be divided into two major categories: natural toxins and industrially-produced toxins. Since the time of human evolution, people have always been exposed to natural toxins. Before the advent of agriculture, all human beings were hunters and gatherers of plants and animals for the purpose of sustaining life. The discovery of poisonous plants and animals must have been by trial and error. The death of a hunting companion after ingesting some recently gathered poisonous fruits soon taught the survivors which plants were toxic. Thus began a mental cataloging of toxic plants with the certainty that the information was handed down to suceeding generations. The same must have been true about poisonous animals. The first fatality from the bite of a venomous reptile along the trail or from the ingestion of poisonous fish by fellow villagers must have been a sobering lesson on how toxic the natural environment could be. Trial and error eventually paid off, however, because almost all the common natural toxic sources were known to human beings before the beginning of recorded history. By the time ancient languages were being scratched on rocks or penned on Papyrus, ancient peoples could recognize a number of different kinds of poisonous plants and animals and knew how to avoid them. Coupled with this, they had acquired the knowledge and skill of how certain plants and animals could be detoxified before use as food or how certain poisonous plants could be used as drugs or medicinals when applied or ingested into the body in the proper concentration. In many societies, this knowledge became vested in the "medicine man." In many areas of the world today, such circumstances continue.

Ancient peoples were never exposed to the second major category of environmental toxins, namely the industrially produced chemicals. The industrial revolution is a rather recent occurrence when viewed in terms of how long people have inhabited the earth. Only the people who have lived within the past 200 years or so have been exposed to a large number of rather sophisticated and highly toxic gases, liquids, and solids. There are hundreds of industrial chemicals, agricultural chemicals, and household products, including medicinals, which are directly toxic or indirectly toxic by absorbing them from contaminated food, polluted water, or polluted air. Much of the research effort in toxicology today is aimed at understanding the mechanisms of toxification and detoxification of natural and industrially-produced toxins in humans plus wild and domesticated animals.

There is not enough time in one general session to cover all aspects of environmental toxins. My best choice under the circumstances was to call upon a small number of experts in the field of toxicology with whom I am acquainted, and have them present a general picture, within their field of research and expertise, of the current status of the effects of natural and industrially-produced toxins.

PLANTS TOXIC TO MAN AND ANIMALS

R. D. LINNABARY,

The University of Tennessee
Knoxville, Tennessee 37916

Toxic plants are ubiquitous and nearly any plant can be toxic. When we think of toxic plants we most commonly think of weeds when, in fact, many cultivated ornamentals and vegetables contain toxins.

There are many variables that determine the toxicity of a plant. Its toxins (phytotoxins) can range from minimal to extremely toxic. Dry or wet conditions during the plant's growth may increase its toxicity. Various stages of growth and certain parts of the plant at these stages may be more toxic than others. Freezing temperatures can cause plants, such as the sorghums, to be more toxic. Sorghums contain a glucosidal combination from which hydrocyanic acid is liberated after the plant has been injured by a frost. There are animal species variation and individual susceptibility often encountered with a specific phytotoxin. The rate of exposure is also an important facet of intoxication and concurrent with but not necessarily determined by exposure, is the dosage of the toxin.[9]

Humans and animals are exposed to much the same plants. Animals, being herbivorous and depending solely on plants for nutrition, have the greatest exposure while humans, being omniverous, have less. Mother nature was kind in making most poisonous plants rather unpalatable due to an undesriable taste or odor. Yet, there are some conditions even with good management, in which certain plants become more palatable or a given animal develops a taste for them. Such is the

*Highlights of presentations made on the 1978 TAS theme of Environmental Toxins at the General Session of the Tennessee Academy of Science Annual Meeting, November 1978.

case where pigs eat young cocklebur and cattle eat sweet clover. However, most toxic plants are consumed because the animal is hungry due to poor pasture management.

In the past two years the Tennessee Animal Disease Laboratory has encountered numerous toxicities due to unusual weather conditions. The summers of 1976 and 1977 were drouthy over the State. This, coupled with unusually cold and wet winters, reduced the forage crops to an inadequate level. Many of our livestock entered the winters in poor body condition with little nutritional reserve. It is understandable that these animals were attracted to and consumed plants such as oak leaves and acorns, mountain laurel, taxus and purple mint plants. These are plants not in the animals normal diet. The oak leaves and acorns are nephrotoxic; laurel and taxus are neurotoxic and the mint plant has pulmonary toxicity.

Indirectly, last year's drouth presented another problem. With a drouth stunted crop, insect damaged corn was much more common. This damaged corn was often infected with molds. Three toxic genera encountered were *Aspergillus, Penicillium,* and *Fusarium.* The first two may produce aflatoxins, the last produces zearalenone, T-2, deoxynivalenol and an unclassified toxin. We have been able to incriminate the *Fusarium* toxin in numerous cases of equine leukoencephalomalacia and porcine pulmonary edema. Dollar losses have been conservatively estimated at ½ million dollars in livestock losses alone. This does not include many tons of corn that were destroyed because of aaflatoxin or *Fusarium* toxin content.

Human phytotoxicosis is often caused by ignorance. This includes ignorance of the toxins present, the amount of toxin needed for irreversible damage, and the preparation of vegetation of known toxicity. Examples of the lack of knowledge of plants are in reports of people, mostly young, who chew henbane, jimson weed and elderberries. Henbane leaves taste like lettuce, the roots like parsnip. Both parts contain toxic atropine.[8] A jimson weed toxicity case was reported by the news media in which at least one child died and several others were sick from the consumption of jimson weed seeds. These children were "told" they could get "high" on the seeds. There are numerous cases of children being attracted to, and eating elderberries.

Some interesting data on plant poisoning came from the National Clearing House for Poison Control Centers, Bulletin of April 1978. This data was for substances more frequently ingested by children under 5 years of age. Plants were the most frequently ingested and accounted for 10% of the total. Soaps, cleaners and detergents were second, with about 6%. Considering the 9,085 plant related poisonings and the types of plants ingested, the dosage-response relationship in phytotoxicosis is obvious since few patients were hospitalized. Ingestion of almost any of the plants involved could result in death if enough were eaten.

Persons who take part in fad diets such as strictly potato, cabbage, banana, etc., have a real danger of toxicosis.[6] Potatoes contain several potent toxins, the most potent being solanine. Cabbage contain a goitre producing agent and bananas contain a vaso pressor amine, which in combination with certain tranquilizers (monoamine oxidase inhibitors), can be very toxic.

The "back to nature" herb diets can be quite dangerous. Ewell Gibbons may have eaten pine needles for breakfast, but he freely admitted the potential dangers of some of the plants used in his herbal cuisines. Examples are: anemia from onions; oxalate nephropathy from beet tops and rhubarb; photosensitizaton from buckwheat; raw elderberries or elderberry stems containing cyanide; poke berries or improperly cooked poke shoots causing respiratory paralysis and nitrate accumulations occurring in turnips and labs' quarters. Fortunately, these phytotoxins are usually diluted by the consumption of other foods which makes them less hazardous. [3, 7]

A major concern is determining the danger of consuming animal protein from animals which have eaten toxic plants. The danger is lessened due to the detoxifying enzymes found in the organ tissues, primarily the liver. These enzymes, grouped together, are called the *mixed function oxidases.*[1] They convert many toxins to non-toxic metabolites which are harmlessly excreted by the animal. Most of our animal protein is harvested from the ruminant in the form of red meat and milk. This group of animals (sheep, goat and cattle) have a large digesting vat, called the rumen, which is a part of the stomach. In the rumen many toxic agents are acted upon by the bacteria and protozoa present and are rendered harmless. If these systems are overwhelmed or by-passed, the animal usually dies. Consequently, there are few residues found in the meat and milk. The aflatoxins are found in both milk and meat, while toxins of white snake root, Astragalus, alkaloids of the *Senecio* and aromatic onions and garlic are found only in milk.[2, 4, 5] However, the dosage of any of these toxic residues is low both in actual amounts and diluted amounts and usually cause little concern.

Phytotoxicology is a relatively unexplored scientific field but by combining primitive societies' herbal lore and today's analytical techniques, many things of substantial worth are being found. Maybe now is the time for multi-disciplinary efforts of the botanists, biochemists, physicians, veterinarians, analytical chemists and others to examine the plants growing around us. With proper data perhaps we can add beneficial drugs to our medical armamentarium.

LITERATURE CITED

Brattsten, L. B.; Wilkinson, C. F.; Herbivore-Plant Interactions: Mixed Function Oxidases and Secondary Plant Substances. Science, 196 (1977): 1349-1352.

Dickinson, J. O.; Cooke, N. P.; King, R. R.; Mohamed, P. A.: Milk Transfer of Pyrrolizidine Alkaloids in Cattle. JAVMA, 169(1976): 1192-1196.

Evers, R. A.; Link, R. P.: *Poisonous Plants of the Midwest.* University of Ill. Special Publication 24 (1972).

Jacobsen, W. C.; Harmeyer, W. C.; Jackson, J. D.; Armbrect, B.; Wiseman, H. G.: Transmission of Aflatoxin B, into the Tissues of Growing Pigs. Bull. Environ. Contam. Toxicol. (1978): 156-161.

James, L. F.; Hartley, W. J.: Effects of Milk from Animals Fed Locoweed on Kittens, Calves and Lambs. AJVR, 38 (1977): 1263-1265.

Larkin, T.: Natural Poisons in Food. FDA Consumer (Oct. 1975): 5-7.

Muenscher, W. C.: *Poisonous Plants of the United States.* The McMillan Co., New York, 1957.

Sands, J. M.; Sands, R.: Henbane Chewing. Med. Jour. Australia 2, (1976): 55-58.

Smyth, P. J.: The Toxic Hazards of Some Pasture Plants and Vegetables on Ruminant Animals. Vet. Serv. Bull., 6 (1976): 49-56.

TOXICOLOGY OF THERMODEGRADATION AND COMBUSTION PRODUCTS OF MAN-MADE MATERIALS

W. HOMER LAWRENCE AND JOHN AUTIAN

University of Tennessee Center for the Health Sciences Memphis, Tennessee

Death and injury from fires are common occurrences. Certain types of fires, e.g. aircraft, night club, hotel, large apartment house, home for the aged, etc., tend to be sensational and gain much publicity, but are less frequent and result in less total loss of life than those in small businesses or private homes. Fire victims are not always burned by the fire. In 1971 Thomas indicated that 80% of the victims of fire are not touched by the flame, but die as a result of exposure to smoke—the term "smoke" is used here to include toxic gases. Based upon autopsies of fire casualties in a Maryland study, combustion products were indicated to be the most frequent cause of death.

More recently, and closer to home, was a jail fire in Columbia, Tennessee in June of 1977, in which 42 people died (8 visitors and 34 inmates). Polymeric padding materials within the jail were blamed for release of heavy smoke and toxic fumes which were picked up and circulated through the air duct system. According to newspaper accounts, some of the victims showed relatively high blood levels of cyanide and carbon monoxide. Somewhat comparable tragedies in building fires have been attributed to pyrolysis or combustion of polyurethane insulation, furniture padding, carpet, etc.

Flammability of materials is a factor intimately associated with hazards from fires. Not long ago action was taken to reduce accidents in which children were seriously burned by ignition of sleepwear or other clothing. Federal legislation required certain children's garments to be non-combustible; this necessitated treatment of many fabrics with flame retardant chemicals. Interestingly, this created another furor unrelated to fire or combustion products, when it was found that one of the most widely used flame retardant chemicals, Tris, caused mutagenic changes in bacteria. The implication was that the compound might be mutagenic or carcinogenic to humans, and thus pose an insidious, long-range health hazard.

Aside from the possible alteration in pyrolysate toxicity, sometimes additives to fabrics or other materials may present a potential for other adverse or toxic reactions as a result of leaching or possible vaporization of one or more of the additives. Several years ago we examined some materials for possible leachability by conducting tests designed to evaluate biological activity of substances that are leached from the sample material. Results varied tremendously from one material to another; however, some of the materials (fabrics, in this case) released substance(s) to a biological environment that produced local necrosis of tissue. The material was also extracted with polar and non-polar solvents and the extracts evaluated for the presence of toxic leachables. Some of these extracts contained substance(s) which were cytotoxic to cells in culture, were irritating when injected intradermally in rabbits, and occasionally produced death when administered (orally or intraperitoneally) to mice.

While treatment of fabrics or other materials with flame retardants will decrease their tendencies to burn and to support combustion, if these materials are exposed to sufficient heat they will degrade, either through combustion or pyrolysis. Thermodegradation of such flame retardant-treated material often produces gaseous products which are more toxic than those produced from a similar quantity of the untreated material. Again one sees that resolution of one problem (flammability) may create or aggravate another (toxicity of pyrolysis products).

As indicated earlier the relative toxicologic importance of thermodegradation and combustion products *vs.* heat generation produced in a fire environment is highly variable. Major factors which influence this interrelationship relate to the *kinds* of materials involved, the *quantity* of such materials, degree of oxygenation at the combusion site, smoldering *vs.* flaming combustion, proximity of individuals to heat source, and type and degree of ventilation for exposed individuals.

Heat. Excessive temperatures can be harmful *via* three modes: (1) severe burns on the surface of the body; (2) inhalation of hot air or other gases can produce thermal damage to trachea or lungs and pulmonary edema; or (3) prolonged heated environment can raise body's temperature and produce death.

Smoke. It is a common feature of almost all fires, but varies greatly in its density and composition. Smoke may irritate the eyes and respiratory tract; it may interfere with vision and promote irrational behavior leading to injury or preventing safe escape.

During pyrolysis/combustion a variety of gaseous compounds are formed; a number of these exert marked toxicologic activity. Some of the more important pyrolysis/combustion products are CO, HCN, CO_2 and oxygen depletion. With some materials HCl, HF, NO_X, SO_2, H_2S, etc. are also formed and may contribute significantly to the toxicity of these pyrolysis/combustion products. While the toxic effects are understood for many of these compounds, the role played by each in an actual fire may be quite variable; other components of the mixture may not be well characterized toxicologically. Even if one knew the actual qualitative and quantitative composition of the pyrolysis/combustion

products, it would be difficult or impossible to predict the exact toxic liability because of potential interactions involving additive or potentiating effects upon the biological system produced by the various combination. Therefore animals are almost indispensable for evaluating the total toxic liability of such diverse mixtures.

Oxygen. Oxygen is necessary to support combustion and is also involved in oxidative pyrolysis. A relative deficiency of O_2 during pyrolysis/combustion tends to increase formation of carbon monoxide at the expense of more complete oxidation to carbon dioxide. Oxygen deficiency can produce various symptoms in man, but does not become life-threatening until it is reduced to about 6-8% or less.

Carbon Dioxide. Respiratory stimulation is probably the most important toxicologic role of CO_2 in a fire situation. This will tend to increase respiratory minute volume, and hence exposure to other toxic gases.

Carbon Monoxide (CO). CO in varying quantities is found in almost all pyrolysis/combustion products. It is dangerous to man in concentrations of 0.15% or more, when inhaled for an hour. CO exerts its toxicity by combining with hemoglobin (Hb) to form carboxyhemoglobin (COHb) which prevents Hb from transporting oxygen to the tissues.

Cyanide. Cyanide, such as hydrogen cyanide (HCN), is a rapidly acting poison. It's primary toxicologic effect results from its reaction with cytochrome oxidase in the mitochondria of the cell to block energy transfer in such critical areas as the brain and heart (cytotoxic hypoxia).

Studies on the toxicity of pyrolysis or combustion products from fabrics and polymers were made possible by support from groups such as Cotton, Inc. and NASA Johnson Space Center. Four experimental methods were employed.

1. **MSTL Procedure.** An out-of-chamber, controlled heating rate, pyrolysis procedure. Rats are exposed to pyrolysates from various sample weights in a dynamic air-flow system to determine the LD_{50}.

2. **In-Chamber Pyrolysis.** Test samples are rapidly pyrolyzed inside exposure chamber and rats are exposed to the fumes in a static environment (no air flow).

3. **NASA Procedure.** A static exposure system using a modified Vac-Hyperbaric chamber, with out-of-chamber, rapid pyrolysis to a fixed maximum temperature.

4 **MSTL Combustion Procedure.** Test samples are combusted with an infrared gas burner, and rats are exposed to the fumes in a static environment.

Limited data (MSTL) indicate the toxicity of pyrolysis or combustion products from flame-retardant treated materials is generally greater than that from comparable materials without the flame retardant.

When samples are pyrolyzed outside the exposure chamber there is often some condensate formed during transport of pyrolysates to the exposure chamber. Thus, it was some what unexpected to find the toxicity of pyrolysates formed by the MSTL Procedure (out-of-chamber pyrolysis) were as great as, or greater than, pyrolysates produced inside the exposure chamber. This observation was based upon a limited number of samples that were tested by both procedures, and thus may or may not be universally applicable; however, no exceptions were seen in those samples we tested. This would suggest that the condensates which were observed with the MSTL Procedure, contributed little or nothing to the overall toxicity of pyrolysates produced.

SUMMARY

Thus, we have briefly examined some of the toxicological aspects of pyrolysis and/or combustion products of man-made materials. While the data presented were derived from laboratory experiments, they serve to illustrate potential hazards for those in the immediate environment of a fire, and a source for environmental toxins. Hazards presented by such toxins are markedly influenced by circumstances of exposure. For instance, the hazard of acute exposure to vapors of burning materials involves the kind of material being burned, quantity being burned, and space occupied (closed room, out-of-doors, etc.). Of course a heavy cloud of smoke and toxic pyrolysis/combustion products can produce discomfort and possibly death to those in its path.

And a final point concerning this topic is its relation to the theme of this meeting "Environmental Toxins." Not only does inadvertent or accidental burning of polymeric materials, such as during a house fire, office fire, etc., produce many gaseous toxins, these are also produced when 'disposable' plastic items are incinerated for disposal. While this practice may not create any significant short term problems, the pyrolysis/combustion products from the tons of plastics burned each day in this country will be entering the atmosphere, thereby adding to the total pollution burden.

HEAVY METALS: ROUTES OF EXPOSURE AND CONSEQUENCES

HENRY A. MOSES, PH.D.

Meharry Medical College
Nashville, Tennessee 37208

Heavy metals may be added to the atmosphere, water and/or soils by way of 1) insecticides; 2) impurities in fertilizer; 3) rain water from atmosphere; 4) industrial runoffs, and 5) automobile exhaust. Some metals, moreover, are naturally present in soils.

It is therefore apparent that heavy metals may be introduced into the environment from a variety of sources. The extent to which a heavy metal may find its way into human tissues depends upon the physical properties of the heavy metal-containing species that are significant in terms of transport, physical interaction with other substances, deposition in various environmental "compartments," physiological activities, and removal mechanisms. Properties that must be considered, therefore, are particle size and distribution, phase

relations, solubility, surface characteristics, washout efficiency, morphology and crystal structure. The chemical composition of the heavy metal-containing materials is also important; the chemical form of the metal determines its solubility in water and biological fluids, the extent to which it is fixed in soils, and its chemical reactions in the atmospheric, aquatic and soil environments.

I shall attempt to characterize the more prevalent environmental heavy metals in terms of their major sources, the chemical form of these sources, the routes of entry of these forms into the human system, and the consequences or toxicologic effects of the heavy metals in man.

Selenium: Although it was reported in 1957 that selenium is essential for mammals, it is also toxic to mammals. The fact that it is toxic is of more economic than physiologic importance to humans. Where high levels of selenium are present in soils, high levels appear in plants (primarily as methylselenocysteine and selenocystathionine) and hence acute selenium toxicity is observed in domestic animals that eat these plants in what has been classically termed "alkali disease" and blind staggers. Toxicity has not been found in human beings, however, despite extensive examinations throughout the population in those parts of the U.S. in which there is a high Se concentration. Indeed, the deleterious effect of selenium on humans, based upon the excretion of Se through the skin and lungs as dimethylselenide, seems more social than medical: Dimethylselenide has the marked and characteristic order of garlic.

Acute toxicity of selenium compounds affects primarily the central nervous system. According to Schwarz, the minimum toxic dose level in growing rats amounts to 59 3-4 ppm of seleniteselenium in the diet. For most selenium compounds, the index between the minimum toxic dosage and the dose which will effectively present selenium deficiency is about 100:1. This index is better than that for many other nutrients, including amino acid and common table salt.

Copper: Exogenous copper toxicity is very uncommon and in the United States is almost always due to the consumption of acidic fluids which have had prolonged contact with copper pipes or vessels. Ingestion of gram quantities of copper as the sulfate seems to be rather frequently used as a method of committing suicide in India. Several examples reported in the literature involve citrus juices, drinks, or carbonated beverages that have been mixed in, or allowed to stand several hours in contact with copper vessels, plumbing, or check valves. Copper poisoning as an industrial hazard, while possible, appears to be negligible.

Mercury—Toxicity of mercury depends upon whether it is in the inorganic state as the oxide or chloride, or in the organic state, most commonly as alkymercury. There are many inorganic forms of mercury that have been used in medicinals for centuries. A very early notation of mercury poisoning was that of the "Mad Hatter." These persons became toxic from mercury after contact with compounds used in making felt. Many studies on the biotransformation of mercury have been undertaken. Interconversions occur between mercury vapor and mecuric ions. Absorbed mercury vapor may also be converted to methyl and ethyl mercury. One study showed that after the injection of mercuric ions into the rat, approximately one-fifth of the excreted mercury was lost via the animal as volatile mercury vapor or as volatile alkylmercury. Inorganic mercury remains in the kidney for extremely long periods of time even in the absence of renal failure.

The symptoms of alkylmercury poisoning primarily involve the central nervous system and include parethesias, poor concentration, apathy, fatigue, difficulty in swallowing, tunnel vision with abnormal blind spots, hearing difficulties, emotional instability, ataxia with other cerebellar signs, gross and uncoordinated tremors, spasticity, paralysis, coma, and eventually death.

Other forms of organic mercury may be ingested in the body and transformed to alkylmercurials which are more toxic. Phenylmercury, for example, is rapidly broken down to inorganic mercury and then transmethylated to methylmercury.

In Japan, manufacturing plants near two cities (Minamata Bay and Niigata) have discharged methylmercury into the water, and the fish and shellfish eventually accumulated the mercury through the marine food chain. The consumption of these fishes as foodstuffs led to deaths and to the births of infants with cogenital cerebral paresis, whose mothers had shown no signs of mercury toxicity. Other examples of industrial accidents or management accidents account for most of the more recent examples of mercury poisoning in humans.

Cadmium. The body burden of cadmium has been estimated to be about 30 mg for the average adult American. Approximately one-third of this is in the kidneys and about one-sixth in the liver. There is very little cadmium in the newborn infant, however, the metal is accumulated throughout life. There are wide geographic differences in the body cadmium of adults and these differences are probably environmental.

Ingested cadmium is probably the major source of accumulated cadmium in man. Typical daily ingestion is from 25-75 μg per day, but certain foods (shellfish and mammalian livers) may contain more than 100 μg per gram. Furthermore, when large amounts of cadmium are present in the soils, as much as 1μg Cd/gram may accumulate in some plants, including certain staple grains. Such concentration occurs in rice grown in some regions of Japan.

Cadmium concentration in water is less the 1μg per liter; however it may be more concentrated in soft water (acidic water). Drinking water of less than 5μg/liter is not likely to contribute significantly to the total daily cadmium intake.

Inhaled cadmium is usually responsible for relatively little of the body burden of cadmium, except in smokers. Absorption of ingested cadmium is usually 3 to 8 percent; however, absorption from the lungs is 25 to 50 percent. The usual cadmium content in the air is 0.001 to 0.05 μg per cubic meter. Since the average amount of air breathed per day is 20 liters, a non-smoker inhales less than 1μg of Cd per day. A cigarette contains about 2μg Cd of which 5 to 10 percent is inhaled.

Hence, a "pack a day smoker" may inhale 2 to 4μg Cd per day or from 0.25 to 0.5 mg Cd per "pack year."

The lethal dose of cadmium is not known; however, 10mg consumed orally can cause serious symptoms. A self-protective effect with regard to oral cadmium is that it is a very effective emetic.

Again, it was in Japan that the effect of long-term low-level exposure to cadmium on man was first noted. A major effect seen was the development of brittle and easily broken bones. Persons exposed also developed loss of smell, respiratory distress and weight loss.

Less spectacular, but perhaps more important is the relationship between cadmium and hypertension mammals, including man. In laboratory rats, hypertension has been produced by administering cadmium both orally and by injection. In man, however, up to this time the relationship is epidemiologic.

In mature male rats, cadmium has been shown to cause damage to the testes. There is some evidence that certain other metals, (Zn, Co, and Se) may at least in part prevent some of the toxic effects of cadmium.

Lead. Lead is an element ubiquitous in nature and detactable in tissues of man and animals even in environments remote from man-made sources. Although lead has been found throughout the animal kingdom, there is no evidence suggesting lead as an essential trace metal. To date only undesirable effects have been recognized.

The accumulation of lead by vegetation, especially vegetables and forage crops consumed by man and domestic animals, should and must be of great concern. The greatest accumulations are near smelters or in areas where crops are grown on soils near old mines. Storm runoff is the major transport mechanism in the movement of lead.

The combustion of leaded gasoline is currently the largest source of lead that is emitted into the environment (atmosphere). A large fraction of this lead quickly settles out of the air, contaminating soil and dust along streets and highways. Airborne lead is inhaled and may be absorbed into the body via the lungs. Lead-contaminated diets and urban-grown vegetables represent a potential source of ingestible lead.

Human lead exposure results from a combination of sources, including lead in the air, food, water, leaded paint and street dust. It is difficult to determine which sources are the most significant from a health standpoint. Although ingestion of lead-based paint chips is generally regarded as the major cause of overt lead poisoning in children, sources of lead other than paint may play an important role in childhood lead exposure, especially at subclinical levels of lead toxicity.

In normal adults, the average daily oral lead intake is approximately 300 μg, of which 90 percent is promptly excreted. Potential respiratory intake appears to contribute only a small amount, even in congested urban areas, and amounts to only 5 to 50 μg daily in adults. In clinical terms, adults are considered to have an excessive body burden of lead when the daily intake from all sources exceeds the 500 to 600 μg level. For children a comparable figure is 300 μg for toddlers and even less for infants; ingestion of only a few chips of a lead-based paint by a child with *pica* supplies 100 to 200 mg.

While lead-based house paint remains the principal environmental cause of symptomatic poisoning in children, there are other sources. Old putty used in houses of the same era may also contain lead. In adults, lead poisoning may occur from the ingestion of contaminated beverages or food stored in earthenware pottery where the contamination occurs when an acidic material dissolves the lead used in the pottery glazing. In many parts of the United States, the major cause of lead toxicity is home-made whiskey (moonshine) in which the material is contaminated by the lead in the stills, many of which have been made from discarded automobile radiators.

The average individual, child or adult, who has no unusual exposure to lead will have a blood lead level of 15 to 20 μg/100 ml; the upper limit of normal is considered to be 40 μg/100 ml. It must be noted that, in children, some adverse effects may be first detectable only at this upper limit of normal range. Generally speaking, acute lead toxicity is associated with blood level concentrations above 80 μg/100 ml, although there is comparable individual variation.

The major toxicities of lead are a reversible hematologic abnormality and a generally irreversible central nervous system abnormality. In addition there are abnormalities of renal tubular function which may or may not be reversible. Lead acts at three steps of hemoglobin synthesis, the first being the condensation of succinate from the Krebs cycle and glycine to form δ-amino levulinic acid (DALA); lead also interferes with the conversion of DALA to porphobilinogen and the conversion of protoporphyrin and iron to heme. This results in the accumulation of intermediary metabolites, such as DALA, which is excreted in large amounts in the urine. In addition, the presence of coproporphyrin in the urine almost always antedates the onset of symptomatic lead poisoning. Examination of the blood cells in a patient with lead poisoning shows a shortened life span of red cells, a increased proportion of immature red cells in the circulation, and basophilically stippled cells, the hallmark of clinic lead poisoning. This stippling represents the remnants of mitochondria and other cytoplasmic constituents of the red cell precursor.

Lead encephalopathy may be particularly difficult to assess in children. Children with lead poisoning may show only restlessness, short attention span, impulsiveness or withdrawal. These changes may be difficult to assess in a population of low socio-economic status in which there is the greatest chance of exposure.

The renal manifestations of severe lead poisoning relate presumably to the impairment of cellular metabolism. Hyperaminoaciduria resulting from proximal renal tubular injury is a typical manifestation. In the presence of acute lead encephalopathy and blood lead concentrations of greater than 150 μg/100 ml, the chances are very high for the development of a complete Fanconi's syndrome consisting of hyperaminoaciduria, glycosuria and hyperphosphaturia, which may result in hypophosphatemia. Since lead is a bone-seeking mineral and, as a result, can accumulate in large quantities as an inactive form of bone, the hypophosphatemia can result in mobilization of lead as well as phosphate from the bone, thus compounding the problem of lead toxicity.

In this light, it has been anecdotally reported that children in ghetto areas tend to have a marked increase in symptomatic lead poisoning in the spring when they are exposed to increased sunlight and therefore increased vitamin D levels. Vitamin D has the effect of releasing from bone stores accumulated through the winter, when exposure to sunlight is limited and dietary vitamin D intake marginal.

The most effective therapy for lead toxicity is the removal of the source in a preclinical stage. This is especially important in children. Chelating agents may be used on the basis of metabolic evidence of toxicity. Brief courses of chelating agents such as BAL or D-penicillamine are useful in reducing soft tissue lead concentrations quickly and safely, albeit only temporarily. If the source of exposure in children is removed, several years usually pass before the serum lead concentration decreases to the normal range. In the final analysis, however, this is the only effective therapy.

TRIHALOMETHANES IN DRINKING WATER

R. A. MINEAR, J. C. BIRD, D. E. JOHNSON

The University of Tennessee, Knoxville, TN 37916

INTRODUCTION

The perceived problem of haloforms or trihalomethanes in finished drinking waters being supplied to municipal consumers is a relatively recent one, dating back no more than 7 or 8 years. Concern about the relationship between human health effects and trihalomethanes arose from reports of chloroform and other organohalogen compounds being present in New Orleans drinking water[1,2] and observation of these compounds in blood plasma of New Orleans residents.[2] Study of cancer records was reported to relate cancer in white males with consumption of New Orleans drinking water. Though challenged on the basis of statistical methods employed[4] the publicity generated from the New Orelans study and other work in progress on chemical composition of drinking waters and water sources lead to the evolution of national concern over the safety of public drinking water supplies culminating in the passage of the Safe Drinking Water Act (PL 93-523). A section (1442) of the act charged EPA with the conduct of a comprehensive study of public water supplies and drinking water sources for the purpose of assessing the nature and extent of contamination of potentially carcinogenic substances. The results of this study were reported as the National Organic Reconnaissance Survey for Halogenated Organics[5] (NORS or 80 cities study) and demonstrated that 4 trihalomethane species were widespread in USA drinking waters. Generally chloroform was the major constituent of this group. The linkage between chloroform and cancer in mice and rats by the National Cancer Institute[6] obviously spurred efforts to establish regulation of the organic content of the nation's drinking waters and currently this effort resides in the interim primary drinking water regulations.[7] The focus of these regulations is dual, pertaining both to man made compounds representing contaminants in the raw water supply and those compounds, including the trihalomethanes, which result from the conversion of the raw water source to finished drinking water.

CHEMISTRY OF TRIHALOMETHANE FORMATION

That the halomethanes result from the practice of disinfecting drinking water with chlorine has been conclusively demonstrated from the early work of Rook[8] and Bellar *et al.*[9], subsequently confirmed by numerous, other published works [10-13] and the general subject of water chlorination has been the subject of two symposia with subsequent publication.

Generally, the four possible species resulting from chlorine and bromine substitution on methane (chloroform, $CHCl_3$; bromodichloromethane, $CHCl_2Br$; chlorodibromomethane, $CHClBr_2$; bromoform $CHBr_3$) are found decreasing in concentration as the number of bromine atoms increases. However, iodine containing species have been reported.[13]

Examination of most any fundamental textbook on organic chemistry will disclose the oxidation reaction of methyl ketones with hypohalous acid to form the carboxylic acid and a trihalogenated methane. This reaction, referred to as the haloform reaction, is base catalyzed and stepwise in nature, such that a mixture of hypohalous species would lead to mixed halogen products. The base catalysis effect is consistent with observation that high pH waters (naturally or as a result of the lime softening process) yield higher concentrations of trihalomethanes.[11,16,18] Our own work with aqueous solutions of humic acid is in agreement with other work on such model systems regarding the pH effect.

Pure humic acid systems in distilled-deionized water when treated with hypochlorite solution yield only the single trihalomethane, chloroform, as expected. Addition of bromide ion yields the mixed reaction products and substantiates the participation of hypobromite ion resulting from the oxidation of bromide by aqueous chlorine. The relative abundance of the species depends upon the initial concentration of bromide in the reactor.

The choice of humic acids as aqueous organic constituents for study of the chlorination reaction products is not accidental because of the similarity of humic compounds and the organic constituents of natural waters.[19,20] While the exact chemical composition of soil and water humic substances is not known specifically, the polyhydroxybenzenoid carboxylic nature of these complex 3 dimensional polymeric substances has been established in general if not explicitly by soil and water chemists. Given the assumption that the

humic substances are in fact the precursors of trihalomethanes in drinking waters and that the proposed structures are accurate, then the haloform reaction does not adequately explain the observed behavior. Morris[21] has suggested this and proposed several basic molecular structures, likely to be found in natural aqueous organics. These include alpha keto esters, B-diketones (chain and ring), meta dihydroxybenzoid configurations and pyrrole ring structures. Rook[22] has quantitatively studied the rate and reaction efficiency of model compounds testing the behavior of the meta dihydroxybenzoid structures and found both rapid conversion of certain configurations and high percentage yields of chloroform. This work led to a proposed mechanism of fulvic acid (a humic subfraction) reaction with chlorine in the formation of chloroform and other chlorinated species and one that accounted for enhanced reaction at higher pH.

Other factors remain to be sorted out as some of our work and unpublished results of some colleagues have indicated shifts in the distribution of bromine species with changes in ionic strength and non-reactive ion composition at constant ionic strength. Field observations have demonstrated occasional but not exactly rare predominance by the bromine species which is not initially accounted for by high bromide levels in the raw water.

Trihalomethane Analysis

The interim drinking water regulations if passed into final form as they stand will set a standard of total trihalomethanes (TTHM) in drinking water supplies (reaching the consumer) at 100 μg/1. Analysis for these compounds in drinking water will be routine for water treatment plant laboratories and/or commercial laboratories. The analytical procedures available and to be used will take on an importance not previously assigned and are thus worthy of brief consideration.

Basically, trihalomethane analysis is done by gas chromatographic techniques. However, there are essentially 3 different approaches, two of which are in more common usage. These are (1) direct aqueous injection which likely requires venting of the water vapor (essential for electron capture detection), (2) solvent extraction, and (3) head space analysis. Pfaender *et al.* [23] have presented a detailed discussion of this procedure which basically causes problems of on column conversion to trihalomethanes and can yield high results.

Direct solvent extraction under zero head space conditions has been developed by Henderson *et al.*[24] using pentane and different solvents by others.[25,26] Of concern here is variation in extraction efficiency depending on the solvent, the solvent-water ratio, and the individual trihalomethanes (Trussell *et al.*[25]) necessitating use of correction factors in calculations or aqueous standards carried through the process. The primary advanage is simplicity, and use of an electron capture detector without temperature programming affords low concentration detectabilities. Electron capture detectors are not without problems, not the least of which are low tolerance for gas leaks and contamination from solvents and plastic surfaces.

The most widely publicized procedure is the head space or purge and trap procedure of Bellar and Lichtenberg[28] in which gaseous sparging of the sample strips volatile organics into a cold, porous polymer adsorbant column. The column is then heated in a carrier gas stream onto the cold gas chromatograph column followed by temperature programming. The stripping step serves as a 1000 fold or more concentration step and allows detection by flame ionization detection or halogen specific detectors such as the electrolytic or coulometric type detectors in the halogen mode. Decrease in interferences is a distinct advantage but memory effects have been a problem to some analysts.

Trihalomethane Levels In Drinking Waters

Results from the NORS Report[5] typified the mean drinking water concentration of trihalomethanes to be 21 μg/1 $CHCl_3$, 6 μg/1 $CHCl_2Br$, 1.2 μg/1 $CHClBr_2$ and $CHBr_3$ below analytical detection limits. The ranges were 0.1-311, 0-116, 0-100 and 0-92, respectively. Subsequent studies by EPA and others have provided data showing even higher values for TTHM and unusual distributions among the bromo species.[29] A strong association was found between the total trihalomethane concentration and raw water nonvolatile total organic carbon.

Of these 80 cities only two, Chattanooga and Memphis, were from Tennessee. Furthermore, that particular data base did not characterize seasonal variation. For this reason, a survey of the State of Tennessee drinking waters was undertaken which included examination of seasonal variation through quarterly sampling of 27 cities representing 33 separate water supply facilities. A more detailed sampling program was undertaken for the city of Knoxville. Two types of samples were taken simultaneously at each site, one representing the finished product water (chlorine quenched on site) and one representing water which reaches the consumer after prolonged residence in the distribution system (unquenched, analyzed after seven days).

For total trihalomethanes, range, mean and median values on unquenched samples were 0-83, 25, 23 for winter (Feb.-April, 1978), 6-123, 54, 48 for spring (May-June, 1978), and 2-265, 99, 93 for summer (Aug.-Sept., 1978). These values clearly illustrate a seasonal variation attributable either directly or indirectly to temperature (i.e. direct effect on reaction rate or extent of reaction or indirect effect due to increased organic levels in the raw waters). In the spring sampling, 5 cities exceeded the proposed 100 ppb criterion and in the summer, 15 cities exceeded this value. However ,the fall sampling value is necessary to assess the "annual average value." Unquenched samples for spring and summer showed much lower changes, 0-102, 30, 29 and 1-118, 40, 27, respectively, thus indicating the importance of distribution system residence time.

Parallel sampling of 21 cities during the summer interval for NVTOC indicated an association between water organic content and TTHM (unquenched) which has been noted by others. Using the 21 data pairs the regression equation obtained was TTHM (μg/1) = 40.5 NVTOC (mg/) − 78.5, which had an $r^2 = 0.515$. However, observation of the data indicated 4 outlayers and deletion of these values vastly improved the r^2 value to 0.831 and modified the equation to TTHM (μg/1) = 47.6 NVTOC (mg/1) − 105.6.

Similar to other studies and the NORS report, bromine containing species were lower in concentration and generally in proportion to the chloroform, the monobromo species in greater abundance than the dibromo species and bromoform concentration was generally very low or below detection limits. For unquenched samples, the monobromo data (range, mean, median) were 0-9, 4, 4; 0-32, 9, 7; 2-76, 13, 9 for winter, spring, and summer respectively. Corresponding figures for the dibromo compound were as follows: 0-3, .3, .1; 0-11, 2, 1; 5-41, 6, 3. Again the seasonal effect is clearly demonstrated.

There were individual water supplies, usually those that did not have high overall TTHM levels, which consistently had a dominance by the dibromo compound. The exact relationship between water composition, bromide level and the resulting distribution of the trihalomethane species is not yet clear.

The seasonal variation represented by the quarterly sampling program is strongly reinforced by the more intensive sampling from the Knoxville water supply. One to two samples per week were analyzed over a 7 month period, and clear seasonal relationship is indicated. By plotting of the trihalomethane concentrations as a function time and superimposing raw water temperature values over the same time interval one can observe a close tracking of the values. However, a lag is apparent in examination of the data. Allgeier *et al.*[30] on the other hand have reported a linear relationship (TTHM = −28.5 + 1.64 × TEMP) between mean monthly TTHM and temperature values with an $r^2 = 0.86$ for Louisville, Kentucky.

Attempts to relate the TTHM data to other raw water characteristics or water treatment variables did not generally show highly significant correlations for both Spearman and Pearson analyses. The higher values were obtained for raw water turbidity, prechlorination level and residual chlorine level but were not generally of large magnitude.

Summary

In brief summary, the problem of trace organics, especially those generated in the course of treating drinking water for microbiological suitability by chlorination, has only recently received intense scrutiny and regulations are still in the formative state. More data and understanding are evolving daily and should aid in substantiating or dispelling the need for concern. The drinking waters of the State of Tennessee have been partly characterized and work is continuing. State data seem to reflect those of the nation and indicated that areas of the State are not without potential problems, final definition of which depend on final regulations. Clearly, the "problem" of trihalomethanes is variable intensity depending upon the season of the year.

Acknowledgement

Portions of the data represented in this paper resulted from the financial support provided through The University of Tennessee Water Resources Research Center under an OWRT allotment grant #A-051-Tenn. and a research grant from the Church and Dwight, Co., Inc. of Piscataway, New Jersey.

Literature Cited

R. H. Harris and E. M. Breecher, "Is the Water Safe to Drink? Part I, The Problem, Part II, How to Make it Safer, Part III, What You Can Do," Consumer Reports, *39*, 436-443, 533-542, 618-627 (1974).

B. Dowty, D. Carlisle, J. L. Laseter, and J. Storer, "Halogenated Hydro-Carbons in New Orleans Drinking Water and Blood Plasms," Science, *187*, *75* (1975).

T. Page and R. F. Harris, Implication of Cancer Causing Substances in Mississippi River Water. Environmental Defense Fund Report, Washington, D.C. (November 6, 1974).

T. A. DeRowen and T. E. Diem. American Journal Public Health, 65:10, 1060-1062 (1975).

James M. Symons, Thomas A. Bellar, J. Keith Carswell, Jack DeMarco, Kenneth L. Kropp, Gordon G. Robeck, Dennis R. Seeger, Clois J. Slocum, Bradford L. Smith, and Alan A. Stevens, "National Organics Reconnaissance Survey for Halogenated Organics," Journal American Water Works Association, 67, 634 (1975).

National Cancer Institute, "Report on the Carcinogenesis Bioassy of Chloroform," Carcinogen Bioassy and Program Resources Branch, Bethesda, Maryland (1976).

National Interim Primary Drinking Water Regulations, Federal Register, Vol. 43, No. 28, Thursday, February 9, 1978.

J. J. Rook, "Formation of Haloforms During Chlorination of Natural Waters," Journal Water Treat Exam, *23* (Part 2); 234 (1974).

T. A. Bellar, J. J. Lichtenberg, and R. C. Kroner, "The Occurrence of Organohalides in Chlorinated Drinking Waters," Journal American Water Works Association, *66*, 703 (1974).

Johannes J. Rook, "Haloforms in Drinking Water," Journal American Water Works Association, *68*, 168 (1976).

Alan A. Sevens, Clois J. Slocum, Dennis R. Seeger, and Gordon G. Robeck, "Chlorination of Organics in Drinking Water," Journal American Water Works Association, *68*, 615 (1976).

Robert L. Morris and Lauren G. Johnson, "Agricultural Runoff as a Source of Halomethanes in Drinking Water," Journal American Water Works Association, *68*, 492 (1976).

W. E. Coleman, R. D. Lingg, R. G. Melton, and F. C. Kopfler, "The Occurrence of Volatile Organics in Five Drinking Water Supplies Using Gas Chromatography/Mass Spectrometer (gc/ms)" in *Identification and Analysis of Organic Pollutants in Water*, L. H. Keith, Ed., Ann Arbor Science, 1976.

R. L. Jolley, *WATER CHLORINATION: Environmental Impact and Health Effects*, Vol. 1, Ann Arbor Science, 1978.

R. L. Jolley, *WATER CHLORINATION: Environmental Impact and Health Effects*, Vol. 2, Ann Arbor Science, 1978.

O. T. Love, Jr., J. K. Carswell, R. J. Miltner and J. M. Symons, "Treatment for the Prevention or Removal of Trihalomethanes in Drinking Water," Appendix 3 to *Interim Treatment Guide for the Control of Chloroform and Other Trihalomethanes*, U.S. EPA, Cincinnati.

A. A. Stevens and J. M. Symons, "Measurement of Trihalomethane and Precursor Conceneration Changes Occurring During Water Treatment and Distribution," Appendix 4 to *Interim Treatment Guide for the Control of Chloroform and Other Trihalomethanes*. U.S. EPA, Cincinnati.

A. A. Stevens and J. M. Symons, "Measurement of Trihalomethane and Precursor Concentration Changes Occurring Dur-Water Works Association, *69*, 546 (1977).

R. F. Christman and M. Ghassemi, "Chemical Nature of Organic Color in Water," Journal American Water Works Association, *58* (1966).

M. Ghassemi and R. F. Christman, "Properties of the Yellow Organic Acids of Natural Waters," Limnology and Oceanography, *13*, 583 (1968).

J. Carrell Morris, "The Chemistry of Aqueous Chlorine in Relation to Water Chlorination," in *WATER CHLORINATION: Environmental Impact and Health Effects*, Vol. 1, R. L. Jolley, Ed., Ann Arbor Science, 1978, pages 21-32.

J. J. Rook, "Chlorination Reactions of Fulvic Acids in Natural Waters," Environmental Science and Technology, *11*, 478 (1977).

F. K. Pfaender, R. B. Jonas, A. A. Stevens, L. Moore, and J. R. Hass, "Evaluation of Direct Aqueous Injection Method for Analysis of Chloroform in Drinking Water," Environmental Science and Technology, *12*, 438 (1978).

J. E. Henderson, G. R. Peyton and W. H. Glaze, "A Convenient Liquid-Liquid Extraction Method for the Determination of Halomethanes in Water at the Parts-Per-Billion Level," Chapter 7, pp. 105-111 in *Identification and Analysis of Organic Pollutants in Water*, L. H. Keith, Ed., Ann Arbor Science, 1976.

J. J. Richard and G. A. Junk, "Liquid Extraction for the Rapid Determination of Trihalomethanes in Water," Journal American Water Works Association, *69*, 62 (1977).

J. P. Mieure, "A Rapid and Sensitive Method for Determining Volatile Organohalides in Water," Journal American Water Works Association, *69*, 60 (1977).

A. R. Trussell, M. D. Umphres, L.Y.C. Leong and R. R. Trussell, "Optimizing Trihalomethane Analysis," in *WATER CHLORINATION: Environmental Impact and Health Effects*, R L. Jolley, Ed., Ann Arbor Science, 1978, pp. 543-559.

T. A. Bellar and J. J. Lichtenberg, "Determining Volatile Organics at Microgram - per - Liter Levels by Gas Chromatography," Journal American Water Works Association 66: 12, 739-744 (1974).

J. F. Symons, U.S. EPA, Cincinnati, Ohio (personal communication).

G. D. Allgeier, R. L. Mullins, Jr., D. A. Wilding, J. S. Zogorski, and S. A. Hubbs, "Trihalomethane Levels at Selected Water Utilities in Kentucky, U.S.A.," a paper presented at the Annual Kentucky-Tennessee Section American Water Works Association Meeting, Memphis, TN, November 14, 1978.

BIOCHEMICAL DETOXIFICATION MECHANISMS IN HERBIVORES

LENA B. BLATTSTEN

University of Tennessee
Knoxville, Tennessee

Even though plants have developed a vast array of chemical and other defenses against being eaten, it is probably safe to say that there is not a single plant species that doesn't serve as food for at least one species of herbivore. Most herbivores are insects. At least 10,000 species of insects out of the close to a million identified species are serious pests, that is direct competitors with people for food and in other ways threatening.

Insect herbivores have developed an arsenal of ingenious mechanisms for dealing with toxicants in plants that would be lethal to vertebrate herbivores. Even cacti have insect enemies: There is a complex of seven species of desert fruitflies and one particular cactus, the senita cactus (*Lophocereus schotti*) in the Sonoran desert which contains a steroid and an alkaloid. The alkaloid is toxic and therefore the cactus is toxic to six of the fruitfly species. The seventh species depends upon the steroid to make their molting hormone and has developed a way to avoid poisoning by the alkaloid. In this case the detoxification mechanism is not precisely known; fruitflies are very small and hard to work with.

But the case of nicotine non-poisoning is well known. As all tobacco growers know, tobacco is one of the most insecticide-dependent crops grown in the U.S. Every insect under the sun seems to relish the tobacco plants even though they contain high concentrations of the very toxic alkaloid nicotine. Despite the fact that nicotine is used as an insecticide, it is really much more toxic to vertebrates than to insects. Insects have at least four different methods for avoiding nicotine poisoning. First there is an aphid, the green peach aphid (*Myzus persicae*) which has developed a behavioral defense. Nicotine is synthesized in the roots of the plant and translocated to the leaves in the xylem, and the aphid feeds selectively on the nicotine-free phloem sap which transports nutrients downwards. Second, there is the tobacco hornworm (*Manduca sexta*) which has an exceedingly high rate of excretion. The ingested food, leaf material, passes so rapidly through the gut that a damaging dose cannot accumulate. This is augmented by a specialized active transport system in the malpighian tubules. Nicotine is a nerve poison. It binds to acetylcholine receptors in the insect central nervous system. That is, if it can reach that target. The insect CNS is protected by an ion-impermeable sheath, and at physiological pH values in most insects nicotine is up to 90% ionized. A third physiological defense mechanism against nicotine poisoning is thus operating. The neural sheath in the nicotine resistant tobacco hornworm is very effective whereas that in the relatively nicotine susceptible silkwork (*Bombyx mori*) was found to be fairly leaky to ions.

The most important defense against nicotine though, in both insects and vertebrates, is a biochemical mechanism, namely rapid metabolic degradation to nontoxic metabolites. Nicotine can undergo several degradation reactions all of which are catalyzed by certain enzymes, the so-called microsomal mixed-function oxidases.

There is in fact a veritable battery of enzymes ready to work on any chemical that enters the interior environment of organisms. The metabolism of foreign compounds, that is chemicals without nutritional value and sometimes with detrimental biological activities such as insecticides, drugs, plant allelochemicals, etc., is often described in two stages, primary and secondary metabolism. The major effect of both stages is to make foreign compounds more easily excretable for organisms with water-based excretory systems. Of all the enzymes involved in the primary metabolism, the mixed-function oxidases (MFOs) are the most important. This is due to the three major characteristics of these enzymes; first, they are capable of catalyzing a large variety of different reactions; second, as a result of this capacity they can attack a large variety of molecules as long as they have a certain degree of lipophilicity; and thirdly, the MFOs are very sensitive to environmental chemicals which either inhibit them or induce them to higher specific activity.

Not all MFO-catalyzed reactions are detoxifications. In fact, several kinds of compounds undergo bioactivation to toxic metabolites by the MFOs. This is the case with the organothiophosphonate insecticides, the polycyclic aromatic hydrocarbon procarcinogens, the fungal aflatoxins and with the hepatotoxic pyrrolizidine alkaloids.

The MFOs are found firmly attached to the smooth endoplasmic reticulum membranes of cells. They consist of a terminal oxidase which is a cytochrome, and a flavoprotein reductase which specifically utilizes NADPH. The terminal oxidase is called cytochrome P-450 and interacts with the substrate molecule. Through a complex series of events the cytochrome-substrate complex interacts with first one electron, then with molecular oxygen which by interaction with the second electron is activated to a superoxide radical. The result of these interactions is a hydroxylated substrate molecule and a molecule of water. The cytochrome will in its reduced state bind carbon monoxide and the complex shows an absorption maximum at 450 nanometer. This is the reason for the name and is also the standard method for measuring cytochrome P-450 concentrations. We also measure the activity of the MFO system by using three model substrates, p-chloro N-methylaniline, aldrin, and aniline. We have convenient, sensitive and reliable methods for measuring the oxidations of these compounds. Our standard experimental animal and source of MFO enzymes is the larva of the southern armyworm moth (*Spodoptera eridania*). To get an active enzyme we use the midgut tissue and make a microsomal preparation by centrifugal fractionation. The highest specific activity is in the microsomes which are vesticulated fragments of the smooth endoplasmic reticulum. And the gut has by far the highest activity not only in armyworms but in most lepidopterous larva. This seems to be a logical place for a biochemical defense system. This high MFO activity in the gut explains the effective metabolic degradation of nicotine ingested along with tobacco leaves. Armyworms have no difficulty in devouring tobacco plants at all.

There is thus a highly active and very diverse enzyme system, the MFOs, present in the guts of herbivores. It is well established that the MFOs degrade naturally occurring potentially toxic plant allelochemicals such as nicotine. Other examples are rotenone, pyrethrin, the opium alkaloids, the pyrrolizidine alkaloids, and the aflatoxins.

This raises the possibility that the MFOs have evolved as an adaptation to plant chemical defenses. The crucial property of the MFOs would be their ability to adapt rapidly enough to the presence of potential toxicants to provide survival value for the herbivore. I mentioned before that the MFOs are extremely sensitive to the presence of chemical inducers or inhibitors in the environment, that can enter the organism by ingestion, absorption through skin, or inhalation and subsequent absorption. We have shown that numerous plant allelochemicals are indeed MFO inducers. More detailed studies with α-pinene, trans-2-hexenal, and sinigrin revealed that the armyworm gut MFO enzymes are induced to higher activity by the presence in food material of very low concentrations of these compounds. The increase in MFO activity proceeds very rapidly in the armyworm. Already after half an hour is it possible to measure significantly higher activities when the larvae ingest food containing either α-pinene or trans-2-hexenal. Prolonged feeding, however, does not cause an unlimited increase in activity. Rather, it appears that there is an adjustment of the activity level that perhaps marks an equilibrium condition with the chemical load of the diet. In another set of experiments done with pentamethylbenzene, a synthetic chemical, we showed that when the inducer is removed from the diet the activity quickly reverts to the control level. This indicates a tendency of the MFO system to adjust quickly to any dietary level of chemical inducer. This is to some extent dependent on the nature of the chemical compound. It has been shown that with metabolically very stable compounds such as for instance DDT which are only very slowly degraded, the elevated level of activity persists longer after the inducer has been withdrawn from the diet.

We also showed that armyworms are indeed better able to withstand poisoning after their MFO enzymes have been induced. We showed that induction by α-pinene results in an increased tolerance to nicotine. But this is not dependent on the chemicals involved in any particular case. We also showed that after induction by dietary exposure to pentamethylbenzene, armyworms had acquired a dramatically increased tolerance to the synthetic insecticide carbaryl.

I mentioned before that the MFO enzymes are also sensitive to inhibition by chemical components in the ingested food. The pyrrolizidine alkaloids as exemplified by monocrotaline appear to be MFO inhibitors. I did an experiment with three species of insect larva and three species of plants. I had larvae of the black swallowtail (*Papilio polyxenes*) and of the bella moth (*Utetheisa bella*) and the armyworm. The black swallowtail larvae are specialist feeders on umbelliferous plants such as the carrot. Umbellifers contain a number of different coumarins in relatively high concentrations. The bella moth larvae are specialist feeders on plants that contain pyrrolizidine alkaloids and are associated with the highly poisonous showy crotalaria (*Crotalaria spectabilis*). The armyworm, on the other hand, is a very broad-range generalist feeder. About 50 different plane species have been identified as an acceptable food source for armyworm larvae. I found that when armyworm larvae are feeding on the carrot they acquire extremely high MFO-activity levels, similar to those normally found in the black swallowtail larvae. On the other hand, when armyworms are raised on seeds of the showy crotalaria, their MFO-activity levels drop drastically to approach the very low levels normally found in the larvae of the bella moth. This not only shows the flexibility of the armyworm MFO-system, but also proves that you are what you eat.

In conclusion, I would like to suggest that these enzymes, the microsomal mixed-function oxidases, play a major role in the feeding strategies of herbivores as we see their specializations, preferences and avoidances today, although this was not necessarily the ancestral role of these enzymes.

ECOTOXICOLOGY AND THE TOXIC SUBSTANCES CONTROL ACT

WALTER G. ROSEN

U.S. Environmental Protection Agency
Washington, D.C.

THE BIRTH OF TOXIC SUBSTANCES CONTROL ACT

Mid-October 1978 marked the second anniversary of Public Law 94-469, the Toxic Substances Control Act (TSCA). TSCA is intended to be a culminating chapter in environmental legislation. It covers all chemicals not regulated by other laws. It addresses both domestic and imported chemicals, and new chemicals as well as those already in commerce. It emphasizes scientific assessment, rather than legalistic approaches to regulation. And TSCA makes clear that human health is not the only regulatory objective. The environment, *per se*, is to be protected against "unreasonable risk."

Implementation of TSCA by EPA's Office of Toxic Substances (OTS) is agreed by all who are knowledgeable about it to be a complex and massive task. Massive, indeed, are the difficulties inherent in creating a new office which must review existing chemicals, of which there are an estimated 70,000, and new chemicals, which are expected to be reported at a rate of perhaps 1000 per year—and a growing number of which are known or suspected toxins. What follows is a summary of efforts to date to develop the testing rules and evaluative procedures which, we hope, will enable us to predict when a chemical might be expected to cause unreasonable risk to the environment.

Under TSCA, the collection of data on chemicals is the responsibility of the manufacturer. Chemicals already in commerce are to be tested only when there is reason to suspect that they may be toxic, at which time the EPA will stipulate the testing to be performed. For all new chemicals, the manufacturer must submit evidence of his own choosing to demonstrate that a chemical should be permitted to enter commerce. The EPA cannot stipulate the nature of the tests to be performed but plans to offer "Guidelines" which will indicate the kind of information it deems important for evaluation. These draft guidelines form the basis for the rest of this discussion. OTS plans to publish them in the Federal Register for public comment early in 1979. The selection of tests has been guided by these criteria: applicability to a wide range of chemicals and organisms; standard, accepted, state-of-the-art methodology; economy in terms of personnel, time and facilities; sensitivity in terms of chemical concentration and organism response; and usefulness in the assessment of environmental risk.

CHEMICAL FATE

If we want to know the effects of a chemical on the environment we first must know where it can be expected to travel in the environment, and whether it will persist. Movement and persistence are the critical components of "Chemical Fate," which should be predictable on the basis of certain properties of chemicals. The fate of a chemical, combined with information on its anticipated production, use, distribution, disposal and related factors, should permit us to predict which environments it might enter, and the levels to which it might accumulate. This information, in turn, should permit the selection of bioassays to establish the chemical's potential for causing adverse ecological effects in the environmental compartments in which it might persist. The properties of a chemical which we consider most important for predicting its movement in the environment are: water solubility; octanol/water partition coefficient; vapor pressure; adsorption isotherm; boiling/melting/sublimation point; density; dissociation constant; particle size: pH.

In order for a chemical to have an ecological effect in a particular environmental compartment it must not only possess the physical and chemical properties which will cause it to be transported to that compartment or medium, but it must also be able to resist those degradative forces which would prevent its persistence in that compartment at levels which could cause significant impairment of biological processes. The pathways of degradation which we recognize as critical in determining the persistence of chemicals in the environment are: chemical degradation (hydrolysis: oxidation/reduction); photochemical degradation; and microbial degradation. Standardized and widely accepted procedures for making these determinations are not always at hand. The rate of photochemical degradation, for example, will vary over time, and vary both between and within environmental compartments. Can a single, cost effective standardized test be developed to measure this process?

Microbial degradative pathways are numerous and vary from place to place according to the micro-environment. We cannot reasonably hope to test all of the possible conditions under which a chemical might be biodegraded in nature, and we therefore will propose a few simple screening tests in the hope that they will reveal whether the test compound is rapidly biodegraded or relatively persistent. If the chemical does not fit either category it may have to be tested by more sophisticated procedures. Once again, however, it must be stressed that even as we recommend a particular test or group of tests, we seek improved alternatives. Assuming that we will be able to predict where a chemical is likely to appear in the environment, how do we measure its potential effects on ecosystems?

TESTING FOR ECOLOGICAL EFFECTS

We have begun with some assumptions, the most important of which is that we can identify plant and animal species which are representative of ecologically significant species and/or functions in the natural environment. We further assume that by observing the effects of chemicals on critical functions or activities of these organisms we will be able to extrapolate to the likely impact of the chemical on those organisms or processes, in the natural or man-made environment, for which the test organism is a surrogate.

The development of testing procedures requires, first of all, the identification of the environmental compartments, and those major life forms therein, which must be of concern in evaluating the potential ecological effects of chemicals. Our range of concern must potentially include all major life forms in all major environmental compartments.

In selecting test organisms, the choices have been guided by several considerations. These criteria represent goals rather than standards, and we cannot expect to meet each, in its entirety, for every test organism selected. Having identified test organisms, what effects does one test for? What biological processes does one monitor? The following have been selected:

1. toxicity resulting in lethality
2. impairment of reproduction
3. impairment of growth and development
4. interference with critical aspects of animal behavior (*e.g.* avoidance, mating, feeding, nest-building).

Procedures for evaluation of test results are being developed.

TEST DEVELOPMENT: TIERS AND TRIGGERS

The amount of ecological testing that will be required for the assessment of a chemical will depend on its predicted fate in the environment. The entire range of tests should therefore rarely be required. To make testing requirements maximally cost-effective, we are trying to organize tests, wherever possible, into tiers, with clearly defined "triggers" to indicate what degree of effect will indicate the need for further testing, if any, or for regulation. In this way, if extensive testing is required, the need for it should be revealed in a progression from simple, rapid, broad spectrum tests to more advanced, specific tests.

TESTING STRATEGIES: LEVELS OF BIOLOGICAL ORGANIZATION

The foregoing approach to testing can be termed for convenience the single species approach. It is predicted on the majority of previously-developed tests and the assumption that plants, animals and microbes can be selected, on the basis of their known critical roles in food chains or in other aspects of ecosystem function, to serve as surrogates in testing for large functional and/or taxonomic groups in the "real world" environment.

But test procedures or culture methods for representatives of important taxonomic groups are often lacking. Thus, we have no procedures for testing the various plant taxa between unicellular algae and angiosperms, and some groups which are included in the draft test battery are not adequately represented. For example, while we include angiosperms, we have tests for only a fraction of the life cycle, namely, germination and early seedling growth. Other gaps in the testing scheme will be evident: fungi, gymnosperms and soil invertebrates, for example.

How many surrogates are needed to represent the biosphere adequately? Even the most cursory survey of the literature suggests a discouraging answer. Sensitivity to toxic chemicals can vary widely between species which are taxonomically close, and significant variation is often found between different ecotypes within the same species. Because of inter- and intraspecific variability in sensitivity to chemical toxicity, as well as for other reasons discussed below, other testing approaches are being considered as supplements to single species testing.

IN VITRO TESTING

Although some chemicals might be expected to inhibit biological processes by purely physical mechanisms, it seems reasonable to conclude that most inhibitions result from interference with specific biochemical reactions. Interspecific and intraspecific differences in sensitivity to chemical inhibition may result from the presence or absence of detoxification mechanisms, or from differences in accessibility of sensitive sites to the toxicants.

Procedures which deprive sensitive sites of protection might therefore be expected to result in tests which are more rapid and sensitive than tests on intact single species, and which are more uniform in sensitivity. Cultured cells and tissues, and isolated subcellular components (organelles, enzyme systems) are biological entities which may prove useful in the quest for rapid and sensitive assays at levels of biological organization below that which is represented by the intact, multicellular organism. Protozoa might also be expected to lend themselves to development as sensitive bioassay organisms. These various biological systems have as their common denominator the fact that they can be maintained in the laboratory as cultures. Hence they can be referred to generically as *in vitro* test approaches, for convenience. OTS seeks *in vitro* tests which can be applied to ecological hazard evaluation under TSCA, and approaches to the interpretation of *in vitro* test results in the context of ecological impact.

ECOSYSTEM TESTING

Single species testing fails to reveal species interactions and their vulnerability to perturbation by chemicals. We must therefore find ways to test assemblages of interacting organisms in contained environments. There are two present approaches to such testing. The first is to create assemblages of plants and animals which function as simple food chains. Another approach is to start with an assemblage which has been taken from nature and brought into the laboratory. The sample might be a soil core removed from the field in a manner that preserves the natural stratification. Aquatic ecosystems might be represented by samples of pondwater, including bottom sediment and macroorganisms. Whether natural or man-made, such assemblages, by virtue of being contained (isolated) will be subjected to restricted capacity for exchange of energy, materials and organisms and thereby will de-