Heavy Metals in the Environment

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Preface

Environmental pollution and impairment of human health from toxic metals is now recognized as a serious and insidious problem. Many experts consider this form of contamination of greater potential danger than that caused by synthetic organic chemicals, such as pesticides. Greatest concern recently has centered on mercury and lead. Other metals used commercially, which have been identified as potentially dangerous to health and the environment, are arsenic, barium, cadmium, chromium, nickel, manganese, copper, zinc, beryllium, selenium, silver and vanadium. Rate of use of such metals has increased almost 50 percent in the past 20 years.

Metals are not degradable like most organic substances. Once dug from the earth, they are apt to accumulate unnaturally in the environment and unless controlled or eliminated slowly build up a residue of toxic material which will not disappear. A potential consequence of accumulation is increased intake by the human and animal population. As an example, cadmium is cited by health authorities as being a significant factor in hypertension in the United States.

To examine some of the major facets of pollution by metals, a seminar series was held during Fall Quarter. The weekly seminars were open to the public, faculty, and students of all ages. Representatives of federal and state agencies were in attendance. The lectures are reproduced in this volume to make them available to a wider audience.

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Contents

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Environmental Contamination by Toxic Metals

Pollution by pesticides, radioactivity, smog, etc. has been well known for a number of years. More recently, however, a newly identified and perhaps more dangerous class of environmental contaminants has suddenly moved into prominence.

These are the so-called "heavy" metals such as lead, mercury and cadmium. The toxicity of such metals is well documented. Arsenic, for example, has long been used as an instrument of murder and suicide. Lead, cadmium, mercury, arsenic, antimony and beryllium have all caused accidental deaths in industry. Now plants, animals and even man in their normal surroundings are undergoing a continuous inescapable exposure to increasing concentrations of these toxic metals.

In recent years we have witnessed an immense increase in public interest and concern over the quality of the environment. Evidence for this public awareness can be found in the frequent mention of air and water pollution by the news media, in the appearance of many new citizen-sponsored environmental organizations, in the passage of new laws aimed at eliminating or controlling pollution, and in the creation of new federal or state agencies, such as the Environmental Protection Agency.

In today's climate of public concern, periodic implications of impact, real or imagined, of various types of pollutants or chemicals on human health, fish, wildlife or plants are being promulgated by environmentalists, scientists, or the press. In most cases, these dire pronouncements are directed at only one aspect of the problem and do not generally present the total picture. As a result, the public has been left with a completely distorted viewpoint concerning most environmental contaminants including the metals.

Mercury, cadmium, lead and arsenic are now household words. The public knows that these metals are "bad" and that their continued use will lead to dire consequences. They have forgotten (or have never been told) the many valuable uses of these metals and how our society depends upon them.

METAL POLLUTION

Concern over the metals has developed because: 1) these elements are widely distributed throughout the environment; 2) they are not degraded and hence persist in nature for extended periods of time; 3) generally, they are toxic to living organisms at fairly low concentrations; 4) they tend to be either biologically magnified or cumulative in plants and animals; and 5) certain metals can be converted to more toxic forms in the environment.

While contamination by toxic metals probably affects the entire biota, most attention has focussed on the potential risk of metals to man. Metals thought to have adverse effects on human health are summarized in Table 1, with the most hazardous metals being underlined. While fossil fuels represent a major source for seven of these metal contaminants, Schroeder

Table 1. Trace Metals That May Pose Health Hazards to Man[a]

Element	Sources	Health effects
Antimony	Industry	Shortened life span in rats
Arsenic	Coal, petroleum, detergents, pesticides, mine tailings	Hazard disputed, may cause cancer
Beryllium	Coal, industry (new uses proposed in nuclear power industry, as rocket fuel)	Acute and chronic system poison, cancer
Boron	Coal, cleaning agents, medicinals, glass making, other industrial	Nontoxic except as boran
Cadmium	Coal, zinc mining, water mains and pipes, tobacco smoke	Cardiovascular disease and hypertension in humans suspected, interferes with zinc and copper metabolism
Germanium	Coal	Little innate toxicity
Lead	Auto exhaust (from gasoline), paints (prior to about 1948)	Brain damage, convulsions, behavioral disorders, death
Mercury	Coal, electrical batteries, other industrial	Nerve damage and death
Nickel	Diesel oil, residual oil, coal, tobacco smoke, chemicals and catalysts, steel and nonferrous alloys	Lung cancer (as carbonyl)
Selenium	Coal, sulfur	May cause dental caries, carcinogenic in rats, essential to mammals in low doses
Vanadium	Petroleum (Venezuela, Iran), chemicals and catalysts, steel and nonferrous alloys	Probably no hazard at current levels
Yttrium	Coal, petroleum	Carcinogenic in mice over long term exposure

a. From Chem. Eng. News July 19, 1971

(1970) has suggested that only lead, cadmium and nickel represent real or potential air pollution hazards to man.

The term "heavy metal" is often used to refer to metallic contaminants in the environment. This term is, unfortunately, quite misleading since it has come to include most if not all the toxic metals. While mercury and lead with atomic numbers 80 and 82 are indeed "heavy" elements, the same term "heavy metals" has been applied to chromium and arsenic with atomic numbers of 24 and 33 and even to beryllium with an atomic number of 4.

Unlike most other pollutants, metals and their salts occur naturally in the environment. Over the years, man has also extracted metals from underground mineral or ore deposits and utilized them for his own needs. In his industrial and technological uses of metals, man has discharged quantities of these elements into his surroundings as a result of mining, smelting and processing operations, or from the use or discard of metals or their products. But, other human activities also release metals to the environment. For example, burning of fossil fuels (including gasoline with additives) appears to be the largest source of metal emissions to air. As a result of these various activities, many metallic wastes have either become distributed over the entire earth or excessively concentrated in localized areas, particularly in regions of high population density.

The special difficulty with all metals is their persistence. Unlike organic pollutants, metals are not subject to biological or chemical degradation in nature. Since metals are very stable, they may be transported for considerable distances by air and water or they may pass along food chains,

in which they tend to accumulate, reaching concentrations in the upper trophic levels several orders of magnitude greater than that which originally existed in air or water. Metallic wastes, consequently, tend to remain indefinitely in the ecosystem and can, thus, represent a continuous threat to plants and animals, particularly to carnivorous species such as man.

Plants are especially sensitive to metals such as copper, mercury and silver and contamination of soils can result in damage to plant and/or uptake of the metals. Plants have been shown to accumulate dangerous amounts of cadmium, chromium, cobalt and nickel from the soil. However, plants grown on polluted soils may not be visibly affected by the pollutant. Yet, the concentration of the metal on or in the plant may be a potential health hazard to animals, including humans, consuming these plants.

Regardless of the source, most metallic wastes eventually end up in surface and subsurface waters. Liquid wastes containing toxic metals are produced in many industrial operations. In addition to the direct discharge of such wastes, large quantities of many metals which are released into the atmosphere by industrial plants, incinerators, automobiles, etc., are eventually found as water pollutants after sedimentation or after precipitation from the air by rain or snow. Scrubbers for removing waste products from stack gases also transfer metal pollutants to water. Solid wastes disposed of on land contribute to water pollution by adding metallic substances to runoff from the disposal area and by the leaching of soluble metallic salts by water percolating through the waste. Agricultural runoff, mining and domestic sewage also bring additional quantities of metal into surface water supplies.

As an example of the magnitude of such contamination, the Texas Water Quality Board has found that 1,600 pounds of lead, 7,900 pounds of zinc, 5,000 pounds of cadmium, and 300 pounds of chromium were discharged daily directly into the Houston Ship Channel during 1969. The resulting concentrations in the channel were grossly in excess of those occurring naturally -- more than 63,000 times for lead, 15 for cadmium, and 108,000 for chromium.

Since metals are naturally occurring substances, the geological characteristics of the rocks and soil in a watershed also play a major role in determining the metal content of the water and the sediment in a particular stream. Thus, the concentration of metals in waters varies naturally according to region, season, water temperature, river flow, etc. For example, Silker (1964) has shown that over the period of a year the zinc concentration of the Columbia River in the vicinity of Hanford, Washington varies 9-fold, iron by 15-fold, copper by 38-fold, cobalt by 65-fold and manganese by 414-fold, reflecting differing contributions of these metals from the major tributary streams.

Such factors account for the tremendous variations in metal concentrations observed in U.S. rivers (Table 2). In the case of arsenic and manganese, the mean concentrations observed exceed the permissible criteria set by the U.S. Public Health Service for public water supplies. For many of the other toxic metals, the observed upper concentration values are substantially higher than the permissible criteria.

Fish and other aquatic species are especially susceptible to metal ions in the water. Table 3 summarizes the relative toxicity data of metals for

Table 2. Summary of Metals in Waters of the United States[a]

Element	Concentration (ppb) Low	High	Mean	Surface water criteria for public water supplies[b]
Arsenic	5	336	64	absent[c], 50[d]
Barium	2	340	43	absent[c], 1000[d]
Beryllium	0.01	1.22	0.19	none
Boron	1	5000	101	absent[c], 1000[d]
Cadmium	1	120	9.5	absent[c], 10[d]
Chromium (Cr^{+6})	1	112	9.7	absent[c], 50[d]
Cobalt	1	48	17	none
Copper	1	280	15	virtually absent[c], 1000[d]
Iron	1	4600	52	virtually absent[c], 300[d]
Lead	2	140	23	absent[c], 50[d]
Manganese	0.3	3230	58	absent[c], 50[d]
Molybdenum	2	1500	68	none
Nickel	1	130	19	none
Silver	0.1	38	2.6	absent[c], 50[d]
Vanadium	2	300	40	none
Zinc	2	1183	64	virtually absent[c], 5000[d]

a. For the period Oct. 1, 1962 to Sept. 30, 1967 as reported by Kopp and Kroner (1969).

b. From National Technical Advisory Committee (1968).

c. Desirable criteria.

d. Permissible criteria.

Table 3. Relative Toxicity of Metallic Ions to Fish in Soft Water

Metal Ion[a]	Approximate concentration producing toxicity (ppm) Schroeder[b]	Jones[c]	Pickering and Henderson[d]	AFS Ad Hoc Committee[e]
Antimony	20			
Arsenic	10			1.0
Barium		500		
Beryllium	0.2			
Cadmium	0.1	0.3	0.63 - 1.05	0.003
Chromium (Cr^{+3})		1.3	5.1	
Chromium (Cr^{+6})			17.6	0.05
Cobalt	10	15		
Copper	0.1	0.02	0.022	0.01
Iron	1.3			
Lead	2	0.2	5.6 - 7.3	0.01
Manganese	2400	50		
Mercury	0.2	0.02		0.005
Molybdenum	70			
Nickel	4	1.0	4.6 - 5.2	
Selenium (SeO_3^{-2})	100			
Silver	0.004	0.004		
Tin	1			
Titanium	8			
Uranium	3			
Vanadium	5			
Zinc	2	0.4	0.78 - 0.96	0.01
Zirconium	14			

a. The most hazardous metals to fish are underlined.

b. Reported for assorted fish species by Schroeder (1965). Data taken primarily from McKee and Wolf (1963).

c. Concentration intoxicating large stickleback after 1 week exposure (Jones, 1938, 1939).

d. The 96 hr TL_m concentration for fathead minnow (Pickering and Henderson, 1966).

e. American Fisheries Society's Ad Hoc Committee on Heavy Metal Contamination, Winnipeg, Man., Canada, December 17, 1970.

a variety of fish species and is useful for comparative purposes. A more recent report prepared by the American Fisheries Society, however, indicates that under some conditions the lethal levels for the seven most important metals are considerably lower with lethal concentrations as low as: 1 ppm for arsenic, 3 ppb for cadmium, 50 ppb for hexavalent chromium, 10 ppb for copper, 10 ppb for lead, 5 ppb for mercury, and 10 ppb for zinc. Some long-term laboratory experiments have even shown deleterious effects on reproductive capacity of aquatic organisms at toxic metal concentrations below 1/100th of the previously established lethal levels (Brungs, 1969). Other factors such as water quality and temperature, aquatic species, life stage, etc. also moderate the toxic response of aquatic organisms towards metals.

One of the factors determining toxicity concerns the chemical form to which the organism is exposed. I have referred to things like copper, lead and mercury without emphasizing that we are not really concerned about the free metals which generally present no real problems, except in the case of mercury vapor. What we are really concerned about is the large variety of metallic compounds or ions, where more often than not, the solubility, binding affinity and biological activity of one form may be very much different from those of another.

For example, inorganic arsenic compounds are more or less toxic, depending on whether they are in the trivalent or pentavalent state; organic arsenicals vary considerably in their toxicity; and the form of arsenic present in high concentration in shrimp and other seafoods is relatively non-toxic. Similarly, toxicities vary with the various forms of mercury, depending on whether it is mercury metal (quicksilver), inorganic mercury or an

organic compound containing the metal. Each form of a metal presents its own unique spectrum of toxicity.

ESSENTIAL METALS

A peculiar complication of the metal pollution picture is that some of the metals considered to be dangerous are in fact needed in trace quantities by all plants and animals, including man (Table 4). The metals vanadium, chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and tin are known to be essential to animals for life or maximum growth and nickel is thought to be.

These metals, usually incorporated into proteins, help serve as catalysts which initiate or assist in many biological reactions. Some of the more important metalloenzymes from plants and animals are summarized in Table 5. In addition to enzymes such as these, other biologically important molecules also contain essential metals. In animals, for example, iron is an integral part of the hemoglobin used for oxygen transport while in plants the chlorophyll molecule contains magnesium. No fewer than 51 metals have been detected in the human body and undoubtedly some of these additional metals will eventually be shown to be necessary for life.

Essential metals are therefore, required in small quantities for the well being of living organisms. However, the same metals released to the environment in larger quantities through man's activities or from natural sources, can create toxic conditions. Zinc and copper from mining operations, for example, can easily kill fish and other aquatic organisms at very low concentrations. For essential metals, toxic levels may be 40 to 200 times greater than that required for proper nutritional response.

Table 4. Metals Essential to Life

Element	Symbol	Number	Comments
Hydrogen	H	1	Required for water and organic compounds.
Boron	B	5	Essential in some plants; function unknown.
Carbon	C	6	Required for organic compounds.
Nitrogen	N	7	Required for many organic compounds.
Oxygen	O	8	Required for water and organic compounds.
Fluorine	F	9	Growth factor in rats; possible constituent of teeth and bone.
Sodium	Na	11	Principal extracellular cation.
Magnesium	Mg	12	Required for activity of many enzymes; in chlorophyll.
Aluminum	Al	13	Essentiality under study.
Silicon	Si	14	Possible structural unit of diatoms; recently shown to be essential in chicks.
Phosphorus	P	15	Essential for biochemical synthesis and energy transfer.
Sulfur	S	16	Required for proteins and other biological compounds.
Chlorine	Cl	17	Principal cellular and extracellular anion.
Potassium	K	19	Principal cellular cation.
Calcium	Ca	20	Major component of bone; required for some enzymes.
Vanadium	V	23	Essential in lower plants; certain marine animals and rats.
Chromium	Cr	24	Essential in higher animals; related to action of insulin.
Manganese	Mn	25	Required for activity of several enzymes.
Iron	Fe	26	Most important transition metal ion; essential for hemoglobin and many enzymes.
Cobalt	Co	27	Required for activity of several enzymes; in vitamin B_{12}.

Table 4, Con't.

Nickel	Ni	28	Essentiality under study.
Copper	Cu	29	Essential in oxidative and other enzymes and hemocyanin.
Zinc	Zn	30	Required for activity of many enzymes.
Selenium	Se	34	Essential for liver function.
Molybdenum	Mo 4	42	Required for activity of several enzymes.
Tin	Sn	50	Essential in rats; function unknown.
Iodine	I	53	Essential constituent of the thyroid hormones.

Table 5. Important Metalloenzymes and Metalloproteins.

Metal	Enzyme or Protein	Function
Iron	Ferredoxin	Photosynthesis
	Succinate Dehydrogenase	Aerobic oxidation of carbohydrates
Iron in Heme	Aldehyde Oxidase	Aldehyde oxidation
	Cytochromes	Electron transfer
	Catalase	Protection against hydrogen peroxide
	Hemoglobin	Oxygen transport
Copper	Ceruloplasmin	Iron utilization
	Cytochrome oxidase	Principal terminal oxidase
	Lysine Oxidase	Elasticity of aortic walls
	Tyrosinase	Skin pigmentation
	Plastocyanin	Photosynthesis
	Hemocyanin	Oxygen transport in invertebrates
Zinc	Carbonic anhydrase	CO_2 formation; regulation of acidity
	Carboxypeptidase	Protein digestion
	Alcohol Dehydrogenase	Alcohol metabolism
Manganese	Arginase	Urea formation
	Pyruvate Carboxylase	Pyruvate metabolism
Cobalt	Ribonucleotide Reductase	DNA biosynthesis
	Glutamate Mutase	Amino acid metabolism
Molybdenum	Xanthine Oxidase	Purine metabolism
	Nitrate Reductase	Nitrate utilization
Calcium	Lipases	Lipid digestion
Magnesium	Hexokinase	Phosphate transfer

It is a question of dose or exposure level. Metals, both essential and non-essential, as with all chemicals, are toxic to living systems if ingested at sufficiently high levels and for a long enough period.

With essential metals, it is useful to think of three levels of exposure-response. The first level is associated with physiologic inertness; the second with physiologic benefit; and the third with physiologic damage. For each essential element, a level below that of benefit results in deficiency and harm to the organism. For all other metals, the levels of physiologic inertness and benefit are consistent with safety. A level of excess for either essential or non-essential metals causes toxicity.

Thus, for each of the metallic elements in the environment, to which all living organisms experience a finite level of exposure, the critical question of toxicity cannot be answered with a yes or no, but by how much. What level of exposure to a specific metal is consistent with safety? In order to determine the levels that may be tolerated in air, water and food, it is also necessary to know the level of unavoidable background exposure from all sources.

Organisms are, in general, equipped with some sort of a homeostatic mechanism to regulate the amount of essential trace metals that are incorporated into their tissues. This protective mechanism may not function, however, when the organism is exposed to excessive amounts of an essential metal.

TOXIC METALS

In the case of metals with no biological role, however, plants and animals have generally failed to develop a similar homeostatic defense.

Consequently, even small amounts of such toxic metals as lead, mercury and cadmium are absorbed and readily accumulated by the organism. These metals, therefore, tend to be cumulative poisons.

The tendency for accumulation is reflected in the long biological half-life for these metals in man -- 1460 days for lead, 280 days for arsenic, 200 days for cadmium and 70 days for mercury (Table 6). It should be recognized that the biological half-life has somewhat different meanings, depending on whether one is talking about the whole body, the liver, the bone compartment, or some other tissue. A long-biological half-life can obviously be an important factor influencing the toxicity of a metal.

This table also summarizes data on the mean body burden of the various potentially toxic metals as well as their daily intake via consumption of food and beverage or from inhaled air. In general, oral ingestion is the major route of absorption for these metals while uptake from the lungs provides only a minor intake of the metal.

Lead

Widespread environmental contamination with metals such as lead and mercury is related to the increasing role that these metals play in our modern civilization. Measurement of the lead content of both ancient and recent Arctic snow and ice, for example, have shown that the lead concentration increased 4-fold between 1750 and 1940 and then nearly tripled again since 1940. The first increase reflects the great expansion of lead smelting following the industrial revolution and the second increase reflects the addition of lead additives to gasoline.

Table 6. Metals in the Environment Which May be Harmful to Man[a]

Metal	Average daily intake (µg/day)		Oral dose producing toxicity (mg)	Total body content (mg)	Whole body half-life (days)
	Food and Water	Air			
Antimony (Sb)	100	1.7	100	7.9	38
Arsenic (As)	400-900		5-50	15-20	280
Barium (Ba)	735	30	200	22	65
Beryllium (Be)	12	0.04		0.03	180
Bismuth (Bi)	20	0.76		0.23	5
Boron (B)	4300		4000	48	0.5
Cadmium (Cd)	160	7.4	3	50	200
Chromium (Cr)[b]	245	1.1	200	1.8	616
Cobalt (Co)[b]	390	0.12	500	1.5	9.5
Copper (Cu)[b]	1325	11.4	250-500	72	80
Iron (Fe)[b]	15,000	84		4200	800
Lead (Pb)	300	46		120	1460
Manganese (Mn)[b]	4400	28.8		12	17
Mercury (Hg)	25				70[c]
Molybdenum (Mo)[b]	335	0.6		9.3	5
Nickel (Ni)[b]	600	2.36		10	667
Selenium (Se)[b]	62		5	14.6	11
Silver (Ag)	60-80		60	1	5
Tin (Sn)	7300	0.6	2000	17	35
Titanium (Ti)	1375	1.4		9	320
Uranium (U)	50			0.7	100
Vanadium (V)[b]	116	9.16		22	42
Zinc (Zn)[b]	14,500	16.8		2300	933
Zirconium (Zr)	490			420	450

a. Data primarily from (Bowen, 1966; Schroeder, 1965, 1970; I. H. Tipton, 1969; Underwood, 1971).

b. Elements essential to man.

c. For methylmercury (Aberg et al., 1969).

Lead affords an excellent example of how a metal can be both exceedingly useful and also hazardous to health. Lead has been utilized by man since ancient times because of its malleability, density and corrosion resistance. But absorption of even small quantities of lead into the human body can cause severe illness. Some historians have even suggested that widespread lead poisoning or "plumbism" caused the downfall of the Roman Empire. According to the story, Roman wine became heavily contaminated with lead because of the leaching of the metal from glazed storage containers by the slightly acidic liquid. Consumption of the wine by the Romans then resulted in widespread lead poisoning that contributed to the collapse of the Roman civilization.

There are three reasons for the present concern over the long-term health effects of lead from environmental sources: its ubiquitous occurrence in nature; the precipitous increase in the use of leaded gasolines for automobile fuel; and the alarming report of Patterson (1965) who claimed that "definite indications that residents of the U.S. today are undergoing severe chronic lead insult".

There is no question that the consumption of leaded fuels has increased tremendously, from 100,000 pounds in 1940 to 450,000,000 pounds in 1967--an increase of 4,500 fold in 27 years. If we assume a uniform distribution throughout the country, the potential exposure to each individual approximates 2.21 lb/year -- certainly a frightening thought, particularly when lead distributions are not uniform but concentrated in areas of heavy vehicular traffic.

A series of investigations on various populations throughout the world have shown that: variations in body lead concentrations did not exceed 3.5-fold anywhere in the world; lead values in primitive societies often equalled those found in the cities; lead values had not changed appreciably in three decades; and there was no evidence that, at current lead levels, adverse effects on human health occurred. Blood lead values were measurably higher in certain occupational groups---traffic policemen, tunnel workers, etc.--- and there was a reasonably good correlation in such exposed groups between ambient lead concentrations and blood lead levels.

However, lead values did not approach a level of concern even in individuals receiving substantial exposure (well below 80 mg Pb/100 ml). There is some indication, that biochemical changes may occur in the blood (inhibition of erythrocyte delta-amino-levulunic acid dehydrase) under normal urban conditions of lead exposure. The significance of these changes are not known, however. It must be remembered, also, that human intake of lead from the atmosphere is relatively small in comparison to that from food and beverage.

Plumbism or lead poisoning in children, however, is a significant public health problem in the U.S. today. Surveys show that 10 to 25% of children 12-36 months of age who reside in old deteriorated urban housing show evidence of increased lead absorption, and, surprisingly 2 to 5% have symptoms compatible with lead intoxication. Symptoms of lead poisoning include vomiting, anorexia, apathy, hyper-irritability, incoordination, and eventual brain damage. The principal causative factor of this poisoning is pica--the abnormal craving for unnatural foods, including dirt, paper, plaster,

putty and paint flakes. Much of the interior woodwork, painted plaster and wallpaper of houses built prior to 1940, may contain many layers of lead-based paints which have never been removed. Children consuming flakes of such paint over a period of months then can develop plumbism.

Cases of lead poisoning throughout the world also result from the consumption of acidic foods and beverages (fruit, fruit juice, tomatoes, tomato juice, wine, cider) which have been prepared or stored in improperly lead-glazed ceramic containers. There was a case of this type recently in Oregon, where a group of individuals developed plumbism after consumption of (appropriately) plum wine made in an old bathtub. There is also considerable interest in the relationship between illicit whiskey and plumbism in the southeastern U.S.

Plumbism is not restricted to man alone. For example, consumption of spent lead shot by feeding birds, particularly ducks and geese, in heavily used hunting areas also has caused serious incidents of lead poisoning and resulting mortalities.

Cadmium

After mercury and lead, cadmium is considered next in importance as an environmental pollutant. The investigations by Schroeder (1965) have focussed attention on this metal by linking hypertension to increased retention of cadmium in the kidneys. Cadmium levels in the air of 28 American cities have been closely correlated with the incidence of death from hypertension and arteriosclerotic heart disease (Carroll, 1969). Similar correlations have been made between cardiovascular death rates and cadmium concentrations in

milk (Pinkerton and Murthy, 1969). The ability of cadmium to elevate blood pressure and induce hypertension has also been amply substantiated in experimental animals.

Over 10 million pounds of the metal are used industrially in the U.S. each year, primarily in the photographic, plating, rubber, motor, aircraft, metal fabrication and battery industries. Cadmium is released to the air during smelting of other metals (primarily zinc) and incineration or disposal of cadmium-containing products (for example, rubber tires and plastic containers).

Drinking water and foods grown on cadmium-containing soils are believed to be the chief sources of human cadmium intake. Seafoods, particularly, oysters (3.1 ppm) are also exceptionally rich in the metal and appreciable quantities are also found in coffee, tea, peanuts, kidneys and many grains. Cigarette tobacco also is high in cadmium (1.5µg/cigarette).

Absorbed cadmium is found mainly in the soft tissues, particularly the kidneys. Renal cadmium levels in human subjects have been shown to increase progressively up to about the age of 50 and then decrease. Chronic cadmium poisoning in both man and animals causes kidney damage, proteinuria and ultimately hypertension. Testicular damage also can result following chronic exposure to cadmium.

Recently a chronic form of cadmium poisoning called "itai - itai" or ouch-ouch disease has been described in Japanese women. This episode was apparently caused by pollution of river water by wastes from a cadmium mine. Prolonged consumption of contaminated foods (rice and soybeans) grown in that

area and drinking of river water lead to the disease, occurring primarily in women over 45 years of age who have had multiple pregnancies. In addition to kidney damage, afflicted individuals developed extreme pain in the bones, back and joints, osteomalacia (demineralization of the bones), bone fractures and occasional fatal renal failure. Despite the evidence implicating cadmium, however, other factors, particularly nutritional state, may have played an important role.

Arsenic

Arsenic is ubiquitous in nature, being present in the earth's crust in concentrations of 2-5 ppm. Arsenic, which is used in the glass, pigment, and bronzeplating industries, also appears in the air, especially in coal-burning areas or near smelters involved in the manufacture of iron, copper, silver, cobalt and nickel. Major sources of human intake are from seafoods, particularly oysters, shrimp (42 ppm), and mussels (120 ppm), pork, liver, mushrooms, table salt, certain wines, and wheat. Except in isolated areas, such as parts of the lower Willamette Valley, the contribution of arsenic can be detected in several household presoaks and detergents at concentrations of 10-70 ppm (Angino et al., 1970).

For the most part, airborne arsenic is mainly in the more toxic trivalent form whereas, pentavalent arsenic is the primary form present in foods. The extent to which arsenic is absorbed and retained in the body and its route of excretion vary with the level and chemical form of the arsenic ingested. Pentavalent arsenic is readily excreted in the urine, and, despite its affinity for hair, nails and skin, does not accumulate in man. The trivalent compound is retained in the tissues to a greater extent and is more

slowly excreted, primarily via the feces-factors which contribute to its greater toxicity.

Although there is still some controversy, exposure to trivalent arsenic is thought to cause skin cancer. Exposure to arsenic, especially arsenic trioxide, also has been correlated with the occurrence of cancers of the lung and respiratory tract. Experiments with animals, however, have not conclusively confirmed the carcinogenicity of arsenic compounds, and, in fact, studies in rats indicate that feeding trivalent arsenic caused a significantly lower incidence of spontaneous tumors.

In Taiwan, where soil and water have exceedingly high arsenic levels, blood arsenic values in individuals suffering from a severe type of arteriosclerosis called blackfoot disease, were found to be closely related to the severity of the disease. Arsenic can also cause chromosomal damage in human leucocytes, induce teratogenic changes in hamsters and in small concentrations inhibit wound healing.

Recent work by B. C. McBride and R. S. Wolfe working with John Wood at the University of Illinois, indicate that, like mercury, arsenic can be converted by bacteria in nature to a much more poisonous form. Anaerobic microorganisms in sediments containing arsenic can convert the metal from the inorganic form to dimethylarsine. The resulting methyl arsenic compound is highly toxic and is readily accumulated by fish and other aquatic organisms.

Nickel

About 400 million pounds of nickel are used annually in the U.S., primarily in stainless steel and various alloys, for nickel plating and as a catalyst. It is found in varying concentrations in the air of most cities, primarily derived from the combustion of coal and petroleum and from the operation of incinerators. However, most of the nickel enters the body in the diet, primarily in legumes, tea, cocoa, pepper and grains.

Although several nickel compounds are toxic, the most dangerous is nickel carbonyl, formed by reactions of nickel with hot carbon monoxide. It is an extremely active pulmonary irritant and a potent carcinogen. Workers in nickel refineries, using nickel carbonyl in the refining process, are prone to develop lung cancer and cancer of the nose and sinus. Inhaled nickel dust can also produce bronchial cancer in man and animals. The presence of nickel in tobacco (2-5.4 μg/cigarette) suggests that nickel released in the smoke, possibly as nickel carbonyl, may contribute to the carcinogenic potential of cigarette smoke.

Mercury

Awareness of a dangerous environmental contamination problem with mercury developed very gradually, even though large amounts of the metal were consumed by industry and the toxicity of mercury and its compounds was well known. Total consumption of mercury in the U.S. since 1900 is about 10^5 metric tons and with world consumption probably several times as great. As much as one-third of this has been lost into the environment (Hammond, 1971).

Mercury is used industrially in two predominant forms (Table 7). The first form is as metallic mercury, the liquid metal or quicksilver that we are all familiar with, which is used mainly for electrical apparatus and in the production of electrolytic chlorine and alkali. The second form is as organic mercury compounds. Pulp and paper mills have used phenylmercuric acetate and related compounds for slime control and to improve the storage properties of pulp. Other organomercury compounds including phenylmercuric acetate and methylmercury derivatives are used in agriculture or industry as fungicides. Most seed crops are pre-treated with mercury fungicides prior to planting.

Additional quantities of mercury on the order of 3,000 tons per year, are estimated to be released by burning of coal (Joensuu, 1971), a quantity comparable to that emitted as waste from all industrial processes. Similar quantities of the metal enter the environment from natural sources, such as volcanic action or volatilization and erosion of rocks and soils containing mercury (Peakall and Lovett, 1972).

The first inkling of a dangerous environmental mercury problem appeared from 1953 to 1960 in Japan when 111 fishermen and their families living along the shores of Minamata Bay developed a mysterious neurological disease and 44 people died. A similar outbreak occurred in Niigata, Japan in 1964 with 26 cases and 5 deaths. The afflicted individuals suffered from neurological symptoms and brain damage and many of the survivors were paralyzed for life. The source of the disease was eventually determined to be from methylmercury that had been discharged into Minamata Bay by a plastics factory. The methylmercury became concentrated in fish and shellfish which were subsequently eaten by the victims.

Table 7. U. S. Consumption of Mercury in 1969[a]

Use	Consumption (thousands of pounds)	Form
Electrolytic chlorine	1,572	metallic
Electrical apparatus	1,382	metallic
Paint	739	organic
Instruments	391	metallic
Catalysts	221	metallic
Dental preparations	209	metallic
Agriculture	204	organic
General laboratory use	126	all
Pharmaceuticals	52	inorganic, organic
Pulp and paper making	42	organic
Amalgamation	15	metallic
Other	1,082	all
Total	6,035	

a. From U. S. Department of Interior, Bureau of Mines.

In the late 1950's and early 1960's, several ornithologists also noted marked decreases in Swedish bird populations. By 1960 evidence had pointed to mercury poisoning in these birds from the organic mercury compounds used extensively in Scandinavia for seed treatment.

In 1965 the Swedes also learned that they had a severe water pollution problem with mercury. Analysis of Swedish fish indicated dangerously high concentrations of mercury to be present. Large amounts of organic, inorganic and metallic mercury were being discharged into Swedish rivers and lakes by electrolytic chlorine plants and paper mills, but the metal was thought to be relatively harmless to aquatic life.

The Swedish researchers learned that microorganisms living in the sludge at the bottom of lakes and rivers were capable of transforming inorganic mercury to the much more toxic methylmercury form (Fig. 1). This latter substance was considerably more water soluble and was readily taken up by smaller aquatic organisms. With each upward step in the food chain, methylmercury became more and more concentrated, so that tissues of some carnivorous fish high up in the chain showed a 3,000 or more fold concentration compared with the surrounding water.

The most common form of mercury in the biological environment is, therefore, methylmercury which also appears to be the most toxic form. It is rapidly assimilated, with almost complete absorption from the diet of both animals and man and slowly excreted with a biological half-life in humans of 70 days and ranging between 8-1,000 days for other species; it achieves a much more uniform distribution throughout the body, including the brain, than

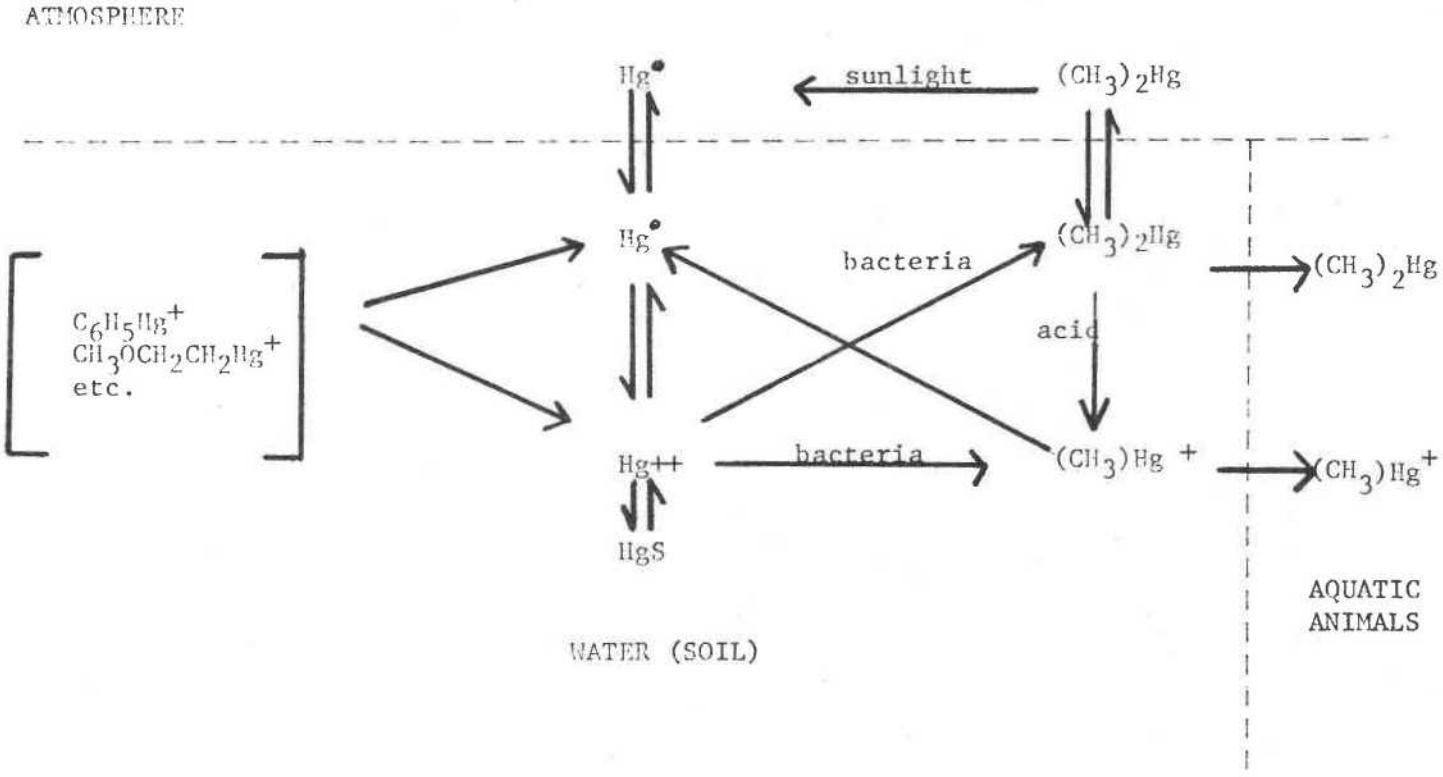

Fig. 1. Conversion of inorganic mercury and some mercury-containing compounds to methylmercury (Wallace et al., 1971).

other forms of mercury; it mainly affects the central nervous system; and readily passes the placental barrier.

Chromosome breakage in lymphocytes in humans following consumption of fish containing methylmercury has also been reported. In contrast, inorganic mercury is not readily absorbed, has a very short half-life, and tends to concentrate primarily in the liver and kidneys where it causes toxic effects.

The major source of the highly toxic methylmercury for humans is from the diet. Consequently, the U.S. Food and Drug Administration (FDA) has established an interim guideline value of 0.5 ppm for mercury in fish and other foods in order to prevent excessive intake of methylmercury with resulting harmful effects on human health. The 0.5 ppm limit prohibits commercial sale of any fish that exceeds this concentration. Bans on commercial or sports fishing or warnings against excessive fish or bird consumption have been issued in more than 20 states on a basis of mercury levels greater than the 0.5 ppm FDA safety limit.

The FDA guideline value was derived following a consideration of amount of fish likely to be eaten by Americans and an estimate of the maximum amount of methylmercury that could be ingested without harmful effects. However, this "no effect" dose estimate was aimed at avoiding symptoms of acute poisoning rather than long-term, chronic effects or possible genetic changes. It has also based upon an average fish consumption for healthy adults rather than on the exceptional fish eater or the old, the sick or children. Some scientists have concluded, therefore, that the FDA guideline value of 0.5 ppm may actually be too high.

Another approach at protecting the population from a toxicant present in foods is to establish, on the basis of toxicologic data, an "allowable daily intake" or ADI for the toxic substance. Using this approach, the Swedish Commission on Evaluating the Toxicity of Mercury in Fish (Berglund et al., 1971) has recently set forth recommendations regarding allowable intakes of methylmercury.

On the basis of toxicologic data, the lowest steady state intake of methylmercury producing toxic symptoms was first assumed to be 0.3 mg per day for a 70 kg man. Using a 10-fold safety factor, the Commission then arrived at an ADI for methylmercury of 0.03 mg per day or 0.21 mg per week. If these ADI recommendations were strictly adhered to, much more severe restrictions on the consumption of fish than those presently in force would result.

Considerations such as these have resulted in the recommendation by many states where high mercury levels have been found, that fish not be eaten more than once a week.

The appearance of high mercury levels in certain marine fishes resulted in the recent tuna crisis and in the complete removal of swordfish from the U.S. market. Now halibut and other ocean fish are under suspicion. Because of governmental and public reaction to these and other incidents many commercial fishermen are out of business and a mortal blow has been dealt to sport fishing and tourism in certain regions of the U.S. and Canada. Substantial pressures have emerged to end mercury contamination of the environment, to stop all discharges of wastes containing mercury and to even eliminate the use of this metal entirely.

But this narrow-minded viewpoint will not remove the mercury already present in our rivers and lakes, it will not prevent the entrance of mercury into the atmosphere from the burning of coal and oil or other relatively noncontrollable sources and it won't change the high levels of mercury that appear to be naturally concentrated in many animal species.

GENERAL CONCLUSIONS

Just how should society proceed to weigh potential and sometimes ill-defined pollution hazards, such as with trace metals, against the needs of society or resulting economic dislocations? Are we going to mindlessly ban completely the use of certain metals? Probably not - they are too essential, too widely used, too abundant in the natural environment.

We cannot allow ourselves to be swept into impetuous action with ill-advised restrictions, stopgap measures or new and untried solutions on a wave of emotionalism or popular appeal. Instead we must take calm, deliberate action based on objective research and evaluation of all aspects of the problem - chemical, biological, and technical as well as economic.

Since the problem is extremely complex and there is much data showing dangerous effects of so many metals on living things, the prudent thing to do is to immediately reduce to the absolute minimum all losses of metals to the environment. At the same time we need to rapidly develop new knowledge and technology and apply them to a rational program of pollution protection.

We may want to eliminate certain uses of metals because of the risk of pollution; we may cease to mine certain ore deposits; we may accelerate

our efforts to recycle certain metallic waste products; and we may utilize more expensive but nontoxic substitutes for cheaper but toxic metals. We may even modify our food habits, reducing or eliminating the consumption of some foods that are naturally high in certain toxic metals.

We must set standards for all the toxic metals to protect man and other living systems. We must first decide, however, on what level of protection is to be afforded to the ecosystem and what type of ecosystem is to be maintained. Scientific criteria on which standards are based, must also define the various detrimental effects that occur in the environment and relate them to corresponding levels of pollutants causing the damage.

Mainly motivated by human health consideration, the Federal government is slowly evolving policies for dealing with environmental contamination by heavy metals. Guidelines for airborne lead, beryllium and mercury are under preparation and the seriousness of air and water pollution from metals are being studied by several U.S. agencies. The Environmental Protection Agency and the various states have established restrictions on the disposal of metallic wastes into water. After first setting a 0.5 ppm standard for mercury, the FDA is now developing minimum tolerance levels for lead, arsenic, and cadmium in fish.

Great strides have already been made in reducing metal pollution in the environment. Industrial discharge of mercury into surface waters of the U.S., for example, has been practically eliminated by shutting down certain plants or by installing pollution control equipment in others. Similar controls are being introduced to reduce the release of other metals to the environment.

But much additional research is needed. For example, the overt toxic effects of metals on biological systems are pretty well documented. What is not well understood, however, is the possible effects of exposure to toxic metals over a long term - that is, the health effects of chronic or even lifetime exposure to subtoxic levels of metals.

Consequently, additional studies must be carried out in animals, exposing them for extended periods to low levels of various metallic contaminants. Sensitive and biochemical or physiological indicies of metal intoxication should be looked for. The various factors that determine the susceptibility or tolerance of an organism to a particular metal also need to be investigated. Some of these are already known, such as: age, sex, nutritional factors, interactions with other metals and concurrent disease. But other parameters undoubtedly influence the effects of metals on living organisms and should be identified.

Additional epidemological investigations are needed to correlate the levels of a particular metal in human or animal populations with the incidence of disease or altered biochemical parameter. This approach has already yielded some fascinating clues relating occurrence of nickel, arsenic, beryllium, lead and cadmium in public water supplies with deaths from various cancers. An interesting and as yet unexplained inverse correlation between hardness of drinking water and the incidence of cardiovascular disease has also appeared.

Investigation of the impact of metals on ecosystems must be intensified to locate affected species, identify geographical areas of concern and

to establish normal background levels for the various metals. Research is also needed on biogeochemical cycles so that the mechanisms of transport, concentration and chemical conversion of metals in the environment can be better understood.

SUMMARY

Increasing amounts of metal are being released into the environment by man's activities. Concern over such widespread metal contamination has developed because metals are not degraded and tend to persist in nature for long periods of time; they are generally toxic to plants and animals at low concentrations; they tend to be biologically magnified or concentrated by living systems; and certain metals can be converted to more toxic forms in the environment by natural processes.

Mercury, lead and cadmium are presently the objects of greatest concern since pollution of the air or water by these metals has resulted in human death and disease. But, many other metal contaminants have reached concentrations sufficiently high to represent a potential threat to man and other species.

Since the prospects of eventual harm are so great and the problem so complex, an immediate effort must be made to reduce losses of toxic metals to the environment to an absolute minimum. We also must begin to learn more about the sources, transport, chemical reactions, persistence and ultimate fate of metals in the environment and their effects on the biosphere. Only by such drastic actions, can we begin to cope with this pressing problem.

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Heavy Metals in Estuaries and the Coastal Zone

Metals, as a class, are among the substances man finds most useful. The history of civilization includes periods in time named after metals--the Iron Age, the Bronze Age. Even the Atomic Age might have been called the "Uranium Age".

Each phase of metal processing results in release of metals to the environment. Mining exposes metal-rich rocks to accelerated weathering. Smelting and refining commonly result in release of minor byproduct metals as waste of one form or another. Indeed the recovery or partial recovery of minor metals is often incidental to waste disposal. "Thallium is recovered from cadmium-containing flue dusts-----". (Bateman 1950 p. 628) Selenium "is another minor by-product obtained from the refining of copper ores. -----Much more could be saved from flue dusts if the demand should justify it." (ibid. p. 622) "Some 60,000 to 70,000 tons of white arsenic are normally produced annually as a by-product of smelter smoke from arsenical ores. -----In most places the available material is greater than consumption. Sweden alone could produce enough for the entire world and has difficulty in getting rid of the poisonous material." (ibid. 609-610)

In use, metals are often subject to corrosion and wear which leads to loss into the environment. Some uses involve direct release into the environment either intentional or unintentional. Application of lead arsenate insecticide or phenyl mercuric acetate fungicide introduces heavy metals to the environment. The addition of tetraethyl lead to gasoline and of chromium and zinc to waters used for industrial cooling leads to environmental contamination.

Whether released to air or water or soil, heavy metal contamination is eventually carried into estuarine and coastal water systems. Estuaries serve as funnels, through which land runoff is transported into the coastal ocean. In some cases estuaries appear to effectively trap or delay a good deal of the heavy metal flux passing through them (Turekian 1971). But estuaries are more than funnels or traps. They are also avenues by which anadromous fishes travel from spawning beds to the sea and return. They are spawning and nursery grounds for a wide variety of coastal organisms. Consequently, adverse changes in estuaries may be reflected in the biota of a broad geographic area beyond the estuary itself.

Figure 1 is a highly schematic representation of a coastal ecosystem. Heavy metals from most sources are introduced into either the water or sediment phases of the system. These phases normally exchange material with one another and with the biotic phase in a dynamic partitioning. Added heavy metals are subject to the same environmental partitioning. Concern over heavy metal contamination generally is focused upon effects in the biota. The magnitude and significance of such effects depends not only

upon the nature of the source (quantity released, which metals are released, etc.) but also upon the details of how the contaminant is distributed within the ecosystem.

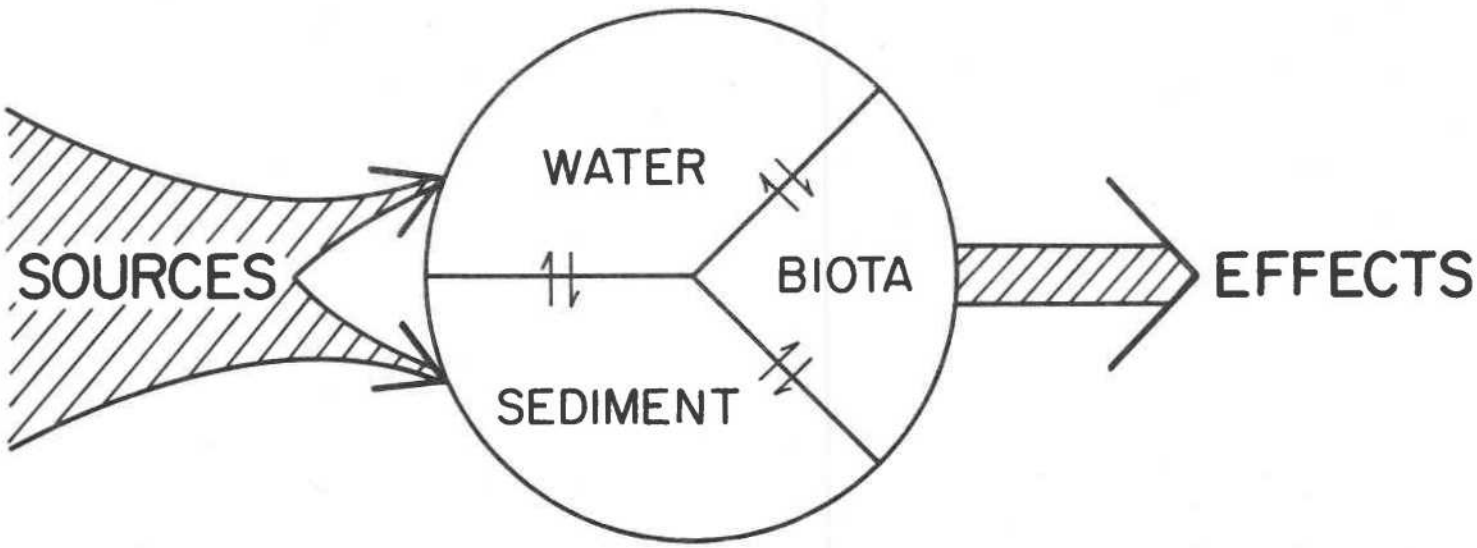

Figure 1. Idealized flow of contaminants through a coastal ecosystem

Heavy metals normally occur at very low concentrations in coastal waters (Table 1). Thus the addition of relatively small quantities of these elements to estuaries can potentially affect the concentrations. As an extreme example, the concentration of beryllium is only 6×10^{-8} grams/liter in seawater. This amounts to about half a cubic foot of the metal in the 200 mile long Chesapeake Bay, the largest estuary in the United States.

TABLE I

Concentrations of Selected Heavy Metals in Seawater[a]

Metal	Concentration µg/liter
Be	6×10^{-4}
Cr	0.5
Cu	3
Zn	10
As	2.6
Se	9×10^{-2}
Ag	0.3
Cd	0.1
Hg	0.2
Pb	0.03
Bi	0.02

a. From Goldberg *et al.* (1971)

Low concentrations of heavy metals in solution are a result of their tendency for sorption on particulate matter including suspended sediment, bed sediment, and small organisms. In some cases, active removal of metals from the water by organisms may also contribute to lowered concentrations in the water. The transport and dilution of heavy metals in coastal systems is therefore affected by the movement of particulate matter as well as by the flow of water. The influence of initial sorption of metals upon their subsequent "availability" to organisms is one of the most significant problem areas in environmental research. How effective are sediments at detoxifying contaminated waters? Under what conditions and to what degree will metals removed into sediments become incorporated into the biosphere?

Mercury illustrates these questions well. Inorganic divalent mercury added to river waters rapidly becomes associated with particulate material (Bothner and Carpenter 1972). It is then susceptible to accumulation in bottom sediments as happened at Lake St. Clair (Chem. and Eng. News, 1970). Jernelöv (1969) showed that mercury thus associated with sediments could be converted to methyl mercury (CH_3Hg^+) through microbial activity.

Mercury in this form appears to be less tightly retained by sediment and more readily taken up by organisms. Jernelöv (1970) simulated the burial of inorganic mercury contaminated sediments with uncontaminated sediment and observed the accumulation of methyl mercury by fish maintained in overlying water. He found that the uptake of methyl mercury rapidly decreased with increasing depth of burial of the contaminated sediment. The addition of benthic macrofauna (tubificids or clams) to the system, however, led to increased uptake of buried mercury.

Apparently the benthic organisms accelerate the transfer of methyl mercury to overlying water thus increasing its availability to fish. Thus one might expect to find both the sediment and biota in the vicinity of a mercury source to contain elevated mercury concentrations. Klein and Goldberg (1970) found that the Hg content of sediments in the vicinity of the Hyperion sewage outfall in Southern California was higher than that of sediments away from the outfall. On the other hand, sessile organisms from the outfall area were not significantly higher in mercury than those collected farther away (Young 1971). The expectation from Jernelöv's (1970) experiment has not been fulfilled.

A number of possible reasons are evident: Perhaps the number of samples taken is not statistically adequate. Perhaps the local current patterns near the outfall carry mercury away from the sample sites. Perhaps the natural rates of dispersion at the site greatly exceed the rates of mercury methylation/release. Perhaps the wrong species were sampled. If the partitioning of mercury is to be adequately known so that its fate and effects in similar systems can be predicted, we must learn which of these hypotheses stand scientific test.

COLUMBIA RIVER SYSTEM

Another example may be found in the Columbia River system. Here radioactive ^{65}Zn, formerly introduced into the river at Hanford, Washington, provides a tracer for Columbia River zinc (Osterberg 1962). This radioisotope becomes associated with particulate matter and can be found in shelf sediments off the coast of Washington (Cutshall et al. 1971).

It has also been measured in a wide variety of coastal marine organisms (Osterberg et al. 1964). Carey and Cutshall (1972) compared the distribution and specific activity (^{65}Zn/total Zn) in benthic organisms known to process sediments, with the distribution and specific activity of sediments. They concluded that sediment associated with ^{65}Zn was not always the primary source of ^{65}Zn to sediment processers. They suggested that supply of ^{65}Zn to these organisms might follow a detrital food web pathway.

These examples show the complexity to heavy metal cycling in coastal ecosystems. They also demonstrate that the role of natural partitioning in the effects of heavy metal contamination is substantial.

Experiments in which the lethality of given water concentrations of metals are measured, although relatively easy to conduct, are not ecologically adequate. Our present understanding of the rates and mechanisms of heavy metal partitioning is only adequate to highlight our ignorance of such factors. If we are to become able to predict the effects of heavy metals in coastal systems, even to recognize subtle effects, this understanding must be improved.

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Arsenic and Cadmium in the Environment

Arsenic and its compounds have been known almost since antiquity. Paradoxically, they are widely known not only as useful medications but also for purposes of either homicide or suicide. Presently, arsenic compounds are no longer considered to be appropriate in medical therapy (1), and the use of arsenic for either homicide or suicide while greatly publicized, is probably over-rated in efficacy. Certainly, with our present day abilities in medicine to sustain life by artificial means, these compounds would indeed be a poor choice for either purpose.

Arsenic is ubiquitous in our environment and levels can be found in air, soil, or water. Most water and air levels are in the range of ppb or less, whereas soils contain levels of ppm. The element does not appear to accumulate in the food chain-----and most foods do not contain high levels of arsenic with the single exception of seafoods which can be a major dietary source. For example, whereas an arsenic tolerance in foods has been established as 1 pp 300,000, seafoods normally contain high levels of arsenic ranging from 2 to 30 ppm.

In our normal environment, background levels of arsenic have not, so far, appeared to represent a significant health hazard. However, with an increasing use of arsenic compounds as pesticides or herbicides, potential human health hazards do exist, the determination of which will have to be assessed by future research. The major environmental concern is whether the compounds are carcinogenic. As will be discussed, there is some clinical evidence to support this theory. In addition, arsenic has been shown to cause chromosomal breaks in human leukocytes and teratogenic changes in hamsters. Less likely questions which have also been raised are the possible associations of arsenic with increased arteriosclerotic disease and chronic liver disease.

ARSENIC INTOXICATION

All arsenicals are considered toxic to human beings, dependent only upon amount and duration of dosage. The inorganic trivalent form is more toxic than the pentavalent form. As little as 1 mgm of arsenious oxide has caused symptoms of intoxication in a child.

The fatal dose is usually considered to be 100 to 200 mgm, although recovery from the ingestion of as much as 10 gm has been reported. Arsene gas is unquestionably the most toxic of all forms. Indeed, the Threshold Limit Value (TLV) for arsene is only 0.2 ppm as compared, for example, to 50 ppm for carbon monoxide.

Arsene gas is produced whenever a reducing agent, such as an active metal like iron, zinc, or tin reacts with water or an acid in the

presence of an arsenic compound. In fact, iron ore (iron pyrite) may contain sufficient quantities of trace arsenic to liberate fatal amounts of arsene gas when the ore is moistened and the fumes inhaled. Arsene gas may also be released from arsenic compounds by the action of various fungi, and probably various bacteria.

Clinically, our knowledge of arsenic intoxication must be divided into acute vs chronic categories. Arsenicals are absorbed into the body mainly via the gastro-intestinal or the respiratory systems. The fundamental physiology appears to be an increased permeability and dilation of the small arterioles and capillaries.

Unlike inorganic compounds which concentrate mainly in the skin, liver and kidneys, acute organic arsenical intoxication is also associated with a high concentration in the central nervous system. Acute intoxication is usually related to the accidental ingestion of an arsenic containing compound. Most poisonings today are secondary to inorganic compounds and produce severe hemorrhagic necrosis of the stomach or the small intestine. This eventually leads to gastro-intestinal bleeding, dehydration, secondary electrolyte imbalance, possible renal shut-down, and eventually cardio-vascular collapse leading to death.

Treatment is a combination of support of involved systems plus the use of chelating agents to remove the ingested arsenic. The clinician must have a high index of suspicion or a definite history of exposure in order to establish the diagnosis early. Twenty-four hour urine excretion may be helpful to establish the diagnosis but blood levels do not correlate

well with exposure (2). In acute intoxication, arsenic levels of hair or of nails is not helpful.

The acute exposure to arsene gas deserves special mention as it is a far more serious problem with toxicity related primarily to central nervous system signs and the production of a severe acute hemolytic anemia. There is no known antidote for arsene intoxication and supportive care is the only form of therapy available.

Regarding our potential environmental problems, the use of cacodylic acid as an herbicide raises the potential danger that spontaneous breakdown of this compound to arsene from the action of bivalent metal ions represents a problem which deserves further study. Although no clinical cases of arsene intoxication in industrially exposed workers has ever been recognized, the amount of breakdown to arsene gas is still undetermined and the potential danger to lower forms of life is unknown.

SYMPTOMS OF EXPOSURE

Chronic intoxication to low levels of arsenic or its compounds is much more difficult to recognize and diagnose because of the non-specific subjective complaints and because of the lack of good diagnostic criteria for abnormal arsenic levels in various tissues. Early symptoms of chronic exposure are such general complaints as weakness, malaise, and vague abdominal pains. Eventually, long term exposure leads to hyperpigmentation and/or dermatitis of the skin, chronic ulceration of the skin or of the

nasal septum, and peripheral neuritis. Both protein losing enteropathy and degenerative liver changes have also been described (3,4).

In reference to our environmental concern with arsenic as a potential carcinogen, several points merit discussion. The long term use of inorganic arsenical salts as a therapeutic agent and the development of chronic skin changes eventually leading to skin cancer would appear to be well established (5,6,7).

Studies in occupationally exposed workers, and also of people living in the effluent of stacks from mining operations of nonferrous metals, have demonstrated a significant relationship to the development of skin disease and skin cancer (8,9). A most significant study of the effects of chronic, very low dosage exposure to arsenic is that of Tseng who, in a study of 40,000 inhabitants living in an area of Taiwan where a high level of arsenic is present in artesian well water, demonstrated a definite increase in prevalence of keratosis, hyperpigmentation, and skin cancer in all ages. Furthermore this prevalence increased in an ascending gradient corresponding to the amount of arsenic in the water and the number of years of exposure (10).

These studies point out the dilemma and raise questions with which future investigators must be concerned. The problem of skin cancer and arsenic is obviously complicated by the fact that this disease appears to be latent, that is, the incidence of skin cancer does not begin to occur until 2 to 40 years after exposure to arsenic. Furthermore, one can biopsy the skin of a patient suspected to have chronic arsenic

intoxication and to have been exposed years ago to arsenic but who now has hyperpigmentation or skin cancer, and there will be no elevation of arsenic in the skin.

Although findings of elevated excretion, elevated hair or nail levels do have some significance, there are no exact values at which one can make a definitive diagnosis of chronic arsenical intoxication based upon tissue study. Since arsenic is not considered to be cumulative in the body, we are left with the peculiar finding of an element which is not cumulative but which is believed to react with cumulative effect after exposure!

Arsenic has also been implicated in the etiology of cancer of other organs besides the skin (1). The study by Lee (11) of 8,047 occupationally exposed white male smelter workers who were compared against statistics of the normal white male population is of interest in this regard. Smelter workers had an excess total mortality with a three-fold increase overall in respiratory cancer.

After 15 years, workers heavily exposed to arsenic had an eight-fold increase in respiratory cancer. These findings support the hypothesis that inhaled arsenic is truly a respiratory carcinogen in man, although the authors are careful to point out that there may be important co-existing influences such as sulfur dioxide or other unidentified chemicals.

Finally, the possible relationship between peripheral arteriosclerosis and long term arsenic exposure should be mentioned. In the Taiwan study, a high prevalence of "Blackfoot" disease, that is gangrene of the feet secondary to severe arteriosclerosis, was also noted and this disease also correlated well with the arsenic levels and period of exposure. The theoretical explanation for such a correlation is completely unknown.

Scientifically, the problem of all the above types of epidemiological studies is that, generally, only a single variable is being studied and therefore, as pointed out by Lee, other unidentified factors may be playing a role. Therefore, it must be emphasized that there are many questions which can be raised regarding the danger of chronic arsenic exposure.

The argument against arsenic or its compounds being detrimental to human health is elegantly discussed by Frost who points out numerous reasons against the detrimental effect of arsenic on human health and advocates that small amounts of arsenic may even be beneficial, including its role as a possible anti-carcinogen (12).

In summary, occupational studies and long term follow up of patients previously treated with arsenicals appears to have established a relationship between arsenic and the development of skin disease, including skin cancer. The development of arteriosclerotic disease and chronic liver disease has also been implicated. Questions regarding the amount of dosage required, length of exposure, co-existing or even independent variables have not been answered.

CADMIUM

Cadmium is an element which is receiving increasing attention as a potential environmental hazard. The element occurs naturally in only trace amounts with the exception of a relatively high level found in association with zinc and lead. Until now, most exposure has been from occupational exposure in workers involved in battery manufacture, electroplating, the making of alloys, welding and solders, ceramics, and vapor lamps.

At birth, the human being has essentially no tissue levels and then gradually accumulates cadmium, particularly in the red blood cells, kidney, liver, and bone, reaching a peak elevation in middle age. Normal urine contains only 0.0 to 15 mcgm per liter, although workers in cadmium industries excrete up to 500 mcgm per liter without apparent symptomatology.

Little is known of its physiology, although it is believed that cadmium inhibits the synthesis of iron into hemoglobin and therefore may produce a secondary type of "iron deficiency" anemia. As with arsenic, the compound generally exhibits toxicity via the gastro-intestinal route but, again, the inhalation of cadmium fumes is much more toxic than the ingestion of the cadmium salts. Present Threshold Limit Value for cadmium oxide is 0.1 mgm per cubic meter of air.

Acute cadmium intoxication is not considered an environmental problem. Like arsenic, cadmium primarily causes gastro-intestinal signs which may then eventually lead to cardio-vascular collapse. The

inhalation of the fumes, on the other hand, will produce a severe form of acute pneumonitis and a vague flu-like syndrome of lesser severity commonly referred to as "metal-fume fever".

Like arsenic, the potential environmental problems of cadmium are related to chronic exposure to low dosages. The most severe and classical form of low grade chronic exposure is laconically referred to as Itai-Itai (ouch-ouch) disease. This is a very unusual form of renal disease which leads to secondary softening of the bones resulting in multiple spontaneous fractures. There is no known therapy.

The syndrome was originally described in Japan and occurred as the result of the ingestion of rice which had been grown in water containing cadmium from high amounts of industrial pollution. This disease does point out the fact that cadmium may be accumulated in plants and thereby represent a potential human hazard via this route.

QUESTIONS ON HYPERTENSION

Environmental concern with cadmium is not, however, related to the production of Itai-Itai disease. The problems are related instead to the possible development of cardio-vascular disease -- particularly hypertension, to chronic renal disease, and to chronic lung disease. Of these, the major interest has been in the relationship to hypertension. The data has been ably reviewed by Schroeder (13).

In favor of this attractive hypothesis are the facts that hypertension may be produced in a variety of experimental animals by the long term administration of low levels of cadmium salts. Furthermore, this type of experimental hypertension has similarities in onset to that of the most common form of human hypertensive disease, namely essential hypertension. An important question which has not yet been satisfactorily answered is whether people with essential hypertension have increased levels of cadmium in their kidneys. An important experimental design which has yet to be performed would be the development of a drug which would selectively force excretion of cadmium from hypertensive patients to determine whether their hypertension became improved.

Probably the most significant data opposing the relationship of cadmium and hypertension is that occupationally exposed workers in the cadmium industries do not develop this disease! Much future work needs to be performed regarding the relationship of cadmium to such important systems as renin, aldosterone, sodium -- water excretion mechanisms and effect of cadmium on vascular tone. Further epidemiological studies such as that by Creason (14) demonstrating an acute correlation between the levels of cadmium in the diet and the presence of acute cardio-vascular and/or hypertensive heart diseases also need to be performed.

In spite of the difficulties of establishing a hypertensive-cadmium relationship, there is little doubt that chronic exposure to cadmium will lead to the development of proteinuria in human beings and is associated with chronic kidney changes in both humans and experimental animals (15,16,17,18).

The ingestion of cadmium chloride in a concentration of only 10 ppm has been shown to produce renal changes as evidenced by electron microscopy. Furthermore it is important to recognize that cadmium concentration in the kidney is not homogeneous but is preferentially concentrated at the outer medulla of the kidney. This fact will have to be taken into consideration in future studies regarding not only the development of renal disease but the study of hypertensive disease.

SEVERAL STUDIES PUBLISHED

Finally, we must consider whether cadmium has any relationship to the production of chronic lung disease. As mentioned earlier, pneumonitis in acute cadmium fume inhalation is well documented. It is also believed that chronic inhalation of cadmium fumes may lead to the production of pulmonary fibrosis.

Several studies have also been published which would make one highly suspicious that chronic inhalation of cadmium fumes may be a factor in the production of pulmonary emphysema, although the evidence at this time remains very circumstantial. The finding that cigarette smoking is a major source of cadmium accumulation in man has been pointed out (19). The independent observation that very low levels of cadmium inhalation will adversely affect the alveolar macrophage of the lung, therefore takes on added significance regarding cadmium air pollution and the theoretical association with pulmonary emphysema (20).

In conclusion, from the above discussion, it is hoped that the reader will perceive the difficulties encountered in the diagnosis of both acute and chronic poisoning from either cadmium or arsenic. The chronic diseases especially are a problem and the clinician must beware the trap of either over or under diagnosing these diseases.

Recent environmental concern plus advances in technology which allow easier and more accurate tissue determination of these trace elements than has ever before been possible should lead to rapid advances in our knowledge of the health effects of trace elements. The interested reader is referred to a recent excellent review by Louria, et. al. for further information (21).

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Presented October 19, 1972 by ROYSTON H. FILBY, Nuclear Radiation Center, Washington State University.

Problems in the Analysis of Trace Metals

Trace element analysis is now a separate area of analytical chemistry and many of the problems encountered are peculiar to the chemistry of very low concentrations. Trace analysis developed in response to a need to know more about the geochemical and biochemical behaviour of minor elements. In the 1930's emission spectrography was used extensively for the determination of trace elements in many materials and although the method proved to be a convenient and sensitive technique for many elements it has been largely supplanted today by more precise and sensitive analytical methods. It is not the purpose of this paper to survey the analytical techniques at present in use for trace elements because a great number of methods are available, often for a restricted number of elements. Table 1 shows some of the more important methods in use today.

Concern about trace elements in the environment is related to their short-term and possible long-term effects on man and other ecologically important systems. Many elements present in biological organisms as traces have been shown to be essential to some forms of life and another group of

TABLE 1

TRACE ELEMENT ANALYSIS METHODS

Optical Emission Techniques	Electroanalytical
flame emission	polarography
spectrographic analysis	anodic stripping
x-ray fluorescence (XRF)	voltammetry
charged particle induced XRF	
atomic fluorescence	
	Optical Absorption Methods
Nuclear Methods	flame atomic absorption
mass spectrometry	flameless AA
activation analysis	spectrophotometry

TABLE 2

BIOLOGICALLY ESSENTIAL TRACE ELEMENTS

Essential		Doubtful
Co	Mn	Cd
Cu	Mo	Br
Fe	Se	Sr
I	V	Ba
Cr	Zn	As
Sn		

elements are thought to be essential although this has not been proven. These trace elements are shown in Table 2. Several of the elements listed in Table 2 are toxic to man at higher concentrations, e.g. Se, Cd, As, V, etc. and many elements not known to be essential to man are toxic, e.g. Pb, Hg, Sb, Pu, etc. Many of the toxic elements are discharged through human agency into the environment and it is thus essential that the ecological behaviour of these elements be known. Much of this information can be obtained by studying the distribution of the elements in the environment and in environmental processes. In this area, biochemistry, geochemistry and atmospheric sciences overlap to a considerable degree.

The analyst working with environmental materials may be required to determine a trace element in many different materials and some of these are shown in Table 3.

TABLE 3

MATERIALS ANALYZED IN ENVIRONMENTAL STUDIES

Aquatic Environment	Atmosphere	Terrestrial
sea water	air particulates	rocks
fresh water	aerosols	soils
sediments	gases	fuels
plankton	industrial emissions	plants
plants		mammals
fish		

He will often be faced with very different concentrations of a given element in such materials as is exemplified by the case of Co shown in Table 4.

TABLE 4

COBALT CONTENTS (PPB) OF SOME ENVIRONMENTAL MATERIALS (1-3)

Material	Co (ppb)	Material	Co (ppb)
river water	0.9	air particulates	0.95[1]
sea water	0.27	soil	8000
lake sediments	15	petroleum	100
marine plants	500	human blood	0.33
		human liver	230

Note 1: concentration given as ng element/m^3 air

The purpose of this talk is to discuss some of the problems that may be encountered in the determination of trace elements in environmental materials. When one considers the analytical process from the collection of the sample to the reporting of the final result, it is evident that problems can be encountered at any step in the process. Table 5 lists some of these problems.

A detailed discussion of all these problems is not possible here. The problems I would like to consider are those which are not due to the specific analytical technique used--- but, those associated with sample collection and chemical separation. In addition, I would like to discuss some examples of inter-laboratory or inter-method studies and to point out the advantages of adequate standardization methods. Finally I would like to consider one aspect of trace analysis that presents a significant problem

to the analyst and which will prove to be increasingly important in environmental processes, viz, the determination of the chemical form of an element in natural materials.

TABLE 5

PROBLEMS IN TRACE ELEMENT DETERMINATION

Sample Collection

physical nature of the sample

contamination of the sample

losses by adsorption on container, etc.

change of chemical form during storage

Chemical Separation Procedures

contamination from reagents, etc.

losses due to adsorption, volatilization

losses due to incomplete reaction

Analytical Procedure

inadequate sensitivity

poor precision

interferences (matrix, interelement effects)

PROBLEMS OF SAMPLE COLLECTION AND STORAGE

For many types of materials for trace analysis, collection of the sample constitutes a problem only in ensuring a representative sample. Storage of samples of rocks, soils, plants, etc., prior to analysis consists merely of preventing contamination from such sources as airborne dust, dirty containers, etc. Much more severe sampling problems are encountered with many environmental materials, such as air particulates, aerosols, fresh waters and sea water and the problems encountered depend on the type of material to be analyzed and the information desired.

Trace element measurements in air present some specific problems. The trace elements may be present in air in several forms; a) as particulate matter, b) as aerosols and c) in gaseous form. Several techniques are used for sample collection and most of these rely on filtration of known air volumes through fiberglass, paper or Milliport filters of fixed pore size, or on impactor collection.

Generally the collected air particulates (or aerosols) cannot be conveniently removed from the filter or impactor film and thus the filter plus the sample must be analyzed. For the determination of elements present at low concentrations in air the filter material may constitute a serious blank problem.

Table 6 shows some trace element values for some typical filter materials. For a 47 mm Millipore filter (approx. 0.1g) the trace element is thus 2.1 µg Zn, 15.5 µg Cr, and 0.29 µg Fe. Non-urban air has been

found to contain approximately 0.003 μg Cr/m^3, 0.05 μg Zn/m^3 and 1 μg Fe/m^3 (2) hence the blank problem can be serious. Filter and impactor techniques may also be subject to error in that very fine aerosols may not be retained and these may contain significant amounts of certain trace elements. Thus Pillay and Thomas (7) found as much as 50% of the Se present in urban atmospheres passed through regular filters and impactors but was trapped in a liquid trap.

TABLE 6

TRACE ELEMENT CONTENTS OF SOME FILTER MATERIALS

Element	Elemental Concentration		
	Delbag(4)	Millipore(5)	Nucleopore(6)
Zn	4.0	2.37	2.8
Fe	3.9	0.33	5.7
Co	7.4	0.013	0.028
Cr	0.19	17.6	0.57
Cu	7.1	---	0.57
Au	5.8	---	---
Sb	---	0.039	2.8

The analysis of water samples is perhaps the most difficult problem encountered in environmental analysis. The concentrations of many trace elements in natural waters are present at sub-ppb levels and losses due to adsorption on container walls and contamination from containers may be a serious problem. Adsorption on container surfaces has been demonstrated in

a number of cases. Coyne and Collins (8) have shown that serious losses of Hg from natural waters occurs when the samples are stored in polyethylene. Table 7 illustrates this point.

TABLE 7

LOSS OF MERCURY FROM CREEK WATER SAMPLES STORED IN POLYETHYLENE (8)

Preservative Added[1]	Hg Added (Hg/ℓ)	Time After Addition (Days) 0	3	5	10
None	50	18	3	1	1
HCl	50	41	40	20	13
H_2SO_4	50	40	34	21	16
HNO_3	50	40	37	36	38
HAc:HCHO	50	40	22	21	11

Note[1]: Acids added to give pH = 1

It can be seen that only HNO_3 is effective in preventing serious Hg loss after 10 days storage and even with HNO_3 approx. 25% of the Hg in solution was lost. Robertson (9) has shown that serious losses of In, Sc, Fe, Ag, U and Co occur by adsorption from sea water onto polyethylene. Addition of HCl to give a final pH of 1.5 prevented serious adsorption, with the exception of Sc.

Table 8 shows results obtained in this study. For fresh waters, the problem may be more serious because of the lower total electrolyte

TABLE 8

ADSORPTION OF TRACE ELEMENTS

ON POLYETHYLENE FROM SEA WATER (9)

Element Studied	Percent Adsorbed After 8 days	20 days	35 days	75 days
Co	3	15	12	18
Co pH 1.5	3	3	0	0
Zn	8-	1	-	3
Zn pH 1.5	3	1	0	-
Ag	8	25	20	-
Ag pH 1.5	0	0	0	0
Fe	25	45	70	>80
Fe pH 1.5	0	0	0	-
Sc	10	42	55	75
Sc pH 1.5	30	48	40	-

concentrations compared to that of sea water. The addition of HCl or HNO_3 is commonly employed to prevent adsorption of trace elements on container surfaces and the amounts added do not alter the total metal concentration of the sample, provided the acid is of high purity. The addition of acids, however, will change the chemical nature of the medium. Trace elements may be present as suspended matter (e.g. silicates), finely divided precipitates, colloids (Fe hydroxides; rare earth compounds), bound in organic complexes (e.g. humic acid complexes) or in true solution.

The treatment selected for water samples will depend on whether the total content of the element is required or the distribution of the element among different species of the sample.

To overcome the problems of adsorption and contamination, several authors have proposed immediate rapid freezing in liquid nitrogen of water samples to prevent adsorption (10,11).

CHEMICAL SEPARATIONS AND PRECONCENTRATION

The concentrations of many trace elements in environmental materials are often too low to be measureable by certain analytical techniques. It consequently becomes necessary to pre-concentrate trace elements or perform chemical separations for the elements of interest prior to the analytical determination. Such procedures are in themselves sources of error and the problem is most acute for water samples. Sea water poses special problems because of the high Na, Cl and Br contents.

Several techniques have been proposed for pre-concentration of water samples. These include ion exchange, solvent extraction, evaporation and freeze-drying. Ion exchange and solvent extraction have been used extensively for the preconcentration of trace elements in sea water and fresh waters. In addition to the problems of contamination and losses by adsorption, there is also the problem of chemical form of the element in aqueous solution.

The assumption that a given element is present in a cationic or anionic form may not be justified. Nelson et al. (12) have shown that radionuclides of Zn, Sb, Sc and Mn in the Columbia River are distributed among particulate material and cationic, anionic, and uncharged species in true solution. The distribution of the different forms is shown in Table 9.

TABLE 9

DISTRIBUTION OF CHEMICAL FORMS OF

ZN, SB, SC, AND MN IN COLUMBIA RIVER WATER (12)

Element	Percent Particulate	Percent Soluble Fraction in:		
		Cationic	Anionic	Uncharged
Zn	80	83	12	5
Sb	1	0	16	84
Sc	89	4	79	17
Mn	70	48	43	10

There is also considerable evidence that elements such as Cu, Az, Fe, Co, Cr, etc. are present partially as stable organic complexes in water. Hence care must be taken to ensure conversion of all forms of an element into a single chemical form before carrying out separation procedures. For water analysis the best procedure appears to be immediate freezing of the water in liquid nitrogen followed by freeze-drying. This technique has been used at the NBS and by us at WSU as a preparation technique for neutron activation

analysis. Preliminary results indicate no losses of trace elements during the freeze-drying process and contamination is greatly reduced since surface contact by the water is minimized.

Most geochemical, biological and other environmental materials must be decomposed before separations and determinations can be carried out (except for NAA, XFR, etc.). This is commonly done by dry ashing, acid decomposition (H_2SO, $HClO_4$, HNO_3, HF, etc.), or fusion (Na_2O_2, KOH, Na_2CO_3, etc.). For biological and environmental materials, dry-ashing is generally avoided because of the loss of volatile elements; 100% of the Hg, Se, As, etc., are lost. Wet ashing of biological materials provides a convenient decomposition technique but volatile elements such as Hg, Se, and Br may still be lost unless precautions are taken. Filby (13) found that wet ashing of crude oils with H_2SO_4 resulted in significant losses of Hg, Sb, As, Se, and Cu.

In any decomposition procedure followed by chemical separations of either the element of interest or of interfering elements, the addition of reagents increases the contamination problem. In the case of acids and bases, considerable amounts may be added relative to the weight of the sample and the amount of a trace impurity may exceed that amount present in the sample.

Table 10 shows some trace elements in high purity solvents and distilled solvents. In the cases where 100 ml HCl/gm sample are used (e.g. in a decomposition step), the "blank" concentrations added would be 2.2 ppm Zn, 8.2 ppm Cu, 0.1 ppm Fe, and 0.11 ppm Cr. For sub-ppm analysis it is

thus essential to carefully purify the reagents used. Table 11 shows some trace elements in some reagents and common laboratory materials.

Although complexing agents used in colorimetric analysis (e.g. dithizone and TTA) may be used in small quantities, they may contribute significantly to the Fe, An, Cr and Cu values of the blank as can be seen from Table 11. Care must also be taken to ensure that the glassware is scrupulously trace-element free and that neoprene be avoided because of the very high Zn and Co contents.

TABLE 10

TRACE ELEMENT CONTENTS OF SOME SOLVENTS (5,14)

Solvent	Concentration (ppb)					
	Zn	Fe	Co	Cr	Cu	Sb
H_2O (1)	1	0.2	0.02	2	--	0.01
H_2O (2)	0.04	0.05	--	0.02	0.01	--
HCl (3)	22	1	0.09	1.1	82	0.20
HCl (4)	0.2	3	--	0.3	0.1	--
HNO_3 (5)	13	2	0.02	72	1.3	0.03
HNO_3 (6)	0.04	0.3	--	0.05	0.04	--

Notes:

(1) double distilled (ref. 5)
(2) sub-boiling distilled (ref. 14)
(3,5) reagent grade (ref. 5)
(4,6) sub-boiling distilled (ref. 14)

TABLE 11

TRACE ELEMENT CONTENTS

OF SOME REAGENTS AND MATERIALS (5)

Reagent or Material	Zn	Fe	Co	Cr	Cu	Sb
KOH (1)	1250	2700	1.7	<10	--	1.8
Na_2CO_3 (2)	74	1400	1.8	0.76	--	5.1
Dithizone (3)	1150	7000	1.2	<2000	420	0.8
TTA (4)	329	11300	4.9	192	16	6.2
Polyethylene (5)	28	10400	0.07	76	6.6	0.18
Neoprene (6)	-10^7	--	2300	--	--	290

THE ANALYTICAL METHOD

A large number of analytical methods exist for the determination of trace elements in environmental materials, and it is beyond the scope of this talk to consider the problems of each of the most important trace element methods. All methods suffer from poor sensitivity for some elements, interferences, matrix effects and inter-element effects to some degree. Some methods are relatively free from interference, e.g. NAA, but require sophisticated and expensive equipment.

A useful method of evaluating trace element methods is to compare results for a given set of elements determined in the same material by

different methods. Several such studies have been performed and illustrate very graphically the magnitude of the problem of comparing results from different laboratories. Several government agencies (e.g. EPA, USGS, etc.), in addition to many universities and other groups, make large-scale surveys of environmental quality. Much effort will be wasted if results from different laboratories cannot be directly compared, and this may be the case more often than is realized.

The problem of Hg pollution has been extensively studied in the past few years. Unfortunately many of the data are of doubtful validity because of severe losses of Hg during sample analysis in some methods. Table 12 presents data obtained for Hg in fish tissue and soil in two different method-evaluation studies (15,16). These results clearly show great differences in laboratories using the same method and differences among methods. NAA methods for Hg have also been subject to error due to volatilization of Hg from standard solutions during reactor irradiation. In an attempt to develop a standard plant material Bowen (17) prepared a kale sample which has since been analyzed for more than 30 elements by many different analysts. The results obtained are rather disconcerting.

The Cu values shown in Table 13 indicate generally good agreement among methods, except for the last two colorimetric values. These clearly represent a contamination problem. The results for Na shown in Table 14 are surprising. Sodium is readily determined by several methods and is generally considered an easy element to determine by NAA, AA, and flame photometry. The values obtained for kale, however, range from 1220 - 3250 ppm Na. The ranges of values obtained for several other elements in the

kale are shown in Table 15 - clearly the situation is far from satisfactory. Prior to this study, a U.S.G.S. initiated comparison of results for some rock standards has obtained findings very similar, although worse, to the plant study.

TABLE 12

TWO INTER-LABORATORY COMPARISONS

FOR HG IN FISH AND SOIL

[A] Hg in Fish Tissue (15)

Lab. No.	1	2	3	4	5	6
Hg (ppm)	0.10	0.21	0.08	0.44	0.23	0.50

[B] Comparison of Methods (16)

Method	Fish Tissue (ppm)	Soil (ppm)
Flameless AA (acid digestion)	0.93 - 1.80	0.2 - 1.46
Flame AA	0.70 - 1.80	0.56 - 29.0
Dithizone	1.31	2.70
NAA	1.77	1.47

TABLE 13

COMPARISON OF METHODS FOR CU IN STANDARD KALE (18)

Method Used	Values Obtained (ppm)
activation analysis	4.4, 3.6, 4.1, 5.6, 6.5
atomic absorption	4.7, 5.1, 5.2, 5.8, 6.3
colorimetric	3.7, 4.9, 6.5, 9.5, 17.0
polarography	4.5, 5.5
spectrochemical	4.6, 6.4

It is customary for an analyst to try his method on "idealized" samples. Commonly a "spike" is added to a simple solution and the effects of some possible interfering ions are studied. This approach does not test the method under the conditions in which it will be used. A far more meaningful evaluation of a method can be made by analyzing standard materials for which elemental concentrations are known.

The U.S.G.S standard rock samples (G-2, W-1, GSP-1, BCR-1, etc.) have been invaluable in testing methods of analysis of geochemical materials. Few materials of environmental interest have been standardized such that they provide a ready means of method evaluation. Fortunately the National Bureau of Standards has chosen to standardize some materials of environmental interest, and these are shown in Table 16. It should be emphasized that problems of analysis in the field of water pollution and air pollution require standards comprised of air particulates and waters and it is noteworthy that the NBS is working on this problem.

TABLE 14

COMPARISON OF METHODS FOR NA IN KALE (18)

Method Used	Values Obtained (ppm)
activation analysis	1930, 2160, 2500
atomic absorption	2280, 2170, 2970
flame photometry	1920, 2600, 3250
spectrography	1220

TABLE 15

RANGES OF VALUES OBTAINED FOR SOME TRACE ELEMENTS IN STANDARD KALE (18)

Element	Range (ppm)	Element	Range (ppm)
As	0.11 - 1.8	Hg	0.012 - 0.18
B	21 - 56	Mn	10 - 29
Br	23 - 29	Mo	0.59 - 3.1
Cd	0.38 - 1.0	Pb	1.6 - 5.4
Co	0.05 - 2.0	Se	0.017 - 0.64
Fe	59 - 157	Zn	20 - 38

TABLE 16

STANDARD REFERENCE MATERIALS

FOR ENVIRONMENTAL TRACE ANALYSIS

Material	Source	Elements Certified
Orchard Leaves (SRM 1571)	NBS	K, Ca, Fe, Mn, Na, Pb, B, Se, U, Zn, As, Cu, Rb, Ni, Hg, Cd
Bovine Liver (SRM 1577)	NBS	Na, K, Fe, Cu, Zn, Rb, Mn, Se, Pb, Cd, Hg
Coal (SRM 1630)	NBS	Hg
Kale	Univ. Reading	Not Certified
Fly Ash[1]	NBS	Hg, Be, Cd, As, Pb, V, Ni, Cr, Mn, Se, U
Coal[1]	NBS	Hg, Be, Cd, As, Pb, V, Ni, Cr, Mn, Se, U
Residual Fuel[1] Oil	NBS	Hg, Be, Cd, As, Pb, V, Ni, Cr, Mn, Se, U

Note[1]: Provisional Standards

SUMMARY

Trace analysis presents many pitfalls to the analyst used to working with higher concentrations and the combination of low elemental concentrations with the complexing of environmental materials results in several serious sources of error. The problems of sample collection and storage, separation techniques and analytical bias discussed in this talk only represent some of the sources of error possible in environmental trace analysis. It seems evident that more investigation of the sampling, storage and treatment of environmental samples is urgently required. Also new methods of analysis should be carefully checked through analysis of appropriate standard materials, if available. Careful consideration of all sources of error is even more necessary where large scale programs are to be carried out.

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Transport of Heavy Metals Through the Air Environment

Distribution, in terms of size and chemical composition, determines the effect of the atmospheric aerosol on visibility, health, and climate modification. These distributions are actually integral functions of a more general variable, the size-composition probability density function (Friedlander, 1970). The relationship between source characteristics and atmospheric particulate pollution, defined in terms of size and composition distributions, is complex. Chemical and physical processes occurring in the atmosphere, including chemical reaction, condensation and evaporation, coagulation and sedimentation, lead to major changes in the source emissions before they reach a receptor site. As a result, figures on tonnage emissions of particulates by themselves are of limited value in linking sources to what is actually in the atmosphere.

The goal of this work was to develop a method for relating atmospheric aerosol properties to source characteristics and to apply the method to data gathered from a 1969 study in Pasadena (Whitby, et al, 1972b). This method should be of value in establishing control strategies and in setting standards for particulate pollution.

At present, it is possible to collect data on a routine basis for (1) the size distribution of atmospheric aerosols in the range above 0.2μ in diameter, and (2) the chemical element compositions for many species integrated over all sizes. For research purposes, it is possible to measure size spectra for particles larger than 0.01μ and to fractionate chemical element composition rather coarsely with respect to particle size.

The calculations described in this paper, based on chemical and physical information about the aerosol, were carried out in the following way: Miller, Friedlander and Hidy (1972) and Friedlander (1971) have used a chemical element tracer method to estimate the contributions of the major primary sources to the aerosol at a given location in Pasadena, California. Scaling of emission inventories permit estimates of the contributions of other primary sources for which chemical tracers are not available. Secondary source contributions were calculated from the concentrations of sulfate, sulfite, nitrate and organics.

From the contributions of the separate sources and measured source size distributions and chemical compositions taken from the literature, the predicted size and chemical element distributions of the atmospheric aerosol have been calculated as shown below. In carrying out the calculations, certain models have been assumed for the secondary conversion processes.

The calculated spectra were then tested by comparison with measured size and volume distribution data. Calculated chemical composition

distributions with respect to size were also compared in the experiment, although only a few data of this type were available at the time the calculation was carried out.

Mathematically, the characteristics of the atmospheric aerosol can be related to the emission sources as follows: If dm is the total mass of particulate matter per unit volume of air in the particle size range between D_p and D_p+dD_p,

$$dm = \Sigma_i dm_i \qquad (1)$$

where dm_i refers to the mass contribution from the ith source.

Next, the following assumptions are made:

First, it is assumed that there is no interaction (coagulation) among the primary sources which means that the number of particles, dN_i, from the ith source in the size range D_p to D_p+dD_p is directly related to the mass as follows:

$$dm_i = \rho_i \pi \frac{D_p^3}{6} dN_i \qquad (2)$$

where ρ_i is the density of the particles from the ith source. Substituting in Equation 1, the result is:

$$dm = \rho \pi \frac{D_p^3}{6} dN = \Sigma_i \rho_i \pi \frac{D_p^3}{6} dN_i \qquad (3)$$

or
$$\rho\, dN = \Sigma_i \rho_i\, dN_i \qquad (4)$$

where ρ is the average density of the particulate matter in this size range and dN the number of particles per unit volume of air.

Second, it is assumed that homogeneous, gas phase nucleation processes do not lead to the formation of significant numbers of new particles in the size ranges of interest. While such particles may form in the size range below 100 Angstroms, there is evidence that the concentrations and size distributions of the primary emissions in the Los Angeles atmosphere are sufficient to relieve the supersaturation (Goetz and Pueschel, 1967; Husar and Whitby, 1971). With the primary particulate matter serving as condensation nuclei for the products of conversion processes, there is a shift in size from the point at which the particles are emitted to the point at which they are measured. If this shift is from the size D_p' to D_p, then:

$$n_i(D_p) = \frac{dN_i}{dD_p} = \frac{dN_i}{dD_p'} \Bigg/ \frac{dD_p}{dD_p'} = n_i(D_p') \Bigg/ \frac{dD_p}{dD_p'} \qquad (5)$$

The ratio dD_p/dD_p' represents the slope of the curve relating particle size at any time, D_p, to the initial size, D_p'. The secondary processes responsible for the conversion processes, hence this shift, are discussed in the next section.

SECONDARY CONVERSION PROCESSES

Three cases have been considered in the calculation of the size distribution and chemical composition changes resulting from the secondary formation processes. Each case involves a heterogeneous process in which material originally present in the gas phase is distributed among existing primary particles. In the first two cases, the concentration of the converted species is independent of particle size; in the third, it is a strong function of size.

1) Homogeneous, Irreversible Chemical Reaction in the Particulate Phase.

In this case, a gaseous species diffuses from the air and dissolves in the particles where it reacts chemically. When gas to particle transfer rates are much faster than chemical conversion rates in the particles, the dissolved gas is in approximate thermodynamic equilibrium with the gas phase. The volume concentration of the reacting species in the aerosol is independent of particle size in the absence of a curvature (Kelvin) effect. If the rate of conversion of the species in the particulate phase is determined by its concentration, all particles grow at rates proportional to their volumes as material is transferred from the gas to particle phase to make up for the amount converted. The fractional volume increase is the same for all particles. Since the total amount of species converted is proportional to the volume, the concentration of product will be independent of particle size.

2) Evaporation Equilibrium

The case of equilibrium between a pure salt such as sodium chloride and air containing water vapor has been discussed by Junge (1963). Starting as a small crystal, the sodium chloride does not go into droplet form until a relative humidity of about 75% is reached. This corresponds to the vapor pressure above a saturated solution. At this point, the diameter of the droplet about doubles; further increases in relative humidity lead to additional growth. If now, the humidity is decreased, it is found that the behavior is not reversible. When the humidity falls below 75%, the salt solution droplet does not crystallize but becomes supersaturated. The droplet size changes little until a relative humidity of about 40% when crystallization occurs.

3) Diffusion in the Gas Phase

In this case, which has been discussed by Husar and Whitby (1972), the rate at which the condensable species deposits on the primary nuclei is calculated from the theory of diffusion to single spheres. If the condensing substance is forming as a result of chemical reaction, its concentration in the gas is determined by the relative rates of formation and diffusional loss.

The time rate of change of particle volume at arbitrary Knudsen number ($Kn = 2\lambda/D_p$, where λ is the mean free path of the bulk phase) is given by Fuchs (1969):

$$\frac{dV}{dt} = \frac{2\pi D D_p}{1 + \ell Kn} \frac{c_\infty - c_o}{\rho} \qquad (6)$$

where D is the diffusion coefficient of the diffusing gaseous species, c_∞ and c_o are the concentrations far from the particle and at the surface and ρ is the density of the condensed gaseous species. The value of the constant ℓ ranges between 1.15 at Kn = 1.43 and 0.71 at Kn = 0. Considering the uncertainities in Equation (6), it is appropriate to set $\ell = 1$ for the following calculations. For the mean free path, λ, the value for air, $\lambda = 0.066$ was chosen.

If the concentration difference is independent of particle size, the diameter at time t is given by:

$$D_p = -2\lambda + \left[(D_p'+2\lambda)^2 + 2B(t) \right]^{1/2} \qquad (7)$$

where D_p' is the initial diameter, and B(t) is given by:

$$B(t) = \int_o^t \frac{4D}{\rho} (c_\infty - c_o) dt \qquad (8)$$

When the Kelvin effect must be taken into account, the concentration difference is given by:

$$c_\infty - c_o = \frac{P_v S}{RT} M - \frac{P_v M}{RT} \exp \frac{4\sigma M}{\rho RTD_p} \qquad (9)$$

Where S is the saturation ratio. The expression for the rate of change of particle diameter then becomes:

$$\frac{dD_p}{dt} = \frac{P_v M}{RT\rho} \frac{4D}{D_p+2\lambda} \left[S - \exp\left(\frac{4\sigma M}{\rho RTD_p} \right) \right] \qquad (10)$$

Defining D_{po}, the critical diameter below which diffusion does not occur, by:

$$S = \exp\left(\frac{4\sigma M}{\rho RTD_{po}}\right) \tag{11}$$

leads to:

$$\frac{dD_p}{dt} = \frac{P_v M}{RT\,\rho}\,\frac{4D}{D_p+2\lambda}\left[\exp\left(\frac{4\sigma M}{\rho RTD_{po}}\,\frac{D_p-D_{po}}{D_p}\right)-1\right] \tag{12}$$

If the supersaturation is of the order of a few percent, the exponential can be expanded and all but the first two terms dropped. For this case, the solution of the differential equation is:

$$\frac{(D_p-D_{po})^2-(D_p'-D_{po})^2}{2} + 2\,(D_{po}+\lambda)\,(D_p-D_p') + D_{po}\,(D_{po}+2\lambda)\,\ln\left(\frac{D_p-D_{po}}{D_p'-D_{po}}\right)$$

$$= \int_0^t \frac{4DP_v M}{\rho}\,\ln S\,dt \tag{13}$$

where D_p' is the initial particle diameter. D_p must be determined by a numerical solution. The equation can be put in a dimensionless form by defining the following variables:

$$w = \frac{D_p-D_p'}{D_{po}} \qquad \theta = \frac{4DP_v M \ln S}{\rho RTD_{po}^2}\,t$$

$$y = \frac{D_p'-D_{po}}{D_{po}} \qquad \bar{\lambda} = \frac{\lambda}{D_{po}} \tag{14}$$

Assuming constant supersaturation, the equation then becomes:

$$\frac{w^2}{2} + \left[2(1+\bar{\lambda})+y \right] w + (1+2\bar{\lambda}) \ln \left(\frac{w}{y}+1\right) = \theta \qquad (15)$$

The concentration of condensed material in the particle is given by:

$$c = 1 - \left(\frac{y+1}{w+y+1} \right)^3 \qquad (16)$$

If D_{po} is large, $\bar{\lambda}$ can be neglected, and solutions to the dimensionless equation can be found for various values of y and θ. Calculated concentrations as a function of $(D_p-D_{po})/D_{po}$ for various values of θ are shown in Figure 1 for $\bar{\lambda}$ equal to zero. In each case, the concentration of the condensing substance peaks sharply for small values of $(D_p-D_{po})/D_{po}$.

If the size-independent parameters (D,S,and c_∞) are independent of time, the concentration of converted material in a given size particle at any time will be independent of the initial aerosol size distribution. The same conclusion holds for cases 1 and 2 above.

THE PASADENA AEROSOL: PRIMARY SOURCES

A breakdown of sources for a sample of the Pasadena aerosol has been given by Friedlander (1971) as shown in Table 1. The calculation was made for a sample collected over an eleven hour period on September 3, 1969, at the Keck Laboratory of the California Institute of Technology in Pasadena. The sampling and chemical analysis procedures are described

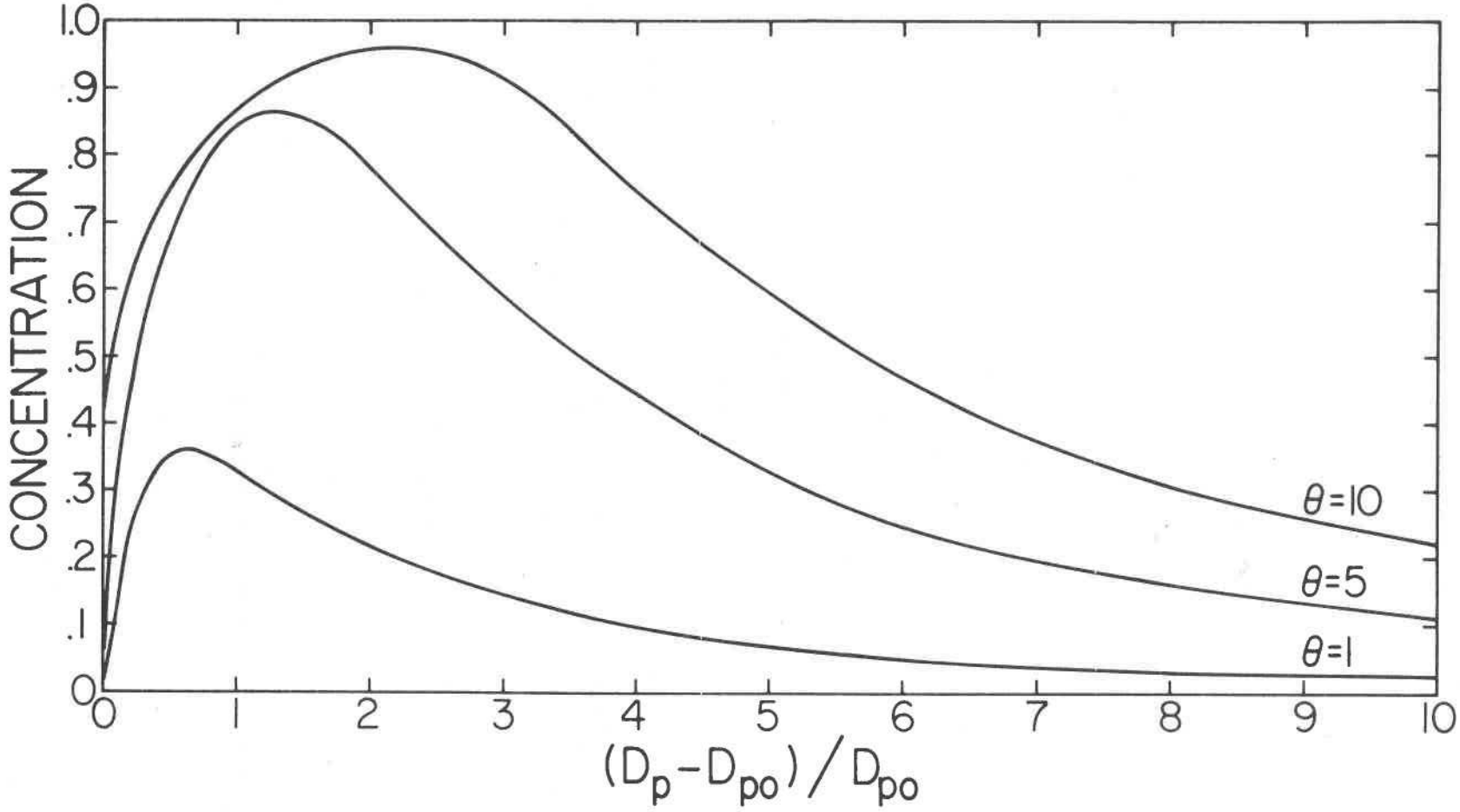

Figure 1. Concentration of condensed material as a function of dimensionless particle size with Kelvin effect present for various values of dimensionless time. Nuclei composed initially of one substance on which a second condenses.

D_p = particle diameter
D_{po} = critical diameter
θ = dimensionless time
$\overline{\lambda} \to 0$

TABLE 1

Source Contributions to the Pasadena Aerosol, 9/3/69 (Friedlander, 1971)

	Mass Contribution ($\mu gm/m^3$)	Density (gm/cm^3)	Volume Contribution ($\mu m^3/cm^3$)
I. Natural Background			
A. Primary			
1. Sea Salt	3.4[a] 2.77[d]	2.66	1.28[a]
2. Soil Dust	11.4	2.6	4.38
B. Secondary			
1. Organic vapors from plants			
2. Ammonia		Unknown	
3. Hydrogen Sulfide			
II. Anthropogenic			
A. Primary			
1. Automobile Exhaust	8.3[b]	1.8	4.51
2. Tire Dust	3.3	1.0	3.30
3. Cement Dust (roads and construction)	1.7	3.1	0.55
4. Fuel Oil Flyash	0.1	--	--
5. Diesel Exhaust	1.8	1.0	1.8
6. Aircraft Exhaust	2.7	1.8	1.50
7. Industrial Emissions	7.3	1.0	7.30
B. Secondary			
1. Sulfur Dioxide→Sulfite and Sulfate	7.2[c]	1.84	3.87
2. Nitrogen Oxides→Nitrate	∿0.1	--	--
3. Organic Vapors→Particulate	23.8	1.0	23.8
	71.1[a,e]		52.3[a,e]

a with chloride replaced by sulfate. Excludes associated water.
b with chloride replaced by sulfate.
c excludes sulfate involved in chloride replacement. Includes only particles larger than 0.6μm (Mueller, 1972).
d without chloride replacement.
e excludes sulfur compounds in particles smaller than 0.6μm.

elsewhere (Whitby, et al., 1972a; Mueller, 1972). The densities of the materials from the various sources are also given in Table 1 and the methods by which the densities were calculated are shown in Table 2.

In Friedlander's estimate, the chloride in the sea salt is replaced by sulfate formed in the atmosphere, so that the salt contribution calculated from the chemical element balance is reduced by the fraction of chloride (55%). In the present calculation, the sulfate was included in the marine contribution. Chloride replacement by sulfate leads to an increase of 0.8% in the sea salt volume contribution.

The calculation was based on three primary sources including the automobile, soil dust and the marine aerosol. These were assumed to have a characteristic average value determined by their mode of formation.

The automobile exhaust size distribution measured by Husar (Whitby, et al., 1969) was used as the automobile source size spectrum. The distribution was measured with the Minnesota Aerosol Analyzing System and covered a particle size range of 0.003 to 6μm.

The automobile exhaust aerosol was produced by an idling car, a 1962 Rambler, 58000 miles, low oil consumption. The exhaust was diluted 1:1000 rapidly so that the size spectrum was frozen at tailpipe conditions. The number spectrum is dominated by the small particles, about 0.01μm in diameter; most of the mass is in the range $0.05<D_p<0.2\mu m$ as shown in Fig. 3.

TABLE 2

Methods of Calculation of Emission Densities

SOURCE	METHOD
Sea Salt	Assuming element concentrations given by Friedlander (1971), with Cl^- replaced by $SO_4^=$ so that mixture of sulfates results.
Soil Dust	Literature value for soil grain density (Plummer and Dore, 1940)
Automobile Exhaust	Assuming element concentrations given by Friedlander (1971), with Cl^- replaced by $SO_4^=$ and a density of tar of 1.0 gm/cm^3
Tire Dust	Based on densities of rubber stocks
Cement Dust	Literature value (Baumeister, 1967)
Diesel Exhaust	Assumed
Aircraft Exhaust	Density of "amorphous" carbon
Industrial Emissions	Assumed
Sulfate	Density of 100% H_2SO_4
Sulfite	Assumed equal to sulfate
Condensed Organic Vapors	Assumed

Thus the primary automobile exhaust aerosol is below the optical subrange of $0.2<D_p<1.0\mu m$ (Ensor et al., 1972). This finding was confirmed by recent nephelometric measurements in the vicinity of freeways.

Data were not available for the size spectra of aircraft and diesel emissions. Particles from these sources are composed primarily of carbon and are formed as a result of incomplete combustion. Since their contribution to the total mass is relatively small, the automobile spectrum (scaled according to mass) has also been used for the aircraft and diesel emissions.

Blifford (1970) measured the aerosol size distribution at 15 meters over Death Valley, California. Samples were collected by an impactor carried by aircraft, and the particles were sized with an automated optical microscope system. These data have been used for the soil dust contribution. In the absence of data for tire and cement dusts, both formed by comminution processes, the Death Valley distribution (scaled to the mass) has been used for these sources.

The marine aerosol spectrum measured by Woodcook (1953) over the ocean near Hawaii was used for the sea salt source spectrum. The data taken at 1550 meters were chosen because the measured mass concentration, $2.3\mu gm/m^3$, was approximately equal to the $2.77\mu gm/m^3$ estimated sea salt contribution without chloride replacement. The measurements were made with an impactor. The particle size information is reported in terms of mass of sea salt in the particles.

The sources contributing to the industrial emissions are extremely varied, ranging from metal working facilities to chemical processing plants. It was decided, therefore, to use the sum of the other scaled primary spectra for the industrial source spectrum. The fuel oil flyash was omitted from the calculation because its contribution was small and good data on the spectrum are not available.

The automobile, soil dust and sea salt size distributions, scaled to their mass contributions, are shown in Figure 2. The sum of the scaled primary volume distributions is shown in Figure 3.

SECONDARY SOURCES: ORGANICS

The secondary sources and their estimated contributions are also given in Table 1. Significant quantities of non-volatile organic substances are produced by photochemical reactions involving gaseous automobile emissions. Three forms of the diffusion model 3 above were applied to the organic condensation: constant driving force for all particles, constant driving force for particles larger than 0.1 microns diameter with no diffusion for smaller particles, and driving force controlled by a supersaturated vapor phase with a critical diameter (Kelvin effect) of 0.09 microns. In making the calculations, the time term in the equations was varied until the volume increase of the size distribution was equal to the estimated source contribution. It was not necessary to make any assumptions concerning the properties of the condensing substances.

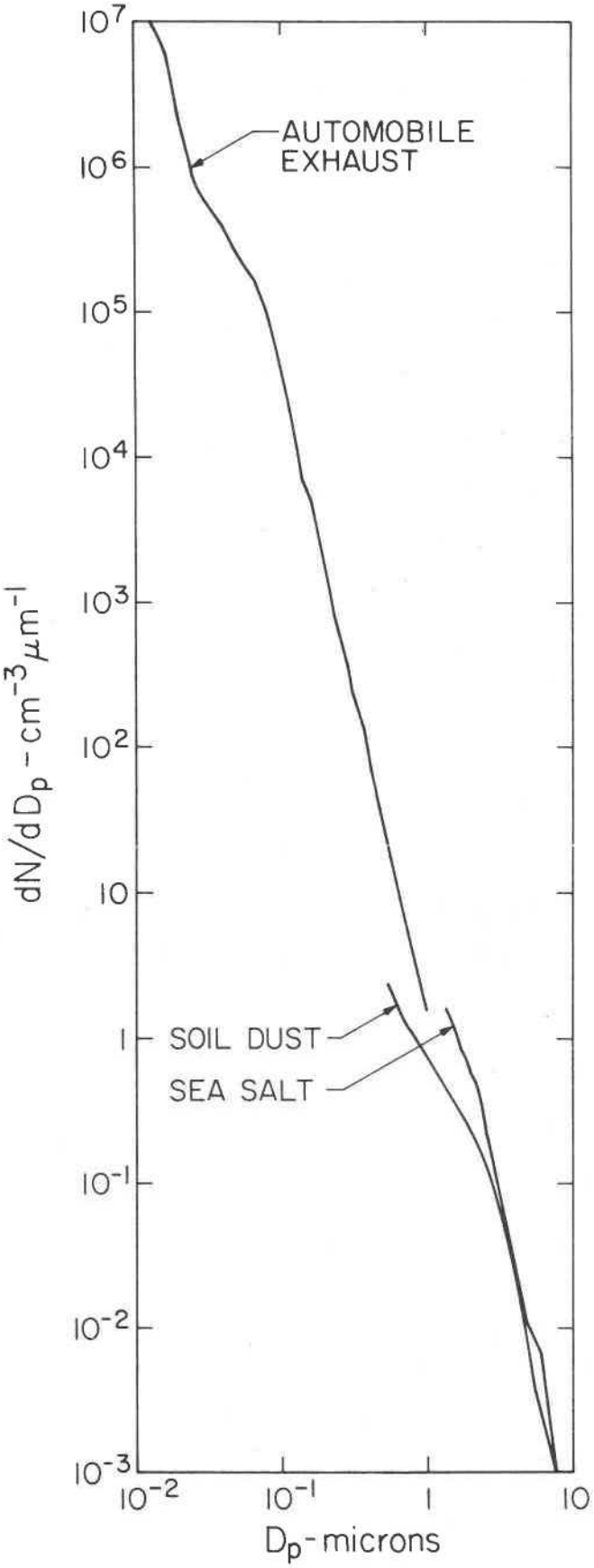

Figure 2

Size distributions of the automobile exhaust, soil dust and marine components of the Pasadena aerosol scaled to their mass contributions. These were used as the characteristic primary source size spectra.

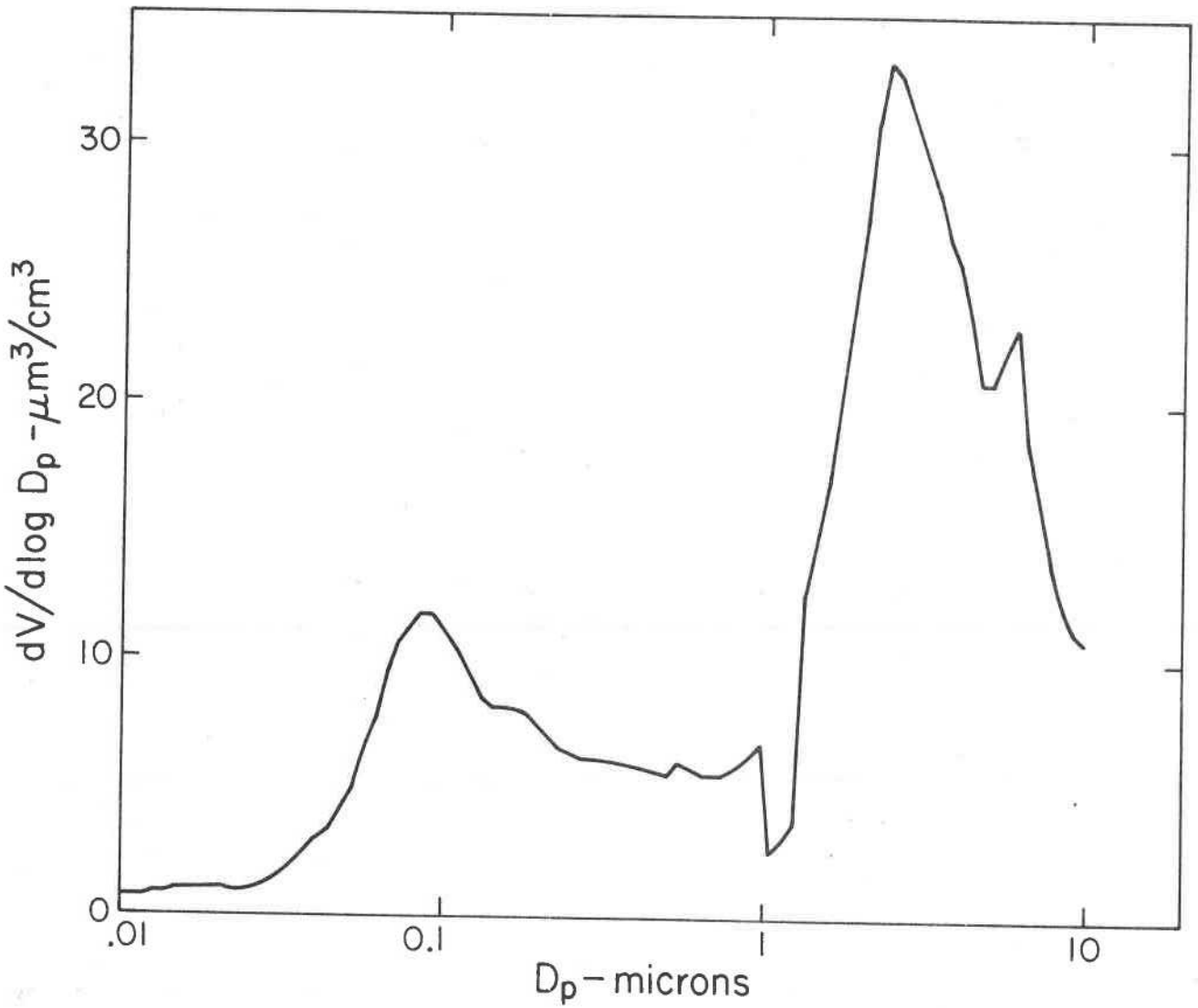

Figure 3. Sum of the scaled volume distributions of the primary sources. Note the lack of a mode between 0.10 and 1.0μm.

The assumption of constant driving force for all particles resulted in a large accumulation of material at the lower end of the size distribution. This accumulation was not observed in the measured size distributions (Whitby, et al., 1972b).

Electron microscope observations of automobile exhaust aerosols suggest that surface effects may prevent accumulation of vapors on particles smaller than about 0.1 microns. Only particles with diameters above this size appear to have a volatile component. Using a diffusion model with 0.1μm cut-off, the calculation showed that material tended to accumulate at about 0.13 microns which was also inconsistent with experiments. In addition, a discontinuity was introduced in the size distribution because only particles parger than 0.1 microns grow in size.

Finally, the supersaturated vapor model with Kelvin effect was applied. This led to a size distribution in good agreement with experiment and these results were used in the calculation.

SECONDARY SOURCES: SULFUR OXIDES

The most difficult substances to account for in this approach were the conversion products of the nitrogen and sulfur oxides. Reported nitrate concentrations were unexpectedly low. The sulfur in the particulate was measured only in particles larger than 0.6μm diameter. The sulfur was found to be largely in the +4 oxidation state with the ratio of sulfur in the sulfite to sulfate form about 2.3, corresponding to $3.5\mu gm/m^3 SO_4^=$ and $6.5\mu gm/m^3 SO_3^=$ in particles larger than 0.6μm (Mueller,

1972). This result is at variance with the usual assumption that sulfate is the dominant sulfur carrying species. The quantities and chemical nature of the sulfur and nitrogen compounds are linked to the water content of the aerosol and its distribution with respect to size.

The following simplified model was adopted for sulfur oxide conversion: All of the chloride in the marine aerosol component is replaced on a molar equivalent basis by sulfate and the resulting droplets are assumed to be saturated. This is consistent with the finding of Junge (1963) and others that salt solution droplets tend to supersaturate as humidity is decreased below the value at which crystallization would be expected to occur. The volume of the supersaturated droplet remains near the saturation value. Chloride replacement in the sea salt together with a small amount in the automobile aerosol accounted for 2.8μgm/m^3, and 6.0μgm/m^3 of water was associated with the saturated droplets.

Sulfite was then assigned to the aerosol with concentration uniform and independent of particle size so that a total of 10μgm/m^3 of sulfate and sulfite were associated with particles larger than 0.6μm diameter. This is equivalent to case 1 above, that is, conversion controlled by homogeneous reaction in the particulate phase. The aerosol volume concentration after chloride replacement and before the additional sulfur oxides were added was 54.6$(\mu m)^3/cm^3$. A total of 14.5μgm/m^3 of sulfite were added which, with the 2.8μgm/m^3 of sulfate replacing the chloride resulted in 17.3μgm/m^3 of sulfite and sulfate in the aerosol. This is about twice the daily average concentration reported for Pasadena by the National Air Surveillance Networks (EPA, 1971).

The sulfite was assigned after the organic conversion and growth calculation. This leads to a somewhat different result for the distributions of mass and chemical composition than if the calculation were carried out in reverse order. The extent of the difference is not known.

The effects of the secondary sources are shown in Figure 4 with the final calculated size distribution. Figure 5 shows the calculated volume distribution of the aerosol. The total measured volume below 5.2μm was 69.0$(\mu m)^3/cm^3$ while the calculated value below 10μm was 62.6.

COMPARISON WITH EXPERIMENT: PHYSICAL PROPERTIES

During the eleven hour time period in which the samples were collected for chemical analysis, the Minnesota Aerosol Analyzing System was used to measure aerosol size distributions at 20 minute intervals over the range of 0.0125 to 5.2 microns (Whitby, et al., 1972a). The average of these measured distributions is shown in Figure 3 along with the calculated average. Figure 4 shows the average measured volume distribution along with the calculated.

The agreement is good, the calculated and measured size distributions differ by a factor of two or less below 0.1μm and above 0.25μm. Both the calculated and measured volume distributions show a mode near 0.25 microns. The agreement at the upper end is not as close, the calculated distribution drops while the measured rises. Experimental error and statistical uncertainties lead to large uncertainties in the sizing of particles for this range of particle sizes.

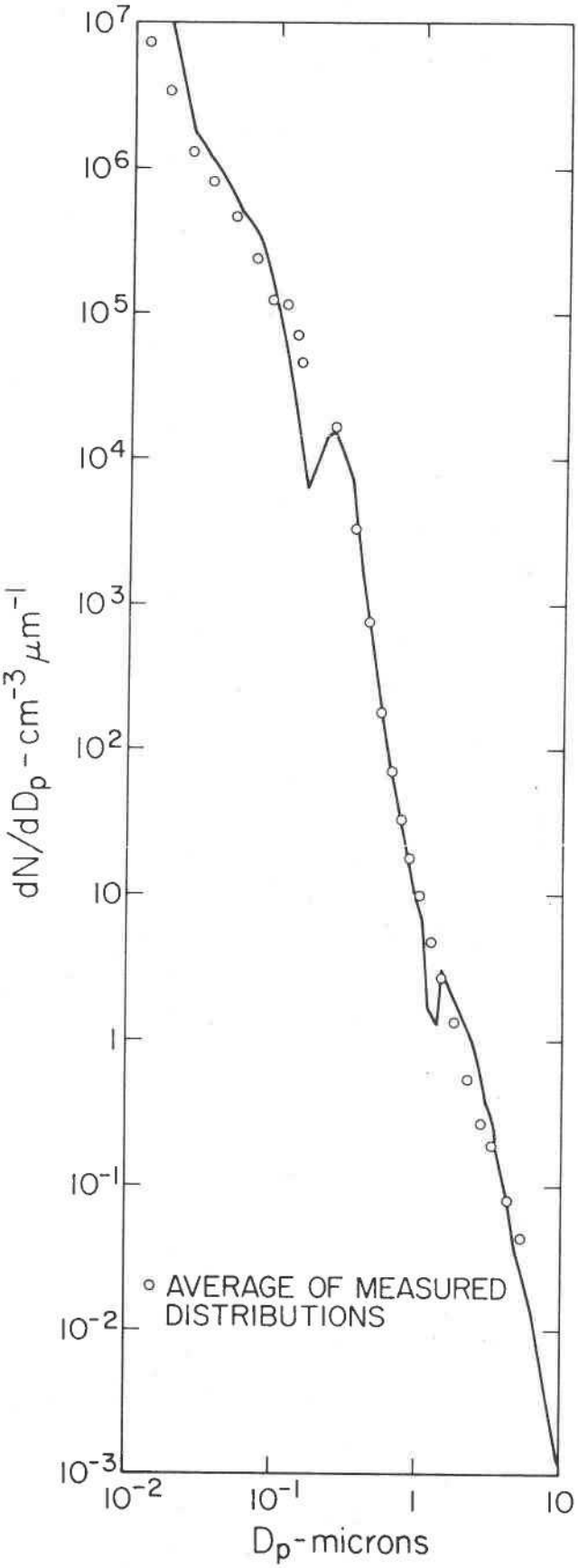

Figure 4.

Calculated and measured size distributions of the Pasadena aerosol on September 3, 1969, averaged from 0900-2000. The calculated distribution is dominated by the automobile exhaust below 0.1μm. From 0.1 to 1.0μm, the condensed organics dominate. Above 1.0μm the marine and soil dust components are of major importance.

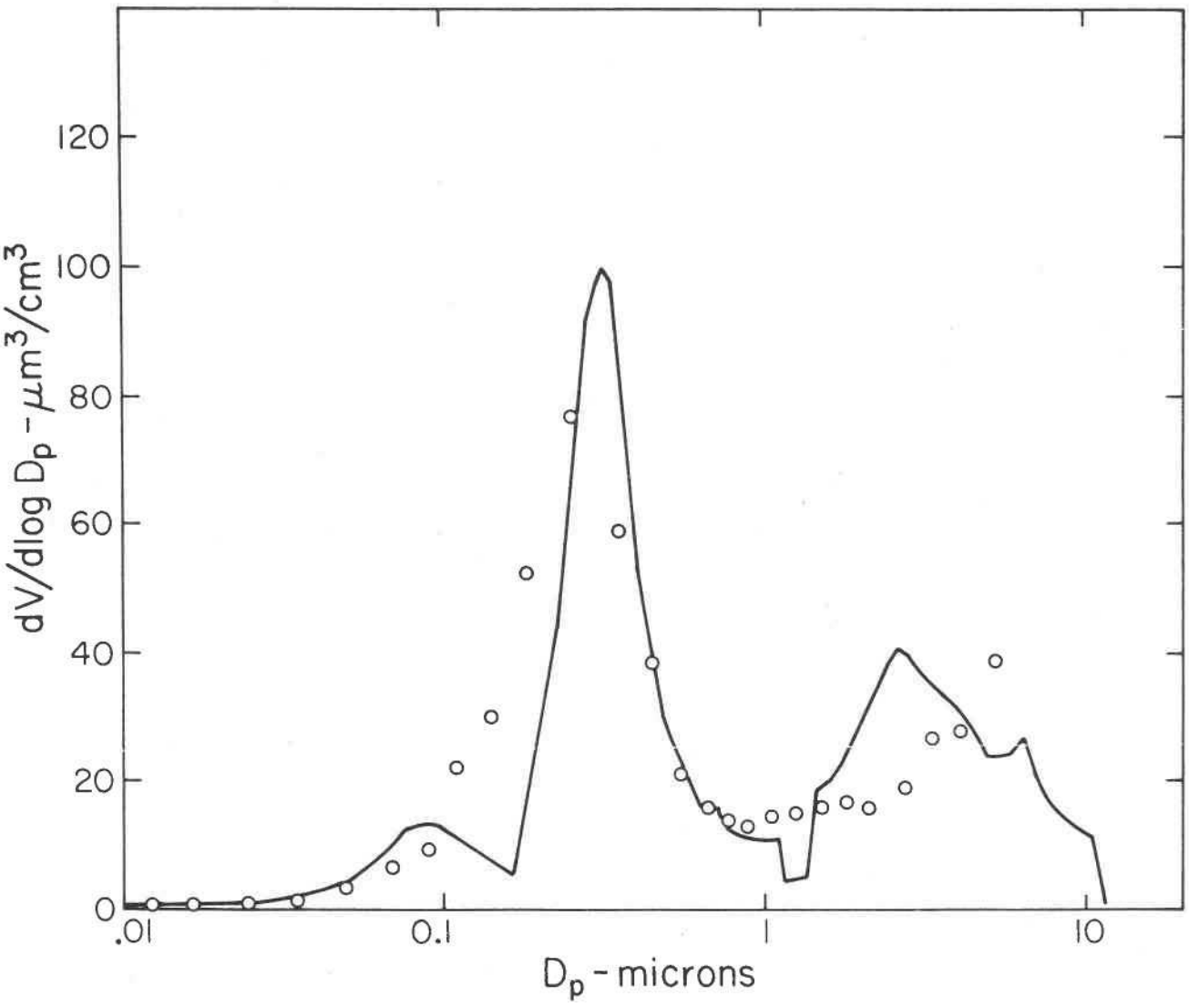

Figure 5. Calculated and measured volume distributions of the Pasadena aerosol averaged from 0900-2000 on September 3, 1969. The peak in the calculated distribution from 0.1 to 1.0μm results from the condensed organics. The soil dust and marine components cause the mode at 2.6μm. The calculated volume concentration was 58.3$\mu m^3/cm^3$.

Over the size range 0.0125 to 5.2 microns, the average of the measured aerosol volume concentrations was 69.0$(\mu m)^3/cm^3$ while the calculated value was 55.3. A possible explanation for the discrepancy is that water was associated with particles other than those of maritime origin. There may also have been other unidentified primary aerosol sources.

The calculated number concentration between 0.0125 and 5.2 microns was $2.02 \times 10^5 cm^{-3}$. The average of the measured concentrations was $8.58 \times 10^4 cm^{-3}$. Since 96% of the calculated number was less than 0.09 microns, it was the shape of the automobile exhaust size distribution below 0.09μm that determined the calculated number concentration.

The mode in the calculated distribution at 0.3 microns results from the condensation of the organic vapors. The agreement with the measured results in the location of the mode suggests that the form of the equations used to account for the conversion is reasonable, although the mechanism on which the form is based is still not proven. The concentrations of most organic vapors in the Los Angeles atmosphere are a few parts per million. This corresponds to partial pressures of only a few microns of mercury and for the atmosphere to be supersaturated, the vapor pressure would have to be of this order. The chemical nature of these substances is at present unknown; mass spectrometeric studies of the kind described by Schuetzle, Crittenden and Charlson (1972) may help shed light on this problem.

The calculated upper mode at 3 microns results primarily from the large amounts of water associated with the marine component of the aerosol. This mode is further reinformced by the soil dust contribution.

Calculations by Ensor, et al., (1972) indicate that much of the visibility reduction by the Pasadena aerosol is due to the mode in the volume distribution at 0.25 microns. The results of the present calculation indicate, therefore, that key factors in the reduction of visibility are the converted organic vapors and sulfur oxides; quantitative estimates of the effects of the various primary and secondary sources on visibility can be made based on the results of this paper.

COMPARISON WITH EXPERIMENT: CHEMICAL CHARACTERISTICS

Chemical element concentrations in the sources are given Friedlander (1972). These were assumed to be independent of particle size and were used in calculating the chemical element distributions. The predicted concentration and mass distributions of lead, aluminum, sodium and the condensed organic vapors are shown in Figures 6 through 9 as a function of particle size. The mass distribution function is defined by:

$$\left(\frac{dm}{d\log D_p}\right) = C_i(D_p)\,\rho\,(D_p)\,\frac{dV}{d\log D_p} \qquad (17)$$

where $C_i(D_p)$ is the mass concentration of species i in a particle of size D_p and the logs are taken to the base 10.

These elements were representative of the three sources of primary particulates on which the calculation was based. The mass distribution of lead tends to fall in the 0.1 to 1μm range while sodium and aluminum are located mostly in the range above 1μm.

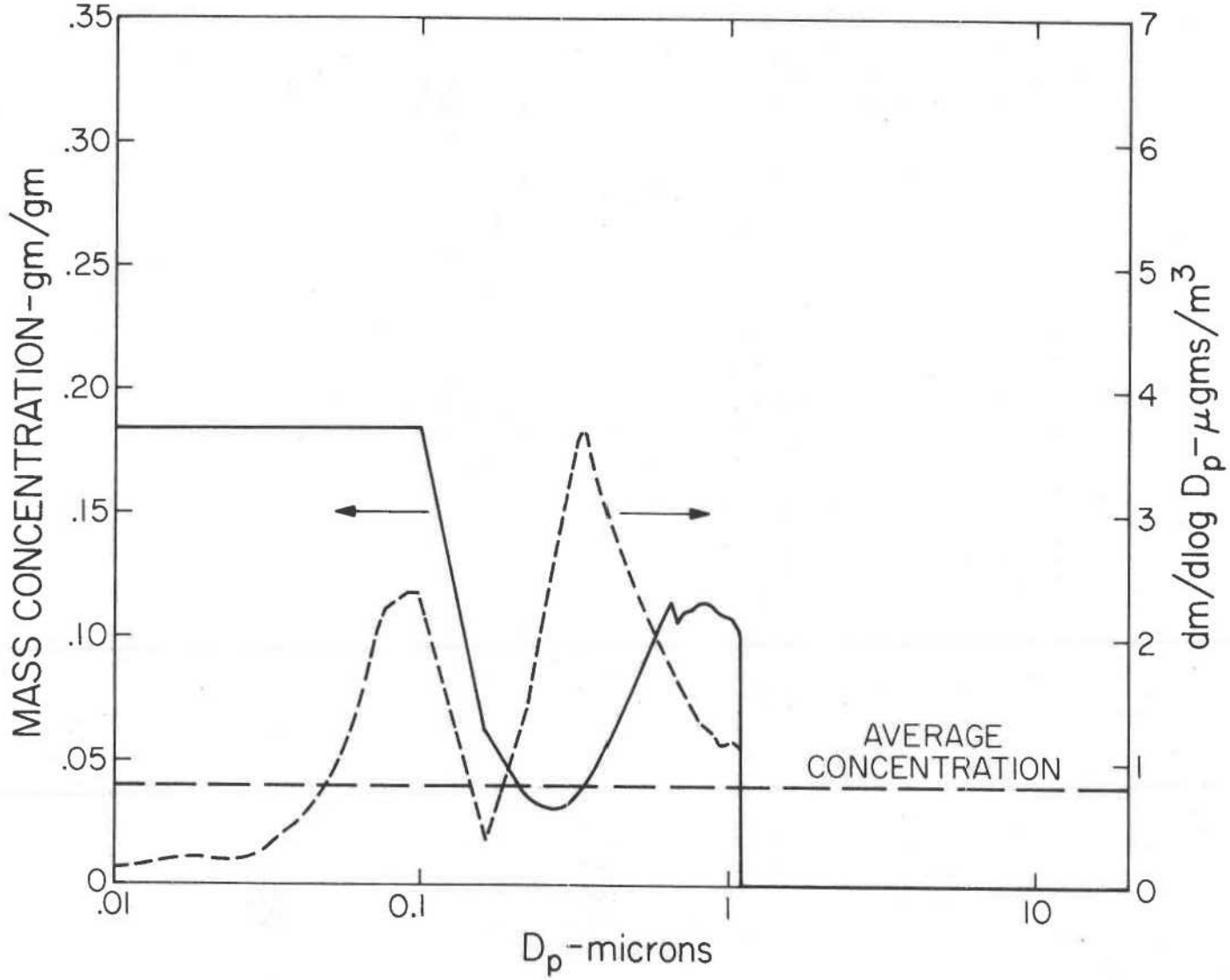

Figure 6. Calculated lead concentration and mass distributions. The high concentration below 0.1μm is due to the primary automobile contribution. The peak in the mass distribution at 0.1μm corresponds to the peak in the primary automobile volume distribution. The drops in the distributions above 0.1μm result from the large amounts of condensed organics. The second peaks above 0.25μm result from the shift to larger sizes caused by condensation. The drop above 1.0μm is due to the absence of the automobile component in larger particles.

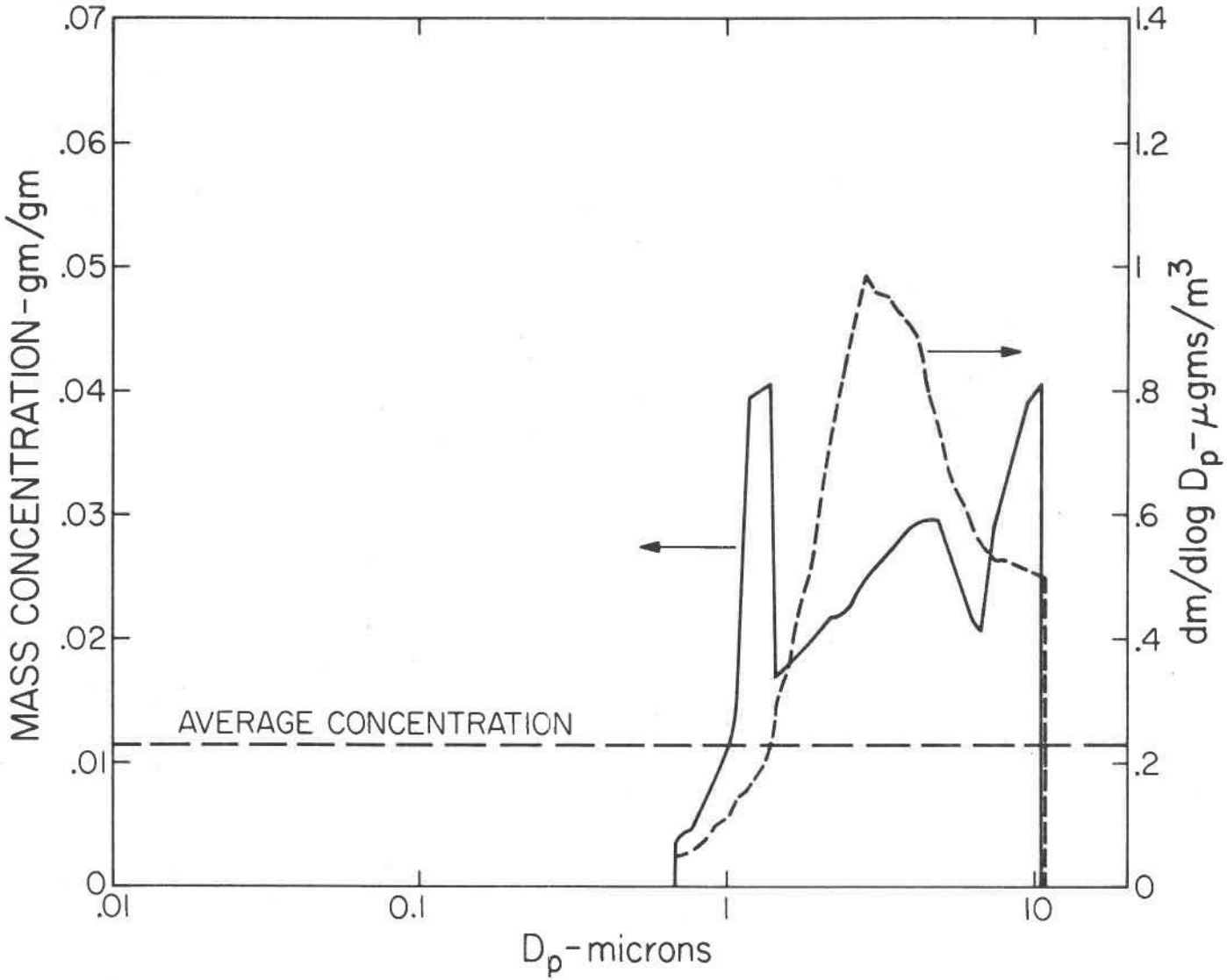

Figure 7. Calculated aluminum concentration and mass distributions. Aluminum is present only in the soil dust and Portland cement components of the aerosol. These sources contribute only to the aerosol above 0.5μm.

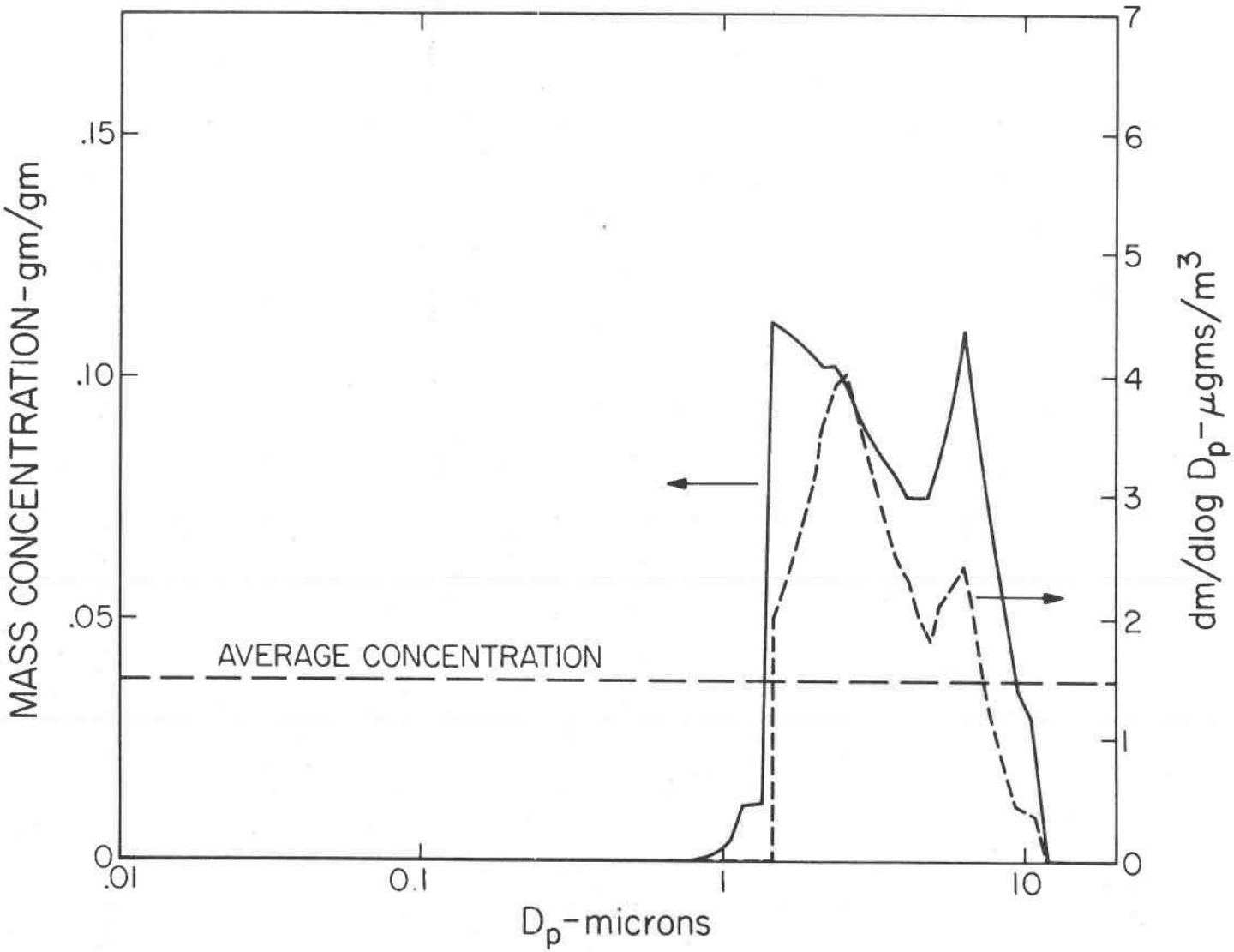

Figure 8. Calculated sodium concentration and mass distributions. Sodium is found primarily in the marine components with smaller quantities in the soil dust and cement.

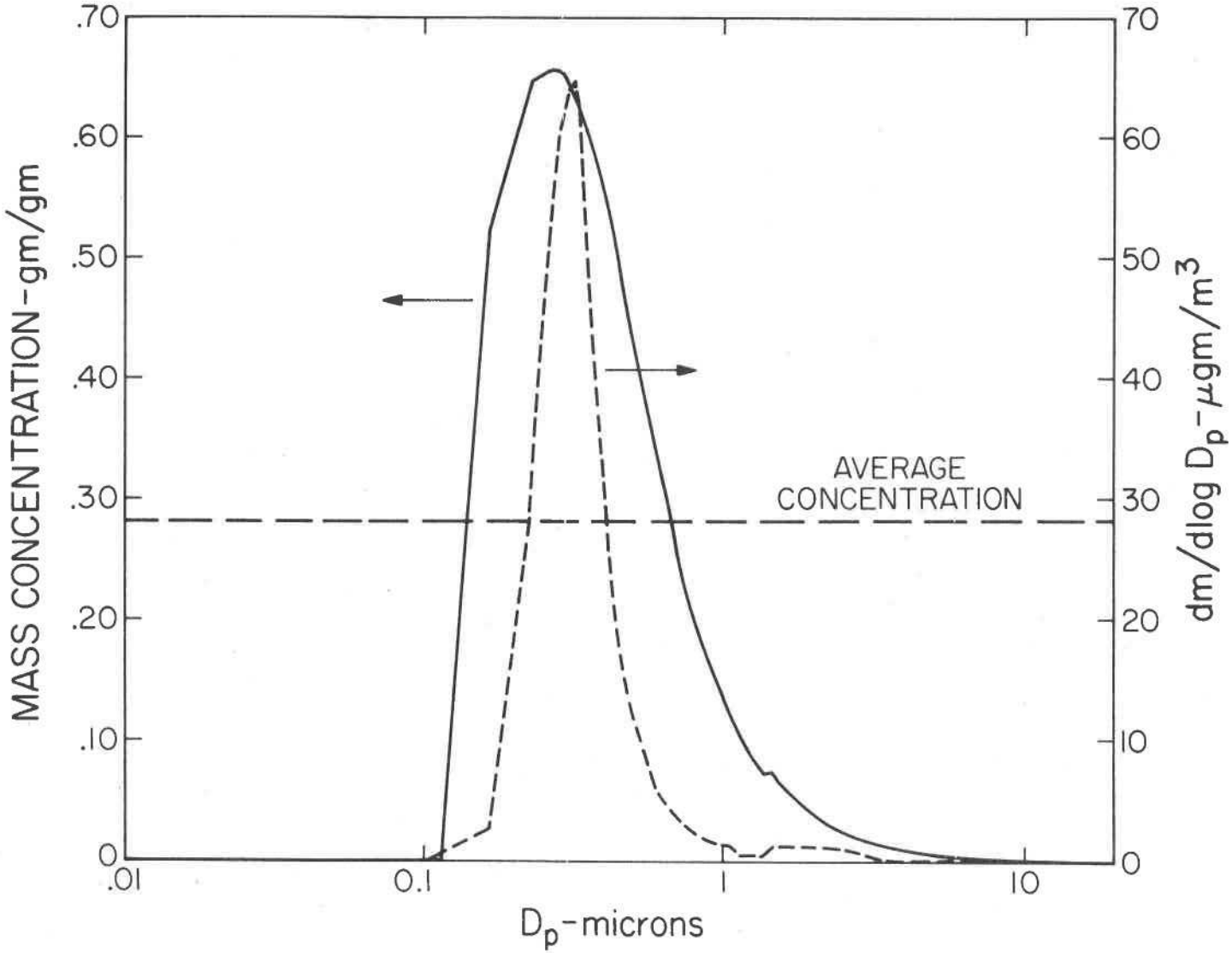

Figure 9. Calculated concentration and mass distributions of condensed organic vapors. The peak in the aerosol volume distribution at 0.35μm corresponds to the peak in the organic mass distribution. The peaked shape of the concentration distribution is a characteristic result of condensation with a Kelvin effect.

The curves of mass concentration vs particle size show the regions of the spectrum which are enriched (or depleted) in the substance under consideration. In the region where the curve falls above the average concentration, the aerosol is enriched.

The lack of experimental data on element distributions with high particle size resolution makes it difficult to compare the calculated and measured distributions. Qualitatively, however, the shapes of the distributions of lead, aluminum and sodium agree well with those reported by Mueller (1971) from the impactor samples used for the chemical analysis. Mueller's measurements and the present calculation show the three species occuring in the same particle size range. Since each of these three species is associated primarily with a single source, the agreement with the measurements add support to the choices made for the size distributions of these sources.

The distribution of the mass concentration of the secondary conversion product with particle size is determined by the mechanism of the conversion process. Hence the form of the distribution can in some cases be used to infer the nature of the mechanism. For example, the sharp peak in the organic distribution (Figure 9) is typical of diffusion with the Kelvin effect. This is also shown in Figure 1. Care must be taken in drawing such inferences, however, when the condensing species is hygroscopic. In this case, the condensation of water will tend to cause the concentration of converted species to distribute itself in a manner between the diffusional result and size independence.

SUMMARY AND CONCLUSIONS

A method has been developed for calculating the number and chemical element size distributions of aerosols in a polluted atmosphere based on measured source characteristics and a chemical element balance for collected aerosol samples. The method assumes that:

1. No interaction occurs among primary aerosol emissions.
2. Certain simplified models can be used to account for the conversion of secondary sources to the particulate phase.
3. No modification of the primary emission size distributions occurs except through dilution and the secondary conversion processes.

The method has been applied to the Pasadena aerosol and good agreement obtained with measured size and chemical element distributions. As our understanding of the mechanisms of the secondary conversion processes improves and as more measured primary source size distributions become available, the method can be further refined and the accuracy improved.

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Presented November 2, 1972 by ROBERT G. TARDIFF, National Environmental Research Center, EPA, Cincinnati, Ohio.

Health Standards for Metals in Drinking Water

In the decade since Silent Spring,[1] the citizens of this country have become more acutely aware of man's exposure to a wide variety of chemicals in his food, air, and water. In general, man has greatly improved the quality of his living as a result of the discovery and application of many synthetic chemicals. Some would argue -- and in some cases justifiably -- that such improvements were achieved at the expense of true quality of life. In some cases the very properties that made certain chemicals highly desirable for human use are now regarded as a threat to man's healthful existence.

As a result of newly-founded concern over possible adverse side effects from the wide use of chemicals, questions such as the following are being asked with increasing frequency: (1) What adverse effects -- direct or indirect -- can synthetic and naturally-occurring compounds in man's environment have on man's health? (2) What deleterious effects can these chemicals produce on the various elements of man's environment? (3) What values are to be applied to the risk-versus-benefit equation in order to decide the extent of man's use and control of chemicals?

The second question is best dealt with by environmentalists; and the third should be discussed by moralists and lawyers. The first question, the evaluation of hazards to man himself, is the subject of this discussion within the limited framework of man's exposure to metallic compounds via drinking water.

The evaluation of hazards from chemicals in drinking water and the setting of standards for these compounds are under the legal jurisdiction of the U. S. Environmental Protection Agency. The actual development of standards for these agents is directed by the Office of Water Programs with assistance of experts both inside and outside of the Agency. The National Environmental Research Center of Cincinnati, Ohio, is the focal point for EPA-directed research on the health aspects of chemicals in drinking water.

The standards presently in force for chemicals in potable water were promulgated by the U. S. Public Health Service in 1962.[2] These standards are listed on Table 1 and are compared with the proposed standards as revised by the U. S. Environmental Protection Agency.[3] In discussing the biologic data which must be evaluated to set standards, and in emphasizing the necessity of current research, mercury and its derivatives will be used as examples.

There are 2 bases for establishing a maximum allowable limit for a chemical in drinking water: (1) Health -- the limit is derived from toxicity data for the protection of human health -- and (2) Esthetics -- the limit is derived from organoleptic data where taste and odor problems occur at a lower concentration than that considered adequate to protect human health.

TABLE 1

Drinking Water Standards for Some Elements

Compound	USPHS 1962 Standard[2] (mg/l)	Proposed USEPA Standard[3] (mg/l)	Basis for Standard
Arsenic	0.05	0.1	Health
Barium	1.0	1.0	Health
Cadmium	0.01	0.01	Health
Chloride	250	250	Esthetics
Chromium	0.05	0.05	Health
Color	15	15	Esthetics
Copper	1	1	Esthetics
Cyanide	0.2	0.2	Health
Fluoride*	1	1	Health
Foaming Agents	-	0.5	Esthetics
Iron	0.3	0.3	Esthetics
Lead	0.05	0.05	Health
Manganese	0.05	0.05	Esthetics
Mercury	-	0.002	Health
Nitrate	45	45	Health
Odor	3 ton	2 ton	Esthetics
Organics (CAM)			
CCE	0.2	0.3	Health
CAE	-	1.5	Health
Selenium	0.01	0.01	Health
Silver	0.05	0.05	Health
Sodium	-	270	Health
Sulfate	250	250	Esthetics
Turbidity	5 Tu	1 Tu	Esthetics
Zinc	5	5	Esthetics

TABLE 1 (cont'd)

Compound	Proposed USEPA Standard (mg/l)	Basis for Standard
Pesticides		
Aldrin	.001	Health
Chlordane	.003	Esthetics
DDT	.05	Health
2,4-Dichlorophenoxylacetic Acid	.02	Health
Dieldrin	.001	Health
Endrin	.0005	Health
Heptachlor	.0001	Health
Heptachlor Epoxide	.0001	Health
Lindane	.005	Health
Methoxychlor	1.0	Health
Silvey	.03	Health
Toxaphene	.005	Esthetics

The 4 predominant factors considered in developing a standard, or maximum allowable limit, for metals and other chemicals are listed in Table 2.

TABLE 2

Factors Important in Developing Drinking Water Standards for Chemicals

1. Exposure: Type and Degree
2. Population Exposed
3. Physical State of Chemical
4. Toxicity to Man and Experimental Animals

The ultimate goal of the evaluation of these factors is to determine a safe intake in one medium (in this case, drinking water) that takes into account environmental exposure via other media. Identification of exposure via food and air, and the extent of such exposure, assists in placing limitations on drinking water standards in order to avoid unnecessarily high exposures. For those chemicals that have an FDA tolerance or an air quality standard, exposure up to those legal levels is assumed as a precautionary measure in developing a maximum allowable limit in drinking water.

In the case of mercury, for example, it is assumed that all fish in the diet contain 0.5 ppm of Hg as methyl mercury; and, consequently, the water standard for Hg is adjusted downward to maintain a safe allowable daily intake. The degree of exposure allowed from different media becomes

more significant with consideration of the degree of absorption via inhalation, ingestion, and skin contact.

Susceptibility to chemical intoxication is known to vary among populations and among individuals within a population. In some cases, hyper-susceptibility is genetically related, as with the increased susceptibility of Eskimos to intoxication by methemoglobin-forming agents such as nitrite (NO_2^-).[4] In some species there has been observed a sex-related response to chemicals, as for example, the greater toxicity of organophosphate pesticides to female rats than to males.[5] Generally, age influences toxic responses, with the very young and the elderly usually more susceptible to intoxication than young adults.

Stress and illness can also be predisposing factors to chemical intoxication. If a standard were applied only to a restrictive group (such as the patients in an Army field hospital), greater control and more precise definition of population differences in response could be exercised. However, in applying a standard across the breadth of a heterogeneous population such as that in the U. S., this factor is considered only minimally because of the relative lack of knowledge of the influence of these factors on chemical toxicity.

INHERENT TOXICITY

The chemical state of a metal is directly related to its inherent toxicity. The toxic potential of alkyl mercurials is much greater than for the other forms. For metals such as arsenic, antimony, chromium, and

selenium, one valence state of each is more toxic than the other valence state (e.g., trivalent arsenic is more toxic than pentavalent species). Questions have arisen about the possibility that a metal covalently bound to animal tissue may be more or less toxic when ingested than the same agent consumed at the same dose but in the more loosely-bound or free forms. Few investigations have dealt with this problem.

Of the 4 factors listed in Table 2, the toxicity to man is generally considered the principal determinant for all health related standards. The standard, or maximum allowable limit, must afford reasonable assurance that no illness will result under the defined conditions of exposure. Although absolute guarantees are impossible, standards have been developed that provide a reasonable low probability of risk to human health.

It would be misleading to assume that all desirable toxicity data have been generated, or need to be generated, before establishing a standard. And by the same token, the setting of a standard does not affix it in legal cement nor should it preclude additional research to discover more of its properties.

For those metals having a maximum allowable limit, the standards were derived by using all available information -- albeit relatively incomplete -- and making the best professional judgments. To date, research of one type or another is continuing on some of these agents.

THREE CATEGORIES

The toxicity data reviewed for a metal fall into 3 categories: (1) human clinical investigations, (2) prospective and retrospective epidemiological studies, and (3) controlled studies with experimental animals. Clinical and epidemiologic data on human exposures to metals originate mostly from occupational studies. In fewer instances, evidence of intoxication as a result of exposure via environmental sources is reported. Mercury exemplifies this point. The vapor and the inorganic salt of Hg have been extensively investigated among individuals exposed to the metal in their work. Detailed information has been developed on the physiologic, pharmacologic, and toxicologic properties of both forms of mercury in man. Mercurial diuretics offer a substantial source of clinical data for various organic forms of mercury. From such studies, much is known about the renal effects - both pharmacological and toxic - of these agents.

By contrast, the toxic effects of the alkyl mercurials were identified mainly from cases of accidental poisoning from contaminated foods. Two of the episodes of chronic alkyl poisoning occurred in Japan and one in the United States. (The illness is often referred to as Minamata Disease for the city in Japan where the poisoning was first recognized.

Chronic alkyl mercury poisoning is characterized mainly by major neurological symptoms and leads to permanent damage. Some of the clinical features include numbness, incoordination, loss of vision and

hearing, and intellectual deterioration. Autopsy findings of the clinical cases reveal severe damage throughout the cortex and cerebellum. The true hazard of alkyl mercurials is emphasized by cases in which children had congential alkyl mercurialism as a result of the mothers' exposure to the toxin during pregnancy. In most of these cases the mothers themselves were asymptomatic.

Although such cases of human poisonings are tragic, they do offer the opportunity to determine how man responds to a given insult. Some of the more important factors which are sought in assessing reports of human poisonings are (1) the dose at which symptoms were observed and the correlation between increasing dose and increasing severity of illness; (2) specific signs and symptoms of intoxication; (3) critical organs and clinical parameters affected; and (4) type of exposure: acute or chronic or intermittent.

Frequently, reports of human chemical poisonings relate acute exposures only, describe mostly non-specific symptomatology, and fail to determine the degree of exposure. In some cases the etiologic agent is questionable.

As a result, few such reports can actually be used in deriving standards for chronic exposure. However, for a few metals such as lead, arsenic, and mercury, much useful data have been generated because of the in-depth study of the toxicity of these agents to man. For a more detailed treatise on the information gained from methyl mercury poisoning in man,

the following should be consulted: Minamata Disease[6], Methyl Mercury in Fish[7], and Mercury in the Environment[8].

USE OF ANIMALS

Investigations using experimental animals to study the toxicity of any metal can offer much useful information about the metal's biological activity. In comparison to human studies, animal experimentation offers greater control of environmental factors that can influence toxicity, more accurate definition of dose-response relationships, and easier accessibility to certain organs and biochemical systems. Although it is known that alkyl mercury is teratogenic in man, animal studies are necessary to determine the minimum effective dose, duration of exposure, times of maximum sensitivity, etc.

With animals, more sensitive parameters can be examined than with man, as, for example, the study of energy metabolism in the brain cortex after alkyl mercury exposure. Correlations between levels of a toxicant in a critical organ (e.g., brain) with that in an available tissue (e.g., red blood cells) and with intake are also possible with small animal studies. Several animal studies involving methyl mercury have demonstrated that these models are good indicators of absorption, metabolism, and excretion of the compound in man.

Thus we find that methyl mercury is absorbed approximately 98% from gastro-intestinal tract whereas inorganic mercury is absorbed only about 2%. Its half-life in experimental animals and in man is approximately

70 days. The excretory processes for methyl mercury have been identified, and this information forms the rationale for the testing of thiol resins as antidotes to alkyl mercury intoxication. The greatest advantage of animal models, however, is in the investigations of the mechanism of action of toxins. The understanding of these mechanisms can lead to a more rational control and study of environmental agents.

The setting of a standard for a metal like mercury requires first, the evaluation of the applicable biological data described previously, followed by judgments as to what value(s) will be used as the basis for application of safety factors. A rough guideline for this judgment is to use information from the most sensitive and functionally significant system tested under the conditions most closely approximating man's exposure.

When there is a choice between "minimum effect" levels and "no effect" levels, the former should be used in order to avoid an unnecessarily restrictive standard, although, extenuating circumstances could temper this choice. For mercury, a "minimum effect" level was chosen based on a study on the toxicity to adult humans. The mercury standard is based on epidemiologic evidence which indicates that the lowest whole blood concentration of methyl mercury associated with mild toxic symptoms is 0.2 μgm Hg^{++}/gm.

This blood concentration can be compared to 60 μgm Hg/gm hair. These values, in turn, correspond to prolonged, continuous exposure of approximately 0.3 mg/70 kg/day (4 μgm/kg/day). By applying a safety factor of 10 to this "minimum effect" exposure level, the maximum allowable

exposure by all routes should be 30 µgm/adult/day (0.4 µgm/kg/day). The mercury standard of 2 µgm/liter of water allows approximately 15% of the maximum allowable exposure to be contributed by drinking water.

In fact, very few water supplies approach this limit of 2 µgm/l. Even if this limit were reached consistently in water supply, additional safety factors may exist by virtue of the possibilities that not all of the mercury may be in the most toxic form and that exposure from other sources may be limited.

Although a maximum allowable limit for Hg has been proposed and may be adopted in the Federal Register, there is no cause to believe that this standard is infallible or immutable. For the reasons that Hg, particularly in the methylated form, has been incompletely studied and that alkyl mercurials are extremely toxic, research must continue on the definition of toxic potential and on the elucidation of the mechanisms of toxicity. Some unanswered questions about methyl mercury toxicity are:

(1) Can methyl mercury alter the toxicity of other environmental chemicals and perhaps create greater hazards?

(2) At what chronic doses does methyl mercury cause initial functional changes in the brain and other tissues?

(3) What factors influence the fetotoxicity and teratogenecity of methyl mercury?

(4) What are the doses of methyl mercury to an expectant mother that will produce minimum effects of functional significance to target organs in the fetus?

The balance of this discussion will recount briefly some of the research at the Water Supply Research Laboratory whose object is to determine if methyl mercury alters the toxicity of other chemicals by influencing their metabolism. Several compounds are metabolized in the liver by enzymes in the smooth endoplasmic reticulum. Alterations of the metabolism of foreign agents can lead to increased or decreased toxicity depending upon the biological activity of the parent compound and its metabolites.

For studies described below, the test systems chosen were the oxodative detoxification of the phosphorothioate EPN and the O-demethylation of p-nitroanisole. Figure 1 depicts the reactions involved. With TPNH as co-factor, both substrates are oxidized aerobically to p-nitrophenol. The reactions are catalyzed by the hepatic microsomal enzymes.

Assay of the EPN Detoxification System was accomplished by the method of Neal and DuBois,[9] and that of the p-nitroanisole O-demethylase by the method of Netter and Seidel.[10] Adult male albino rats of the Charles River strain were used in all experiments. All animals were given standard laboratory diet and water ad libitum. Methyl mercury was administered as the chloride salt. When methyl mercury was administered intraperitoneally, the solvent concentration was adjusted to deliver 1 ml/kg.

To determine the direct effect of methyl mercury on the two enzyme systems, varying concentrations of the agent were incubated with the microsomes of untreated animals, and the activities of the enzymes were measured. At concentration as high as 2.5×10^{-2}M of methyl mercury,

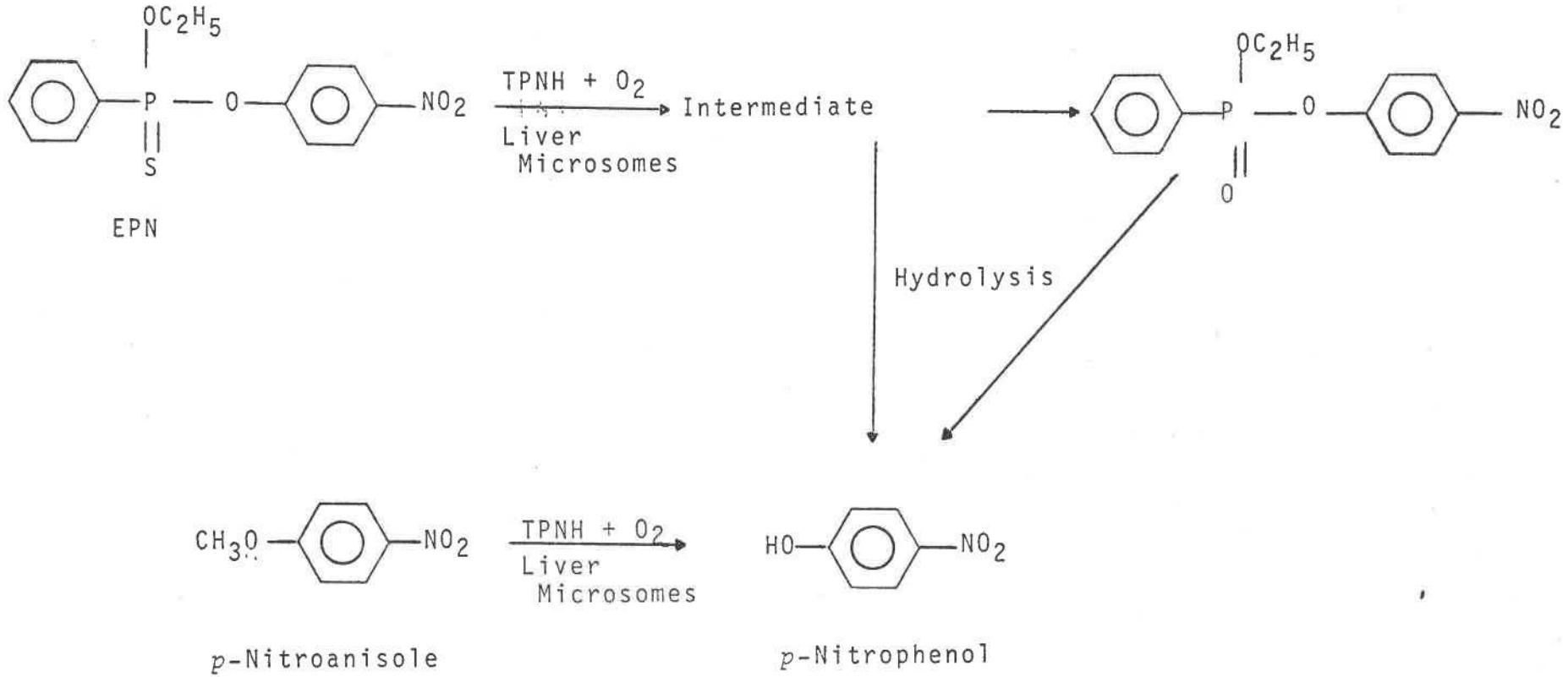

Figure 1. Enzymatic oxidation of EPN and p-nitroanisole by hepatic microsomal enzymes of the rat.

no change in enzyme activities was observed as compared with control incubation media.

ADULT MALE RATS

Since methyl mercury is more of a chronic than an acute toxin, an attempt was made to assess the effect of varying subacute doses of methyl mercury on the activities of the hepatic EPN Detoxification System and the p-nitroanisole O-demethylase. Adult male rats were injected intraperitoneally with methyl mercury at doses of 0.1, 0.25, 0.5, and 2.0 mg/kg/day for 14 days. The agent was administered in two solvents, water and corn oil, for comparison.

The results are presented in Figures 2 and 3. The activity of either enzyme system was unaffected by the administration of methyl mercury in corn oil. However, when methyl mercury was administered in water, the activity of both enzymes was inhibited by doses of 0.25 mg/kg/day and higher. At 2 mg/kg/day the activity of each enzyme was inhibited by approximately 50%.

Another series of experiments was performed to determine the effect of duration of exposure to methyl mercury on the activities of the two hepatic microsomal enzymes. Adult male rats were injected intraperitoneally with 2 mg/kg/day of methyl mercury for 7, 14, and 21 days. The results are presented in Figures 4 and 5.

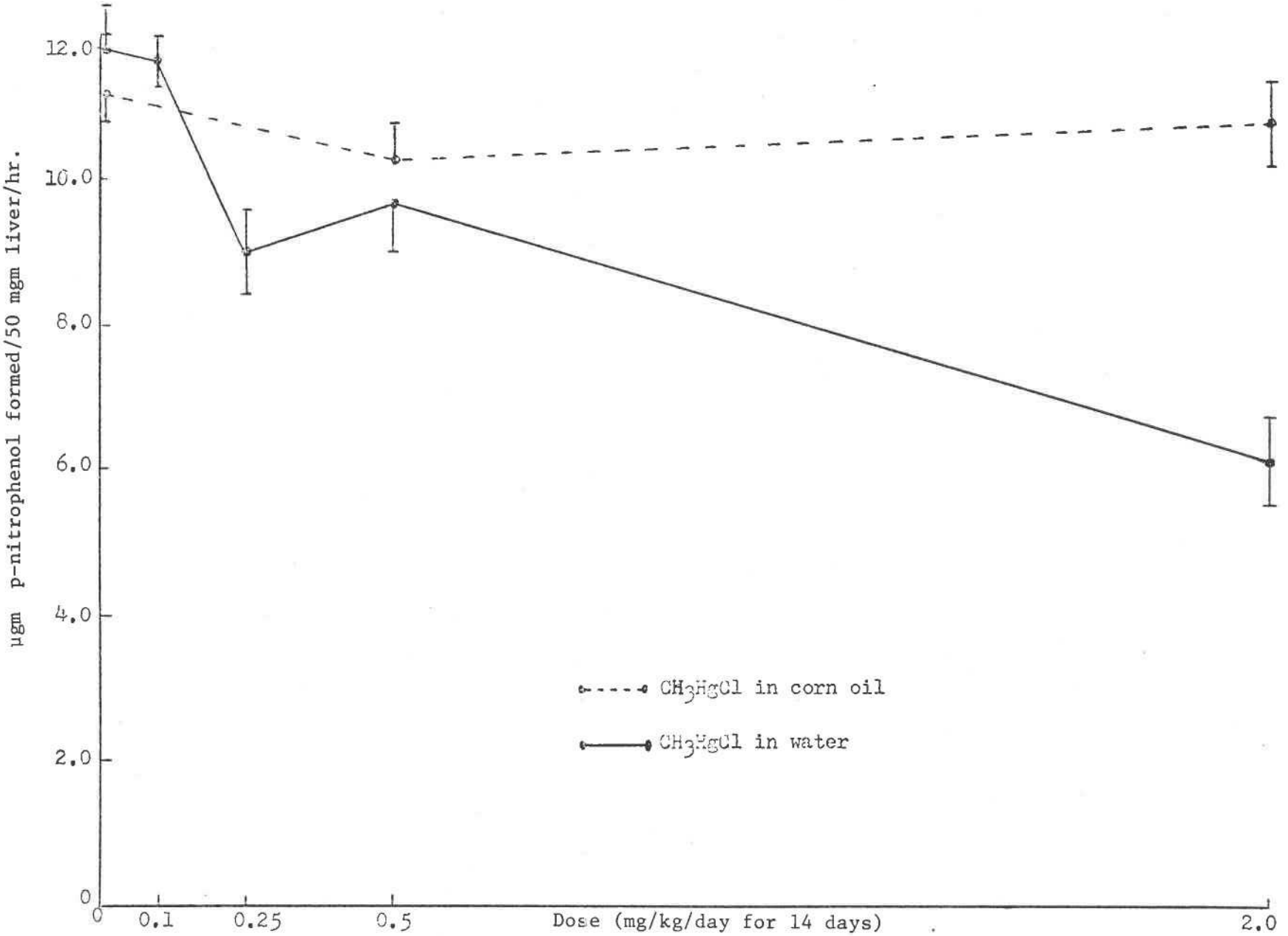

Figure 2. Effect of various subacute doses (ip) of CH_3HgCl on the activity of hepatic EPN Detoxification System of adult male rats. Each point represents the average of at least 3 animals. Vertical lines are standard error of the mean.

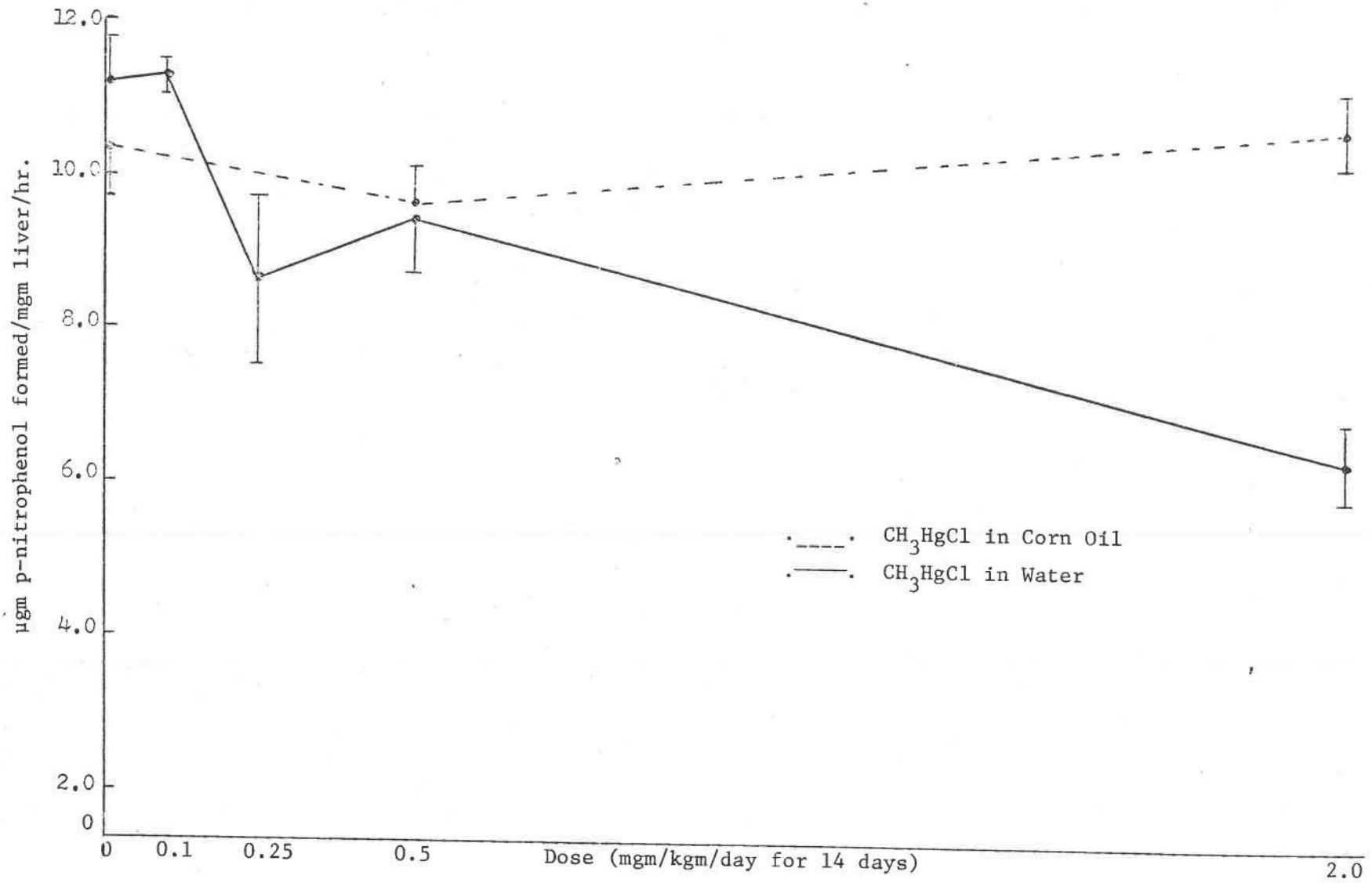

Figure 3. Effect of various subacute doses (ip) of CH_3HgCl on the activity of hepatic p-nitroanisole O-demethylase of adult male rats. Each point represents the average of at least 3 animals. Vertical lines are standard error of the mean.

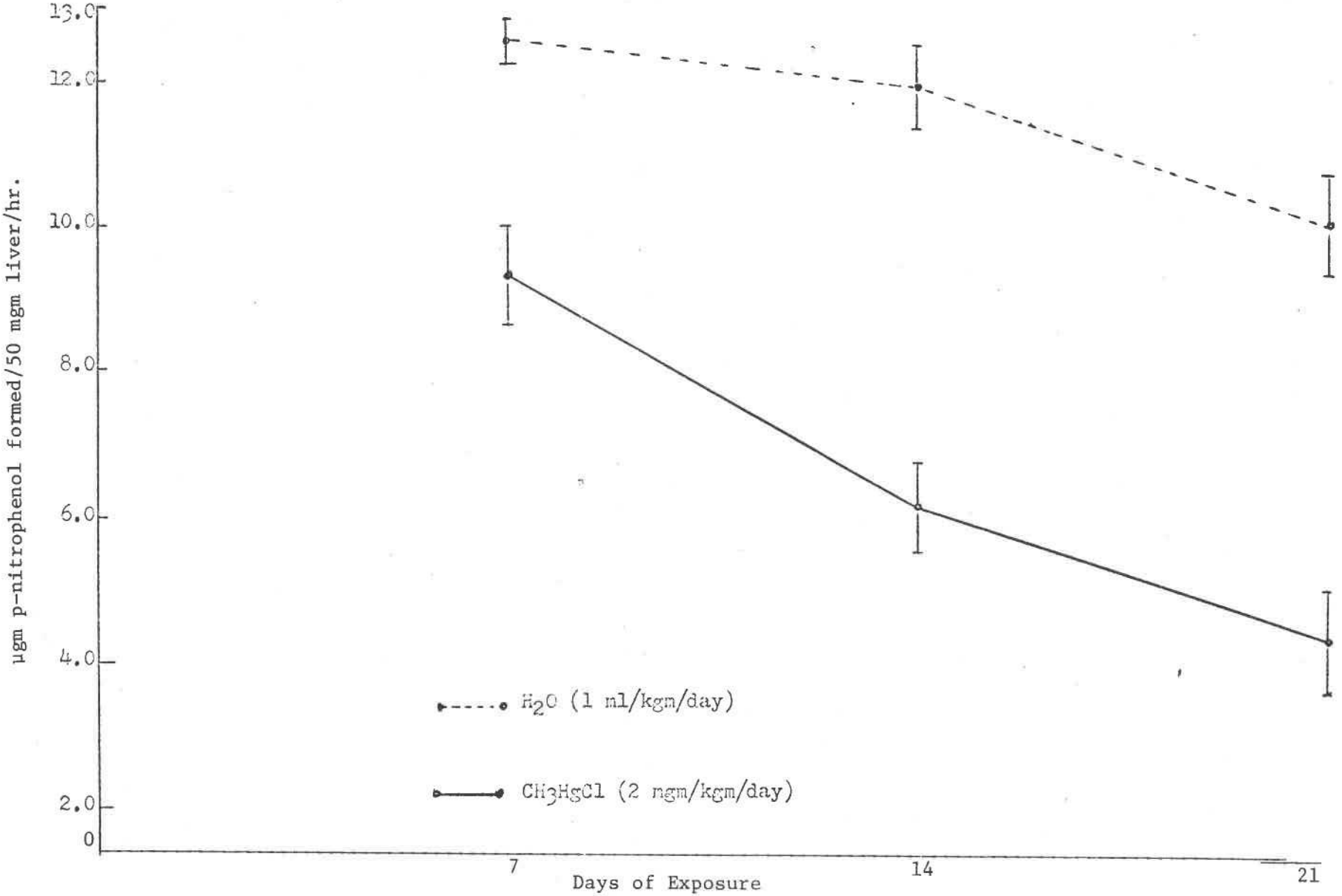

Figure 4. Effect of duration of exposure to CH_3HgCl (2 mgm/kgm/day) on the activity of hepatic EPN Detoxification System of adult male rats. Each point represents the average of at least 3 animals. Vertical lines are the standard error of the mean.

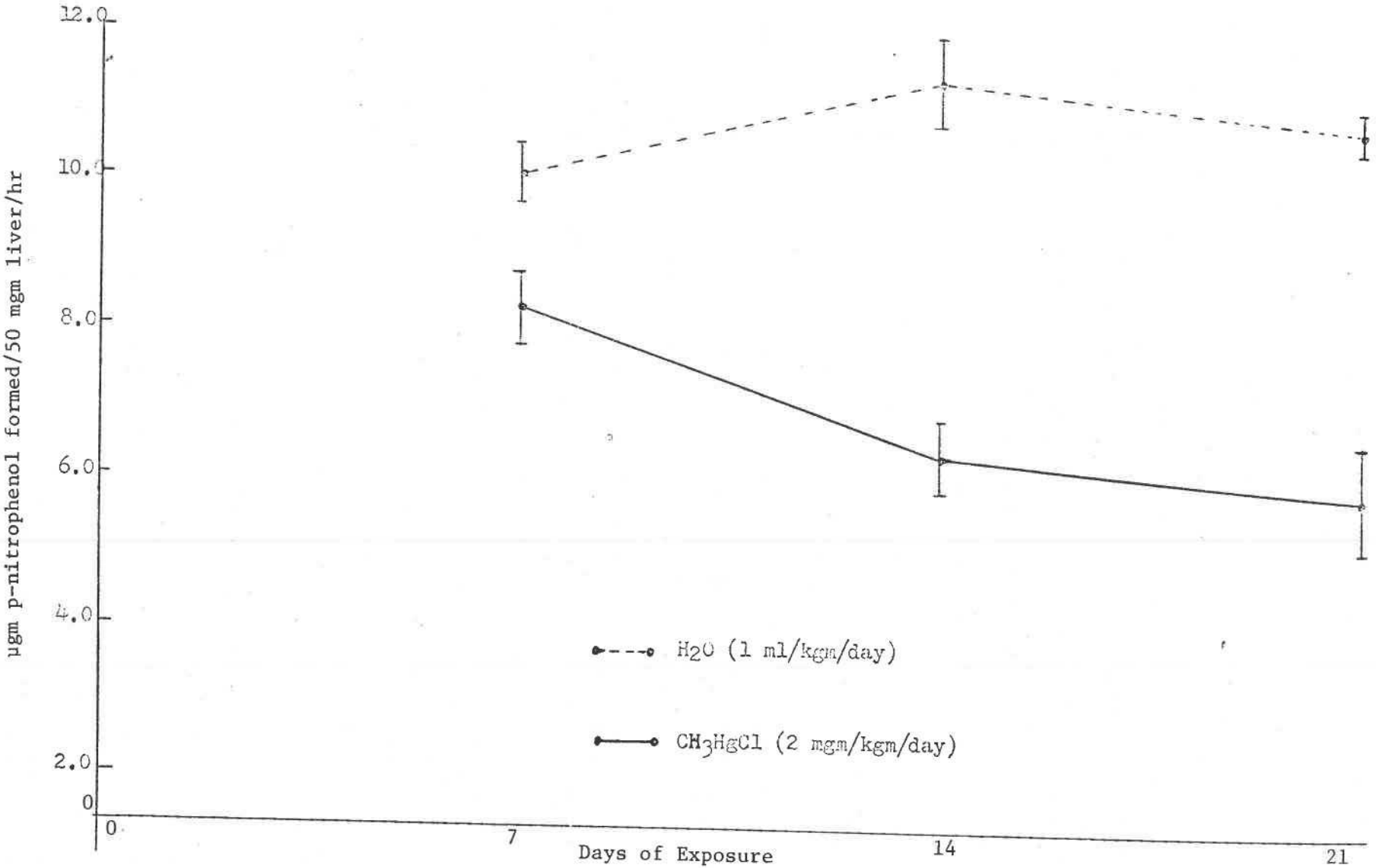

Figure 5. Effect of duration of exposure to CH_3HgCl (2 mgm/kgm/day) on the activity of hepatic p-nitroanisole O-demethlase of adult male rats. Each point represents the average of at least 3 animals. Vertical lines are standard error of the mean.

The activity of both systems was significantly lower than that of control animals after 7 days of exposure. Inhibition of the EPN Detoxification System was progressively greater at 14 and 21 days of exposure, and reached approximately 40% of control with 3 weeks of treatment. However, the activity of the O-demethylase enzyme was inhibited to approximately 50% of control after 14 days of exposure and was not inhibited further even after 21 days of exposure.

It was of interest to ascertain how long it would take for the enzyme activity to return to control levels after cessation of exposure. Animals were injected intraperitoneally with 2 mg/kg/day of methyl mercury for 14 days, and the activities of the EPN Detoxification System and the O-demethylase were assayed 1, 5, and 13 days after cessation of exposure. The results of this study are illustrated in Figures 6 and 7. The activity of the EPN Detoxification System returned to control levels between 5 and 14 days after treatment was stopped. By contrast, the activity of the O-demethylase returned to control levels within 2 or 3 days after exposure to methyl mercury.

EXPOSURE RESULTS

Previous studies demonstrated effects with subacute exposure to methyl mercury. It was of interest to ascertain whether chronic oral administration would have similar inhibitory effects on the enzymes that metabolize EPN and p-nitroanisole. Groups of male rats were exposed to 0.00. 0.01, 0.10, 1.00, and 10.00 mg/l of methyl mercury in their drinking water (corresponds to 0.00, 0.001, 0.01, 0.1, and 1.0 mg/kg/day, respectively).

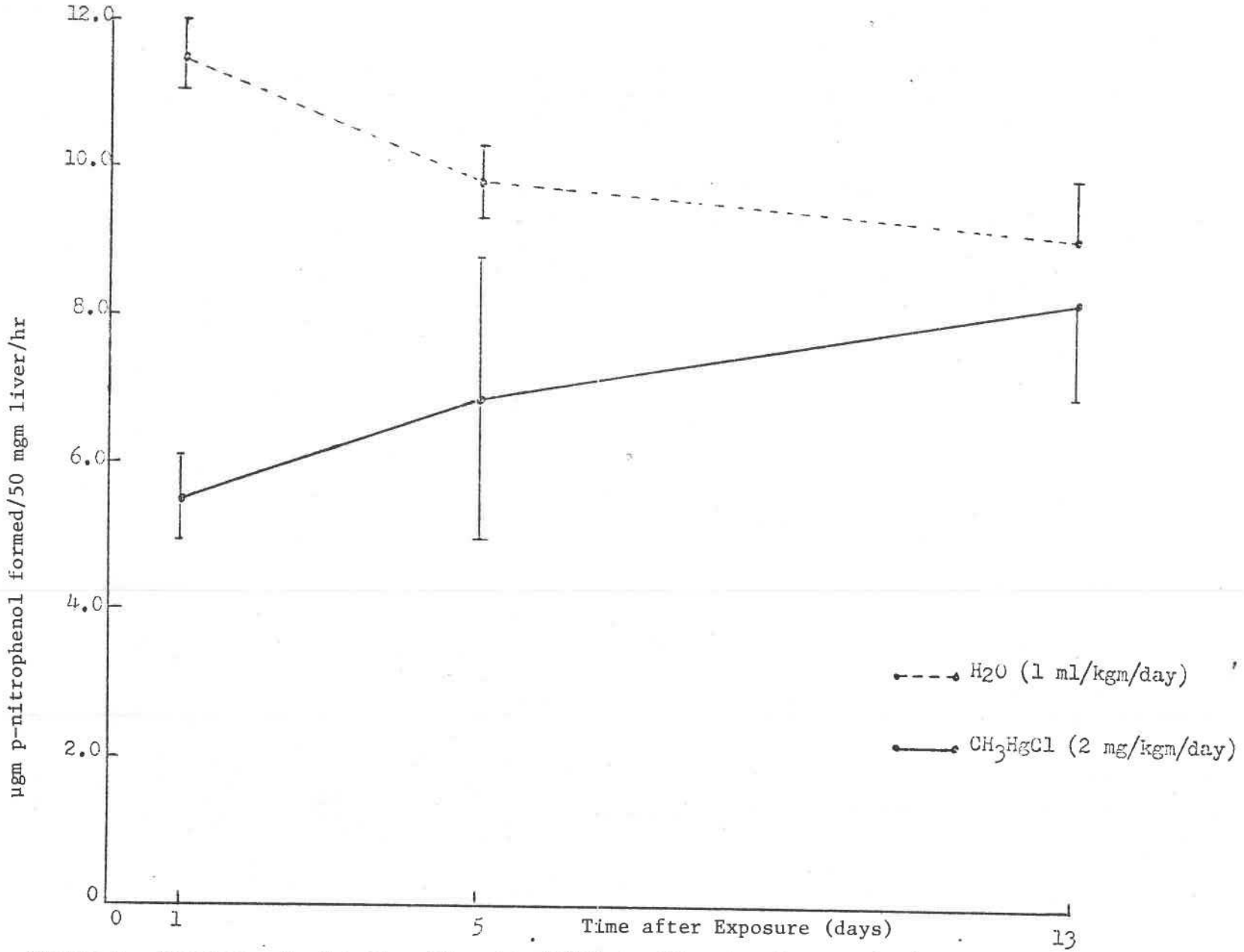

Figure 6. Recovery of activity of hepatic EPN Detoxification System of adult male rats after exposure to CH_3HgCl (2 mgm/kgm/day for 14 days). Each point represents the average of at least 3 animals. Vertical lines are standard error of the mean.

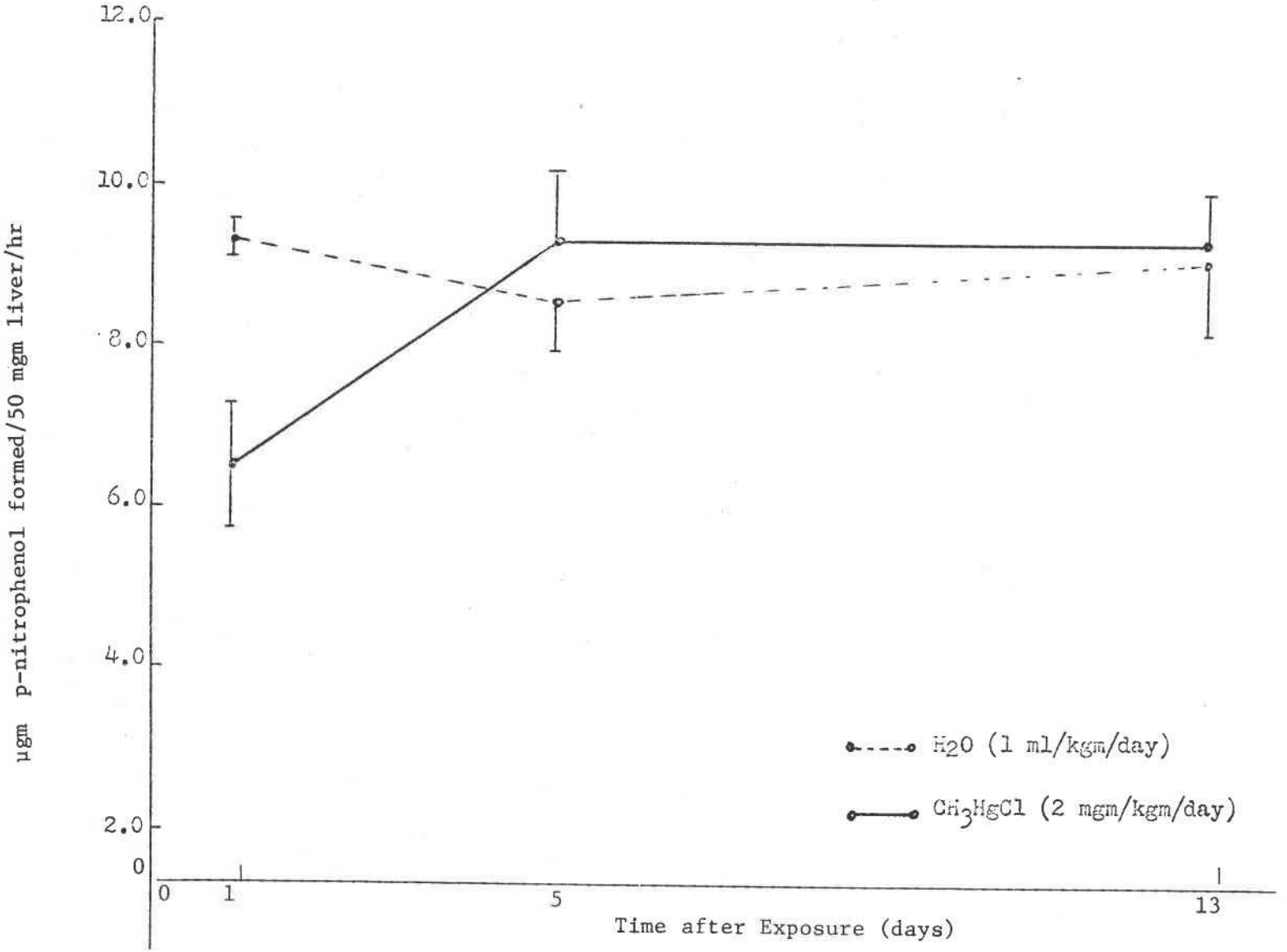

Figure 7. Recovery of activity of hepatic p-nitroanisole O-demethylase of adult male rats after exposure to CH_3HgCl (2 mgm/kgm/day for 14 days). Each point represents the average of at least 3 animals. Vertical lines are standard error of the mean.

After 3 and 6 months of exposure, the animals were sacrificed for assay of the activities of hepatic EPN Detoxification System and p-nitroanisole O-demethylase. Only the 3 month phase is complete at this time; the 6 month assays are being conducted presently. The results in Figures 8 and 9 indicate that the EPN Detoxification System was inhibited to approximately 70% of control values in those animals exposed to 10 ppm of methyl mercury in their drinking water for 3 months. No effect could be observed on the activity of O-demethylase. Figure 10 shows the concentration of Hg in the livers of the animals in this experiment. A concentration of Hg of approximately 40 μgm/gm of liver was associated with the inhibition of the EPN Detoxication System. These results suggest that there may be more than one mechanism for the turnover of mercury in the liver and that the different mechanisms may operate at different doses.

One experiment that is presently in progress is an attempt to confirm the significance of the inhibition of these enzyme systems by determining the LD_{50} of EPN and p-nitroanisole after exposure to methyl mercury.

In conclusion, an attempt has been made to describe the process of deriving drinking water standards for chemicals such as metals. Standards can be developed only with sound scientific evidence and judgment. Useful standards can be promulgated with the judicious use of all available information about the chemical in question, the exposure encountered, and the behavior and effects of the agent in man and experimental animals.

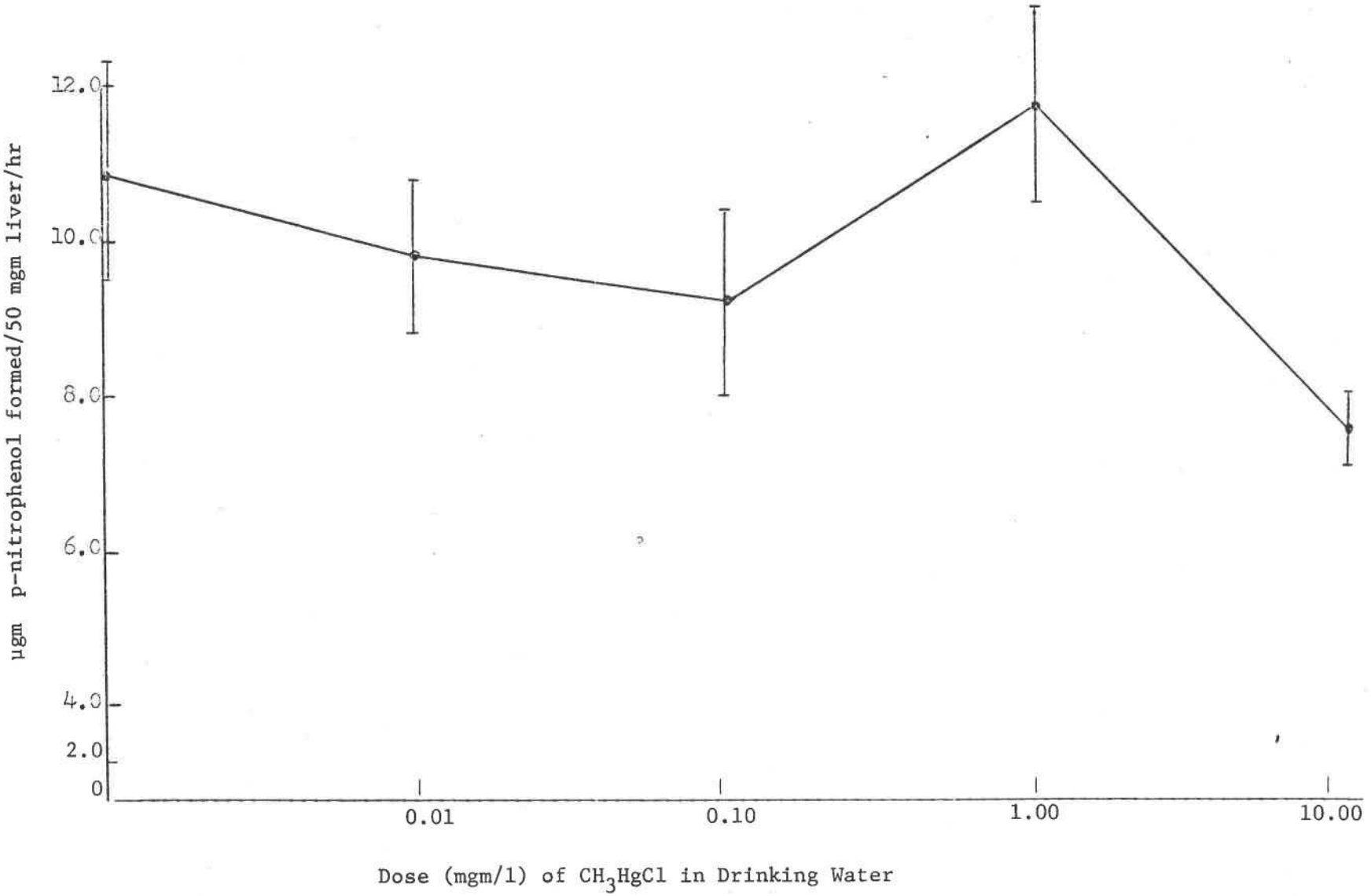

Figure 8. Effect of exposure to CH_3HgCl for 3 months in drinking water on the activity of the EPN Detoxification System of male rats. Each point represents the average of at least 4 animals. Vertical lines are standard error of the mean.

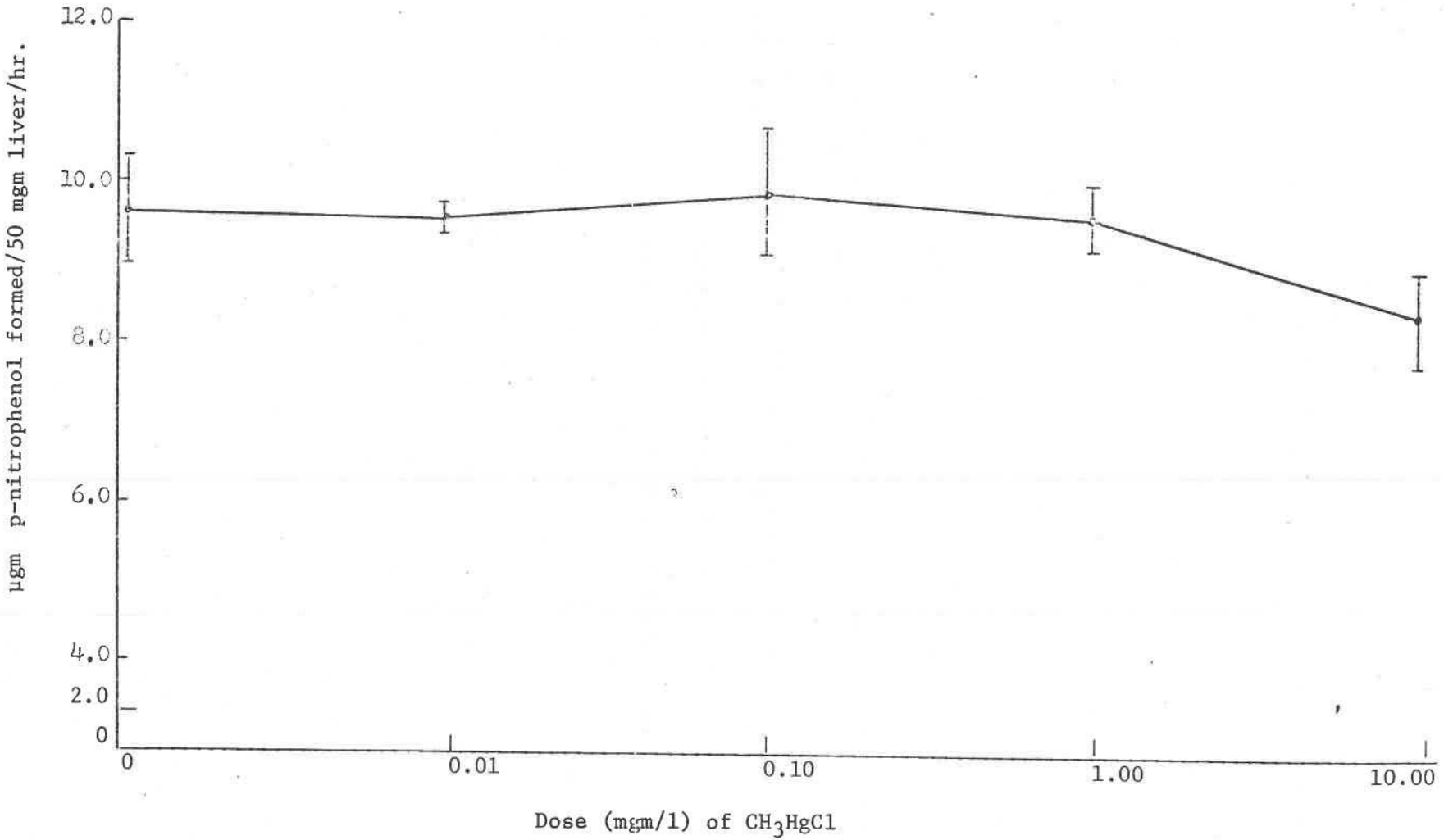

Figure 9. Effect of exposure to CH_3HgCl for 3 months in drinking water on the activity of hepatic p-nitroanisole O-demethylase of male rats. Each point represents the average of at least 4 animals. Vertical lines are standard error of the mean.

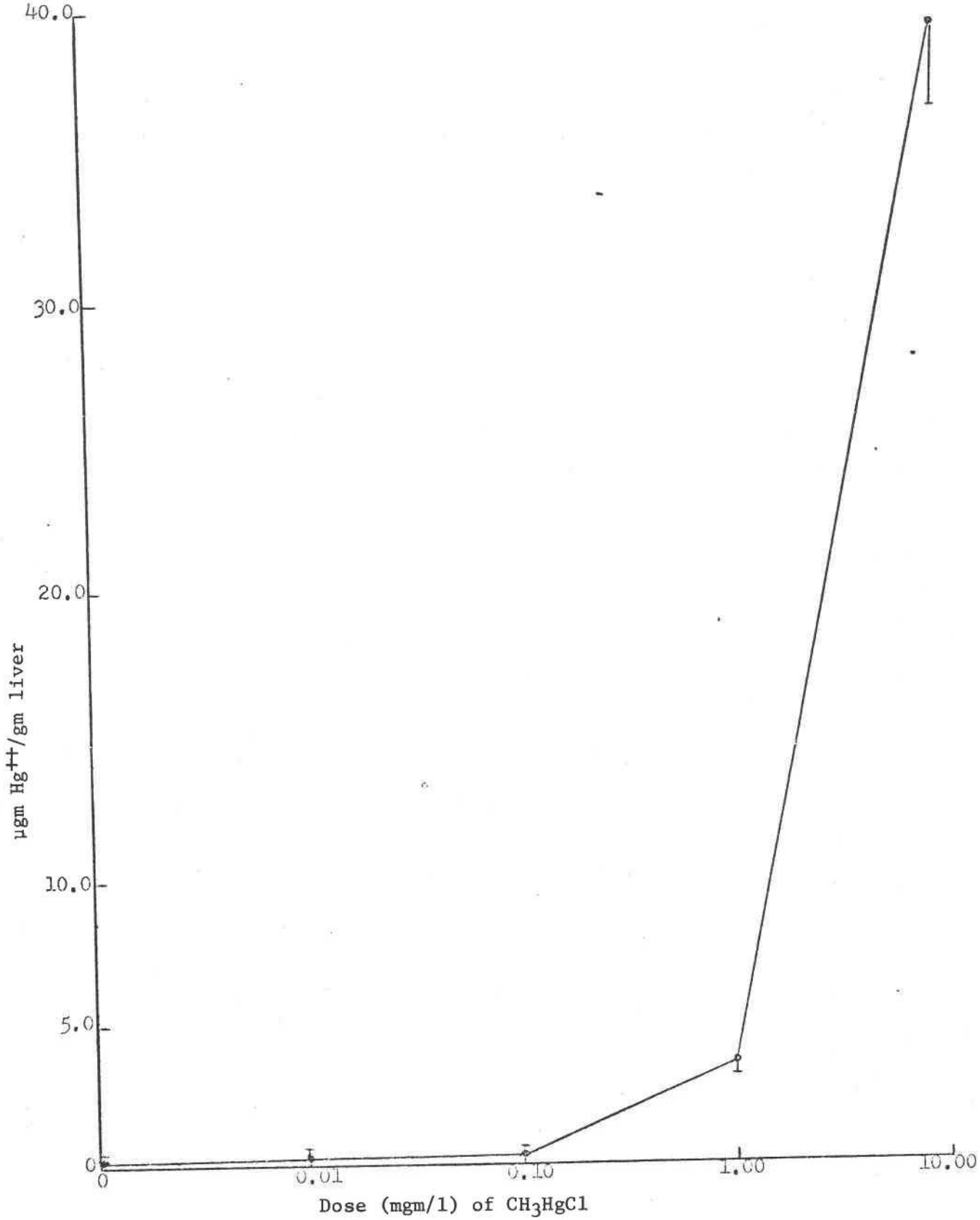

Figure 10. Concentration of Hg^{++} in the livers of male rats exposed to CH_3HgCl in drinking water for 3 months. Each point represents the average of at least 4 animals. Vertical lines are standard error of the mean.

Such standards must be considered dynamic and not static, and thus the compounds for which standards exist require continuing efforts in the elucidation of their biological effects. Our laboratory is illustrating this philosophy by studying the possible effects of methyl mercury on the metabolism of other foreign compounds.

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Effect of Heavy Metals on Fish

The problem of the toxic effects of heavy metals to fish as a result of water pollution has long been recognized and has been investigated since the early part of this century. The early literature concerning this problem came primarily from England where heavy metal pollution of streams occurred because of mining activity. The evolution of investigatory techniques with regard to this pollution is clearly outlined in a series of papers published by Carpenter between 1924 and 1930. The first study surveyed the absence or scarcity of aquatic species in areas of mining activity. The logical extension of these observations was then to test the lethal properties of polluted waters using caged specimens in the field, and subsequently to bring the waters in question into the laboratory and test their lethality or non-lethality under more controlled conditions. Finally, experiments were run by adding known quantities of heavy metals as salts to various clean waters to quantitate the lethal levels.

In the ensuing fifty years a large volume of literature has accumulated in the area of heavy metal toxicity to fish. Zinc is probably

the most completely studied heavy metal with respect to toxicity to fish, and the words "heavy metals" could easily be substituted for "zinc" in the following quotation from Skidmore's 1964 review of the toxicity of zinc to fish:

> "The toxicity of zinc compounds to aquatic animals is modified by several environmental factors, particularly the hardness of the dilution water, the dissolved oxygen concentration, and temperature. The resistance of aquatic animals to zinc poisoning varies with species. It is modified by acclimatization, and possibly age. Survival time is inversely proportional to concentration of zinc. For these reasons concentrations reported as lethal have varied widely."
>
> "The mode of toxic action of zinc is uncertain. At acutely toxic concentrations it probably kills fish by destroying gill tissues. At chronically toxic concentrations it may induce stress resulting in death. The action of zinc undoubtedly differs at different concentrations, it varies with life history, and it is nonspecific."

SUSCEPTIBILITY TO TOXICITY

I have recently completed a cursory review of the literature with respect to the toxicity to fish of the heavy metals, zinc, copper, and cadmium; and I found for each metal a wide range of values which were cited as 96-hr LC50 values, i.e., the concentration which kills 50 percent of the test animals in 96 hours. For zinc this range was 90 to 40,900 ppb; for copper 46 to 10,000 ppb; and for cadmium 470 to 9,000 ppb.

It is not possible to assess these 96-hr LC50 values without delving into the specific nature of each investigation. Categorizing

the various results allows two general conclusions to be drawn with certainty: (1) toxicity varies inversely with hardness; and (2) salmonids (trout and salmon) are more susceptible than most other common test species (primarily bluegills, 'Micropterus salmoides', and goldfish 'Carassius auratus', and perhaps fathead minnows 'Pimephales promelas'). Rather than explore specifically the factors that modify the toxic level of heavy metals to fish, I would like to over-simplify the problem for a moment. Think of a fish as a suspension of cells in a dialysis bag (albeit a rather specialized bag with respect to the transport of certain materials). The rate of entry of a heavy metal into the bag is dependent upon its external concentration, and the death of cells begins when the internal concentration achieves some critical level. All of the factors which influence the toxicity of a given heavy metal affect either the rate of accumulation of the metal or determine the critical internal level.

Differences in the toxicity of heavy metals to various species of fish may be due either to differences in the internal concentrations which they can tolerate and/or due to differences in rate of accumulation of the heavy metal. Since little is known regarding the mode of action of these metals, we do not know what internal "compartment" (e.g. tissue, cellular fraction, enzyme, etc.) to measure for the heavy metal or its effect. The dialysis membrane in the simplified example is analogous to the fish's gills. Differences among fish species with respect to the accumulation of heavy metals could result from different uptake rates through the gills or different rates of heavy metal excretion. The

internal concentration which causes toxicity may differ between species because of any number of biochemical differences.

INFLUENCE OF RATES OF UPTAKE

Disregarding species and age, all of the other generally recognized factors which influence the toxicity of heavy metals to fish largely do so by influencing the rate of accumulation of the metal, and primarily the rate of uptake of the metal. Thus pH, hardness and alkalinity, temperature, and dissolved oxygen level, all affect the rate of uptake of the metal by changing either the biological availability of the metal (by altering its form) or by altering the rate and volume of ventilation of the fish.

Low dissolved oxygen levels create a need for more water to be passed over the gills in order to obtain sufficient oxygen. Increased temperature produces a higher metabolic rate with a greater demand for the uptake of oxygen from the water and loss of CO_2 and metabolites from the blood. In both cases the amount of water passed over the gills increases and the proportion of blood shunted into the thin gill lamellae increases. In effect, this increases the opportunity for the exchange of materials between the blood and the water, and accelerates the uptake of the heavy metal from the water.

WATER CHEMISTRY

The primary problem in investigating the toxicity of heavy metals to fish is that of the effect which differences in water chemistry

produce on the form of the metal and its biological availability. In order to prove toxic to the fish, the metal must be absorbed from the water, transported to, and bound at, its sites of action.

It is generally reasoned that in order for a metal to exert its toxicity it must be in soluble form as opposed to suspended. Suspended metals apparently are not free to pass through the gill membrane. However, several studies (Lloyd, 1960; and Mount, 1966) indicate that suspended zinc contributes significantly to the toxicity of the water containing both dissolved and suspended zinc. Mount theorized that this could be explained by supposing that the suspended zinc is at least partially converted to dissolved zinc because of lower pH values near the surface of the gills due to CO_2 excretion. The toxicity of suspended zinc will obviously depend on the physical-chemical nature of zinc; thus a fine hydroxide precipitate will probably have a different toxicity than zinc silicate or zinc bound to organic matter. At high concentrations the toxic action of heavy metals has been ascribed to suffocation caused by excessive mucous accumulation on the gill surface as a response to irritation. This "coagulation anoxia" theory does not explain the death of fishes at low to moderate concentrations and probably only partially explains the mode of action at high concentrations. However, even this essentially external mode of action requires that the metal be free enough to approach the site of "irritation" at the level of the cell membrane and could reasonably require entry into at least epithelial cells.

When reporting the toxicity of a heavy metal, it is necessary to give a certain quantity of auxillary chemical data. In addition to

measuring both total and dissolved metal concentrations, measurements of other factors including pH, alkalinity, hardness, total solids, dissolved solids, organic matter, temperature, dissolved oxygen, and a general analysis for other major cations and anions should be available. Our present level of ability to chemically measure the biologically effective levels of heavy metals in water is limited by a lack of knowledge about what to look for. Because of this, the bioassay is in some respects the only method available to ascertain whether a specific concentration of heavy metal is safe for aquatic organisms in water of a specific quality.

EFFECTS OF HEAVY METALS

I would now like to discuss some examples of research into the effects of heavy metals on fish. Much of the work which I shall describe has been conducted by biologists at the U.S. Environmental Protection Agency's National Water Quality Laboratory at Duluth, Minnesota, and its satellite laboratories, the Eastern Fish Toxicology Laboratory at Newtown, Ohio, and the Western Fish Toxicology Laboratory at Corvallis, Oregon. Much of the recent work in the literature and essentially all of that from the EPA has attempted to define an incipient minimum effect level by defining a minimum concentration of heavy metal producing known harmful effects and a maximum concentration producing no known harmful effects.

The effect measured in most studies in the literature is death. Death is an easily determined end-point and is especially applicable to short-term studies. In longer studies, growth, reproduction, embryonic development, behavior, tissue residue analyses, disease resistance, or

smoltification (saltwater adaptation) have also been used as criteria of effect. The data from chronic tests has ultimate utility in determining how much heavy metal can be present in the water without producing significant harm to the aquatic organisms. (Man, of course, has the final responsibility and decision as to what he will consider as significant harm).

The utility of no-effect levels measured in short-term bioassays in determining an ultimate safe heavy metal level is limited. The short-term study is valuable in describing the general effects of water chemistry factors and species differences in susceptibility. When and if a fairly constant relationship can be shown to exist between a concentration producing a known effect in a short-term test and an ultimate maximum safe concentration, then an "application factor" can be calculated and applied to subsequent short-term data in order to derive an ultimate safe level. However, adequate data upon which to calculate reliable application factors have been obtained in only a very few studies. Arbitrary application factors (usually 1/10th, sometimes 1/30th, of a 96-hr LC50) have been used, but although these factors often yield good estimates of safe levels, they can also yield estimates which either overprotect or underprotect aquatic organisms.

Table 1 shows some data obtained from bioassays with zinc and fathead minnows (Pimephales promelas). Several points are apparent from this table, namely, the difference in zinc resistance among the different life stages, and the effect of hardness. It appears from this data that fry of the fathead minnow are killed at about 1/10th the concentration of

TABLE I. -- Toxicity of Zinc to the Fathead Minnow (Pimephales promelas).

Stage	Zinc ppb	LC50 period	Temp. C.	Hardness (mg/1)	pH	Source
Fry	870	7 day	20	174-198	7.5	Pickering & Vigor (1965)
Adults	880	96-hr	25	20	7.5	Pickering & Henderson (1966)
Eggs	1600	12 day	20	174-198	7.5	Pickering & Vigor (1965)
Adults	7500	96-hr	25	166	6.2	Rachlin & Perlmutter (1968)
Adults	9200	96-hr	20 (?)	200	7.7	Brungs (1969)
Adults	33400	96-hr	25	360	8.2	Pickering & Henderson (1966)
50% Reduction in egg production	88	10 months	20	200	7.7	Brungs (1969)

Application factor = $\frac{88}{9,200} = 0.0096 \simeq 0.01$

zinc that kills adults, and that eggs are intermediate in susceptibility. It also appears that adult fathead minnows in soft water are killed at about 1/10th the concentration of zinc that kills adults in hard water. These data pose the question, what concentration of zinc will kill fathead minnow fry in soft water? If fry are consistently killed at 1/10th the concentration which kills adults, then the 7-day LC50 in soft water should be about 88 ppb.

The acute and chronic studies conducted by Brungs (1969) on fathead minnows yielded an application factor for zinc of about 0.01 (Table I). This factor is the ratio between the minimum concentration determined to produce undesirable chronic effects and the 96-hr LC50. The 0.01 application factor is subsequently used to estimate safe levels of zinc in other waters based on 96-hr LC50 data only, and eliminates the need for conducting longer and more costly chronic studies each time the safe zinc level must be determined for another water. Conceptually, this is a very reasonable approach and in practice, although not ideal, it can yield good estimates of safe levels if applied with reason.

USING APPLICATION FACTORS

There are three areas which concern me when it comes to using application factors. These are water chemistry, fish species, and methods of heavy metal analysis.

Water chemistry (e.g. pH, hardness, dissolved oxygen, and alkalinity), as well as temperature, affects the toxic level of heavy

metals as determined in bioassays. This complicates the use of the application factor because differences in water chemistry seem to affect the short-term toxicity estimates (e.g. 96-hr LC50) more than they affect long term toxicity estimates. As a result application factors based on studies in hard water, for example, would be expected to be somewhat smaller than those based on studies in soft water. The data in Table IV for fathead minnows seem to bear this out, although the difference between these application factors could be due to normal biological variability. If acute toxicity bioassays to determine the 96-hr LC50 are conducted in a different manner with respect to fish handling, photoperiod, feeding, etc., then that would also produce some variation in the application factor obtained from studies.

Another concern in determining safe levels of heavy metals for fish is the difference in susceptibility between species. A summary of representative data on the toxicity of zinc to various fish species are given in Table II. The range of lethal concentrations listed varies from a minimum of 90 ppb for cutthroat trout (Salmo clarki) to 6,440 ppb for goldfish (Carassius auratus). It is obvious that an arbitrary application factor such as 1/30th of the 96-hr LC50 could not be used to obtain a concentration which would be both reasonable and safe for all these species in a common body of water. One-thirtieth of the goldfish 96-hr LC50 is 215 ppb, which would not be safe for several species of trout; on the other hand, 1/30th of the cutthroat 96-hr LC50 is 3 ppb, which could be an unreasonably low figure when compared to reported natural levels of zinc.

TABLE II. -- Zinc toxicity to various species of fish in soft water.

Species	Zinc Conc ppb	LC50 period	Temp C.	Hardness (mg/l)	pH	Source
Cutthroat trout (Salmo clarki)	90	96-hr	11.5-16	17-32*	6.6-7.6	Sappington (1969)
Steelhead trout (Salmo gairdneri)	110	96-hr	12	20-25	7.4	Western Fish Toxicology Station (Unpublished data)
Rainbow trout (Salmo gairdneri)	240-560	96-hr	14-15	30	7.2-7.3	Colorado Game, Fish & Parks (1971)
Guppy (Lebistes reticulatus)	560	96-hr	24	20	7.25	Chen & Selleck (1969)
Atlantic salmon (Salmo salar)	600	7 day	15	20	7.1-7.5	Sprague (1964)
Rainbow trout (Salmo gairdneri)	410-830	96-hr	6-10	30	6.6-7.1	Colorado Game Fish & Parks (1971)
Fathead Minnow (Pimephales promelas)	880	96-hr	25	20	7.5	Pickering & Henderson (1966)
Sockeye salmon (Oncorhynchus nerka)	1100	96-hr	12	20-25	7.4	Western Fish Toxicology Station (Unpublished data)
Guppy (Lebistes reticulatus)	1270	96-hr	25	20	7.5	Pickering & Henderson (1966)
Bluegill (Micropterus salmoides)	2860-3780	96-hr	16-20	21	---	Patrick Cairns & Schier (1968)
Bluegill (Micropterus salmoides)	4200	96-hr	16-20	20	---	Cairns & Schier (1968)

TABLE II. -- Zinc toxicity to various species of fish in soft water.

Species	Zinc Conc ppb	LC50 period	Temp C.	Hardness (mg/l)	pH	Source
Bluegill (Micropterus salmoides)	4850-5820	96-hr	25	20	7.5	Pickering & Henderson (1966)
Goldfish (Carassius auratus)	6440	96-hr	25	20	7.5	Pickering & Henderson (1966)

*methyl orange alkalinity

Applying the 0.01 factor for fathead minnows and zinc to the 96-hr LC50 which I recently determined for juvenile steelhead trout (100 ppb) yields an estimated safe level of 1 ppb. I think most of us would be reluctant to cite so low a concentration as the maximum safe level for zinc. One reason for such reluctance is that many productive natural waters have zinc levels higher than 1 ppb. The other reason is that there is no inherent reason to assume that application factors would be the same among species. There is little data in this area of research to date, but what is available indicates that the application factor for zinc is similar for fathead minnows and brook trout (Table IV). Further work to determine application factors for other species is needed, certainly for zinc and also for other heavy metals.

The last area of concern regarding application factors revolves around heavy metal analysis of water. Generally, analysis of bioassay solutions and natural waters have measured the total metal concentration (i.e. both dissolved and suspended). Analysis of test solutions has probably been subject to more rigorous methods than general field surveys and the bioassay water itself has been cleaner and contained fewer substances producing analytical difficulties. As a result heavy metals are measured more accurately in laboratory bioassay solutions than in routine water survey samples. Similarly, the sampling of the test solutions for heavy metals is conducted routinely (we test ours daily), whereas most available field data are obtained at much greater intervals in time.

The point I wish to make is that just because natural levels of heavy metals may be shown to exceed a given estimate of a maximum safe

concentration does not invalidate the estimate. One or two final points should be made in this respect. The first is that in measuring total metal concentration, we may be measuring a portion which is, at least temporarily, in a biologically unavailable form. Our data show that from 50 to nearly 100 percent of the heavy metals in our test solutions are dissolved (i.e. they pass through a 0.45 micron filter). In certain natural situations, this proportion may be appreciably less.

A second point is that our bioassays are run under conditions of continuous exposure to a constant concentration. In nature the heavy metal concentrations could fluctuate considerably.

DETERMINING SAFE LEVELS

It seems clear that what is needed in determining safe levels of heavy metals (or any other potentially toxic material) is a long term study of the most susceptible species of fish (or other organisms) in the particular water in question. Even this type of approach has recently been questioned by Lloyd (1972) who concluded that the establishment of water quality criteria can be achieved only if reliable data are available on acute, chronic, and sublethal effects of the material in question, as well as field observations regarding fish mortalities following discharge of the material and analysis for the material in water where fish are known to be present or absent. I think we can and must estimate safe levels for heavy metals in instances where such a complete array of data as Lloyd suggests is not available. Certainly we do not want to wait until

TABLE III. -- Acute toxicity of copper to fish.

Species	Conc ppb	LC50 period	Temp C.	Hardness (mg/1)	pH	Source
Steelhead trout (Salmo gairdneri)	20	96-hr	12	20-25	7.4	Western Fish Toxicology Station (Unpublished data)
Rainbow trout (Salmo gairdneri)	22	unspecified >96-hr	14.1	100	7.1	Colorado Game, Gish & Parks (1971)
Atlantic salmon (Salmo salar)	32	7 day	17	14	7.0-7.4	Sprague & Ramsay (1965)
Adult coho salmon (Oncorhynchus kisutch)	46	96-hr	8.7-11.4	20	7.2-7.4	Western Fish Toxicology Station (Unpublished data)
Fathead minnow (Pimephales promelas)	75	96-hr	25	31	6.9-7.2	Mount & Stephan (1969)
Brook trout (Salvelinus fontinalis)	100	96-hr	?	45	7.4	McKim & Benoit (1971)
Bluegill (Micropterus salmoides)	200	96-hr	?	20	7.4	Tarzwell & Henderson (1960)
Fathead minnow (Pimephales promelas)	470	96-hr	20	198	7.9	Mount (1968)
Fathead minnow (Pimephales promelas)	1,400	96-hr	?	400	8.2	Tarzwell & Henderson (1960)
Bluegill (Micropterus salmoides)	10,000	96-hr	?	400	8.3	Tarzwell & Henderson (1960)

lethal conditions exist in our natural waters before we determine safe levels based on field observations such as Lloyd suggests.

Nevertheless, field data of the type Lloyd suggests is needed from time to time to test conclusions regarding safe levels as determined in laboratory studies. This type of field information can however be obtained in artificial streams or natural test streams without the necessity of waiting for pollution to occur.

Copper has been the second most studied heavy metal with respect to its toxicity to fish. Copper toxicity like that of other heavy metals, is effected by such changes in water chemistry as I discussed earlier. Similarly, species differences are evident among fish with respect to the toxic effects of copper. Table III summarized some of the more pertinent studies regarding the acute toxicity of copper to fish. Acutely toxic levels to salmon and trout in soft water are reported in the literature to be as low as 32 ppb and to approach 20 ppb when exposure to copper is extended beyond 96 hours. Recent tests at our laboratory have shown that juvenile steelhead trout have a 96-hr LC50 of 20 ppb of copper and significant mortality (ca 40%) occurs during two weeks of exposure to 11 ppb of copper.

Chronically safe and unsafe levels of copper have been reported to be 10 and 17 ppb respectively for brook trout (McKim and Benoit, 1971), and 11 and 18 ppb for fathead minnow in soft water (Mount and Stephen, 1969) and 15 and 33 ppb for fathead minnow in hard water (Mount, 1968). Application factors from these three studies ranged from 0.03 to 0.13 for

estimating the safe concentrations and 0.07 to 0.22 for estimating the unsafe concentrations (Table IV). A general application factor for copper is therefore about 0.1 of the 96-hr LC50.

The third heavy metal which I wish to discuss today is cadmium. Cadmium, while not as common as zinc or copper, is of particular interest due to its extreme toxicity. There was little evidence of this high toxicity in the fisheries literature until the study by Ball (1967) on rainbow trout (Table V). Prior to Ball's finding of acute mortality at 10 ppb, the range of reported lethal concentrations was 470 to 8,460 ppb. Studies at the Western Fish Toxicology Laboratory have recently shown a 96-hr LC50 as low as 1.0 ppb for steelhead trout.

Cadmium may tend to act like more of a cumulative poison than do zinc or copper and there is no distinct relationship between survival time and cadmium concentrations. Discussion of these points and derivation of an application factor for cadmium are covered in a recent paper by Pickering and Gast (1972). It is sufficient for me to say at this time that deriving an application factor based on a 96-hr LC50 for cadmium is difficult, but that using a longer period of acute exposure (e.g. one week LC50) yields a tentative application factor for cadmium of about 0.1.

It is interesting that the chronic effects of zinc, copper, and cadmium appear to differ between metals and between species. Thus, the chronic effects of zinc cause poor egg production in fathead minnows. Copper also causes poor egg production in fathead minnows but in brook trout the survival of the young fry is the stage most susceptible to

TABLE IV. -- Chronic toxicity data and calculated application factors for copper.

Species	Hardness	Conc. (ppb) of copper Chronically Safe	Unsafe	Application factor Safe	Unsafe	Source
Fathead Minnow (Pimephales promelas)	198	15	33	0.03	0.07	Mount (1968)
Fathead minnow (Pimephales promelas)	31	11	18	0.13	0.22	Mount & Stephan (1969)
Brook trout (Salvelinus fontinalis)	45	10	17	0.10	0.17	McKim & Benoit (1971)

TABLE V. -- Toxicity of cadmium to various fish species.

Species	Cd Conc. ppb	LC50 period	Temp C.	Hardness (mg/l)	pH	Source
Steelhead trout (Salmo gairdneri)	0.95	96-hr	12 C	20-25	7.4	Western fish Toxicology Stn. (Unpublished data)
Steelhead trout adults (Salmo gairdneri)	2.9-4.95	17 days	6.7-11.9 C	29-90	7.3-7.65	Western Fish Toxicology Stn. (Unpublished data)
Rainbow trout (Salmo gairdneri)	8-15	14 days	11-12.5	290	?	Ball (1967)
Fathead minnow (Pimephales promelas)	470-840	96-hr	25	20	7.5	Pickering & Henderson (1966)
Guppy (Lebistes reticulatus)	480-1720	96-hr	25	20	7.5	Pickering & Henderson (1966)
Rainbow trout (Salmo gairdneri)	3000-5000	7 days	14-18	soft?	7.5	Schweiger (1957)
Brook trout (Salvelinus fontinalis)	4000-5000	7 days	14-18	soft	7.5	Schweiger (1957)
Bluegill (Micropterus salmoides)	3440-7480	96-hr	25	20	7.5	Pickering & Henderson (1966)
Goldfish (Carassius auratus)	5140-8460	96-hr	25	20	7.5	Pickering & Henderson (1966)

copper. Cadmium does not inhibit egg production in fathead minnow, but rather causes poor survival of embryos. Just how diverse the chronic effects of these and other metals will prove to be remains to be seen.

SUMMARY

In discussing heavy metals today, I have touched several times on the problem of water chemistry and what I have termed the "biological availability" of the various forms of the heavy metals. The biggest problem facing those trying to correlate available data on acute and chronic toxicity of heavy metals is the lack of information on the form of the heavy metal measured. I suspect that the proportion of the heavy metal which is "biologically available" tends to be higher in laboratory bioassays than in nature. Much more research should be done in characterizing the toxicity of the various forms of heavy metals which occur in water, and at the same time we need to know better the factors that transform one form into another. Similarly, we need more data on the effects of fluctuating rather than constant levels of heavy metals and the extent to which the toxicity of two-or-more heavy metals (or other toxic materials) is additive, synergistic, or antagonistic.

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Effects of Metals on Fertilization and Development in Fish

The extreme sensitivity of fish to toxic metals in their environment is being demonstrated with increasing frequency. Preliminary results reported by the EPA (U.S. Environmental Protection Agency) (1972a) showed that adult brook trout (Salvelinus fontinalis) that were exposed to methyl mercury at 3 ppb (parts per billion) mercury died within 6 months. Fifty percent mortality of adult steelhead trout (Salmo gairdneri) resulted when separate groups were exposed to 4 ppb cadmium for 14 days, to 60 ppb copper for 96 hours, and to less than 2 ppm (parts per million) zinc for 96 hours. More recently (1972b), the EPA found that most channel catfish (Ictalurus punctatus) that were exposed to 4 ppb methlmercuric chloride died within a 72 hour period.

The treatment levels used in these experiments cannot be considered excessive when they are compared to reported environmental levels. Jenne (1972) reported amounts of mercury in water samples from various locations in the Columbia and Willamette rivers that ranged from less than 0.1 to 35 ppb. The amounts of some of these metals that are found to be toxic for fish challenge the sensitivity of analytical tools.

The potential for deleterious effects of these materials on valuable fish populations is obvious. As in many instances of environmental contamination, the knowledge must now be accumulated, after the fact, to assess the impact on natural systems. The more obvious effects of metals on fish, such as mass mortalities or the rendering of exposed individuals unfit for human consumption through concentrating mechanisms, often can be alleviated with strict controls. Effects on gametes, fertilization and early development are more difficult to define in terms of water quality criteria.

In this report I will briefly review some of the information that is available concerning the effects of toxic metals on fertilization and development, and discuss the potential significance of these effects in the maintenance of our fish fauna as it is known today.

SOME EFFECTS OF TOXIC METALS ON FERTILIZATION

Successful fertilization may be prevented by toxic metals through effects on behavior. In long-term studies of the effects of metals on various life history stages of the brook trout, the EPA (1972a) found that males in exposure levels of 3.4 ppb cadmium were seized by severe muscle spasms as females began to move gravel in redd preparation. These males were generally unsuccessful in fertilizing the eggs and eventually died.

Direct effects of metals on gamete survival may prevent successful fertilization. Results of an acute test in which samples of steelhead trout sperm were held in various concentrations of methylmercuric chloride for 30 minutes before they were combined with eggs indicated that at concentrations greater than 1 ppm. sperm viability was markedly reduced (McIntyre 1972).

Effects of metals on gametogenesis were indicated from preliminary data of the EPA (1972a). They have found that the sexual development of fathead minnows (Pimephales promelas) in methylmercury is arrested at 0.25 ppb. Results from experiments with zebra fish (Brachydanio rerio) that were permitted to spawn in several concentrations of phenylmercuric acetate produced fewer eggs at concentrations of 1 ppb or more as compared to lower concentrations (Kihlstrom et al. 1971).

Results of a study of copper toxicity for fathead minnows reported by Mount (1968) and, Mount and Stehan (1969) showed that 95 ppb killed approximately 50% of the test fish, and retarded sexual development and prevented spawning in the survivors. Survival was not reduced at 33 ppb, but spawning was prevented. Lower test concentrations produced none of these effects. In soft water, 18.4 ppb copper produced 50% mortality, and in the survivors it retarded growth and prevented spawning.

SOME EFFECTS ON DEVELOPMENT

Incubation: Studies of the effects of metals on incubating eggs have been in general restricted to the survival of exposed eggs through hatching. The hatchability of brook trout eggs was reduced at a methylmercury exposure of 1 ppb mercury (EPA; 1972b). Hatchability of incubating steelhead trout eggs in a test with methylmercuric chloride was 35% less for eggs treated at 2.5 ppb mercury than for control eggs (McIntyre, unpublished data). It was also observed that the mean number of days to hatching was 0.5 days greater in the treated eggs. The hatchability of zebra fish eggs in water containing phenylmercuric acetate was significantly reduced at 0.2 and 1 ppb (Kihlstrom et al. 1971).

Larvae: Increases in the occurrence of deformed embryos resulting from exposure to metals have been found by the EPA (1972a,b). Severe embryo deformities were found in brook trout eggs at 500 ppb lead and at 1 ppb mercury. They have also observed significant increases in the occurrence of deformed embryos and the incidence of blacktail syndrome in fathead minnows at 240 ppb lead.

Growth rates of brook trout alevins (larvae) held in water containing 6 ppb cadmium or 1 ppb mercury were retarded (EPA 1972b). It appeared to these investigators that at 6 ppb cadmium the alevins would eventually die.

The sensitivity of steelhead alevins to methylmercuric chloride was demonstrated in two experiments in our laboratory (McIntyre and Blanc 1972). In the first experiment 9600 alevins were held in water containing 8 ppb mercury at 12 C. Within 13 days, 80% of the alevins were dead. In a second experiment, of 1440 alevins exposed to 15 ppb mercury in water at 9 C, approximately 50% died within 96 hours.

POTENTIAL SIGNIFICANCE OF THESE EFFECTS

When the reproductive biology of a fish such as the steelhead is considered it is understandable why the effects of an additional environmental stress could be overlooked. The average female deposits 2500-3000 eggs in the gravels of a stream. Seventy percent or more of these eggs will die prior to emergence of the fry from the gravel. During this period of high mortality the effects of a pollutant could be masked by the complex of other factors that limit survival.

An analogous situation may exist for the gametes. As the volume of sperm produced by a mature steelhead may be on the order of a teaspoonful and the eggs of one female can be fertilized with the spermatozoa contained in less than one drop, it is obvious that considerable sperm mortality could occur with no apparent deleterious effect.

It is clear from the above data that some individuals in specific tests are more resistant to the action of the toxicant than are others. This variability could be explained as the result of slight environmental differences, of genetic differences, or most likely the result of the combined influence of genetic and environmental factors. A determination of the potential significance of the above results lies in an understanding of the contribution of these factors to the observed variability in resistance. Only the presence of a significant genetic component for resistance will permit populations facing these kinds of challenges to adapt to increasing levels of environmental contaminants.

If a stress, such as a toxic metal, causes the death or reduces the reproductive capability of some proportion of the population, the next generation will be produced by the more resistant survivors. If some part of an individual's resistance derives from additive genetic factors, factors that cause offspring to resemble their parents, then we can expect the mean resistance to the effects of the toxicant will be greater than was the mean resistance of the parental generation. If the differences in resistance are based solely on environmental influence, then we could expect that mortalities related to the toxicant would reduce population equilibrium densities and in some cases lead to the extinction of the population. In light of the

demonstrated sensitivity of early life-history stages of fishes to toxic metals and the corresponding potential for drastic changes in the genetic constitution of these populations, it is imperative that the effects of these materials be evaluated in an evolutionary context.

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Presented November 30, 1972 by F. SCOTT KENNEDY, Harvard Medical School, Boston, Massachusetts.

The Role of Heavy Metals in Biology

The study of the role of heavy metals in biology is part of the broader subject which has preoccupied many branches of biology and medicine for years, namely the beneficial and adverse effects of metals on living systems. Interest in this subject dates back at least a century and concerns abnormal amounts of what are termed trace elements, oligo elements or minor elements -- all names which have been arbitrarily applied to a group of elements which occur biologically in exceedingly low concentration. Often they have been identified qualitatively, but not determined quantitatively.

These include aluminum, antimony, arsenic, barium, boron, bromine, cadmium, cobalt, copper, chromium, fluorine, gallium, iodine, iron, lead, lithium, magnesium, manganese, mercury, molybdenum, nickle, rubidium, scandium, silver, strontium, tine, titanium, vanadium, and zinc. Normally they occur in individual concentrations in the range 1 to 10^{-6} ppm, together accounting for less than 0.5% of the body mass in humans.

The term "heavy metals" is non-specific and includes usually row 2 and row 3 transition metals and sometimes the lanthanide and actinide series. Here I would like to concentrate on mercury, cadmium and lead, and discuss

how these metals can influence biological processes at the molecular level.

Briefly, enzymes are large catalytic biopolymers common to all life-forms, and each owes its specificity and catalytic competence to its individual three dimensional structure, which derives from its linear sequence of amino acid components together with other structural determinants such as metal ion and disulfide bridges. It is largely by catalytic participation, structure stabilization, or alternatively, by modifying or disrupting biochemically poised structures such as proteins and membranes that metals express their role in biology.

EARLY STUDIES

Studies as early as 1860 and 1861 demonstrated concretely that plants require trace elements for growth, an observation that was extended to fungi eight years later. Today, metal deficiencies have been demonstrated in microbes, plants, animals and man such that the list of biologically essential elements has grown steadily. Yet, many of these same elements were first studied through their toxic effects. For example, zinc was studied extensively in the 1920's because its fumes were toxic to exposed industrial workers. But, even then the possibility of an essential role, distinct from its toxicological properties, was predicated on the basis of its ubiquitous occurrence in living systems.

The converse is also true in that a toxic response has been discovered subsequent to observation of an essential role. Thus, the discovery of discrete amounts of cobalt in vitamin B_{12} provided the biochemical explanation for cobalt deficiency in microbes, ruminants and man, while its subsequent

use as an anti-foaming agent in beer and therapeutic treatment of anemia led to myocardial damage and blood disease (polycythemia) respectively.

Historically, appreciation of metabolic and enzymatic roles for metals has evolved slowly and at present little information is available regarding the role of mercury, cadmium and lead. Yet the existence of a cadmium protein in nature, the universal occurrence of substantial amounts of lead and mercury in all living matter, and the demonstration that methylmercury can stimulate protein synthesis all signal that these elements may play a discrete and essential role in biological processes quite unrelated to their known toxicity.

For most metals a concentration-response curve can be constructed. Increasing concentrations cause a roughly linear response which reaches a maximum after which further increases cause a decreased response. However, superimposed on this would be a second curve which would show increased biological response, but of a more unpredictable type. The first linear region would describe a deficiency state in which the particular metal is the limiting factor in some essential process. If concentrations increase, pharmacological action and toxicity may result. This holds true for most substances including those regarded as essential elements and metabolites. In most cases, organisms have evolved sophisticated control mechanisms for absorption, metabolism and excretion so as to control metabolite concentrations within discrete narrow limits. Sodium, blood glucose, and uric acid are examples.

BIOLOGICAL INVOLVEMENT

I would emphasize that potentially even elements such as heavy metals have a biological function which can be ascertained against a background deficiency state, and it's quite likely that nutritional requirements for many elements have gone undetected because sufficiently low concentrations required to establish a deficiency state have not been attained.

The known biological involvement of mercury, cadmium and lead can be expressed largely through their ability to form stable complexes with numerous biological ligands. These include SH and imidazole side chains of proteins, nitrogenous bases of DNA and RNA, as well as phosphate groups which occur in phospholipids, DNA and RNA, coenzymes, vitamins and metabolites. Thus, the potential of heavy metals to influence viably poised structures is obvious.

Aside from their widely known toxicity, mercury, cadmium, and lead all induce or enhance enzymatic activity in a large number of enzymes. Thus, when either mercury, cadmium or lead is added to metal-free, bovine pancreatic carboxypeptidase A (a dual specificity zinc metalloenzyme), the reconstituted metalloenzymes all actively catalyze ester hydrolysis, the cadmium enzyme being a more active esterase than the native zinc enzyme. However, unlike the native enzyme, the heavy metal substituted carboxypeptidases exhibit no peptidase activity so that cadmium can be regarded as both an activator and an inhibitor for this enzyme.

Mercury is found virtually everywhere in the environment, and all phyla and species contain varying trace amounts, which undoubtedly can be

influenced by such factors as ore deposits and/or industrial contamination.

Mercury salts and organomercurials complex readily with SH and S-S groups so that the biochemical basis for mercury toxicity has often been sought as a function of mercury-sulfur interactions.

The stability of mercury complexes with protein SH groups has been utilized in studying protein structure-function relationships employing several physical approaches. For example, organomercurials bearing a fluorescent group or spin label reported group have been used. In such studies, the assumed site of attachment is some SH group. However, parachloromercuribenzoate reacts more rapidly with non-sulfur sites of hemerythrin than with its SH groups.

Secondly, mercurials are thought to bind to apo-carbonic anhydrase at the active site which does not contain an SH group and to the single SH group which is 15 Å from the zinc binding site. Also, mercury binds to the active site of apo-carboxypeptidase which has no SH groups. Thus, binding of mercurials to non-sulfur groups must also be considered in studying its toxicity and possible function.

As mentioned earlier, mercury forms strong complexes with nucleic acid components, and it's not surprising, therefore, that many mercurials can produce chromosomal abnormalities and induce teratogenic effects. Significantly, though, chromosomal aberrations have not been reported in humans whose regular diet contained large amounts of methyl-mercury contaminated fish.

Aside from inorganic mercury salts, there is a second, perhaps more important class of mercury compounds. These are the alkyl mercury compounds, of which methyl HG is the most common.

POLLUTANT AND HEALTH THREAT

The alkyl mercury compounds are considered separately because methylation of inorganic mercury salts alters their chemistry, toxicology and perhaps function. Chemically, alkyl mercury compounds are prepared readily by Grignard reactions under fastidiously anhydrous conditions, but recently the ability to methylate mercuric salts has been demonstrated in bacteria, micellia and in human liver. The only known biological mechanism for methylating mercury involves a derivative of the essential vitamin B_{12}, a cobalt containing species. Significantly, aquatic anaerobes are rich in B_{12} and can convert mercuric salts in industrial discharges to the more toxic, recalcitrant methyl-mercury analogues.

In the past three years, public attention has been focused on methylmercury as an industrial pollutant and health threat, and based on analyses of museum specimens of dubious history, claims have been made regarding pollution and levels of methylmercury in previous times. However, conclusions from data on museum species would seem tenuous due to the possible use of a mercurial at some time during their preservation. Recent data, however, show that methylmercury predates museum preservations and that substantial amounts of methylmercury in the environment predate industrial contamination.

Thus, the widespread occurrence of methylmercury is reminiscent of zinc in the 1920's. Indeed, methylmercury has been recently demonstrated to stimulate protein synthesis in vitro, and it seems likely that an essential role for mercury and methylmercury may exist undetected.

Unlike mercury and lead, cadmium is relatively rare in the earth's crust, and only minute traces are found in seawater. While it occurs in a broad range of organisms, it still has no essential biological function.

Cadmium is just below zinc on the periodic table and not surprisingly it exhibits similarities in its chemistry, forming stable complexes with nitrogen, sulfur and oxygen ligands.

The consequences of substituting cadmium for zinc in carboxypeptidase have already been mentioned -- namely, that the cadmium enzyme is a better esterase, but exhibits no peptidase activity. Cadmium enhances activity for no less than 25 enzymes in vitro and/or in vivo, and inhibits an even greater number.

Metallothionein is a small protein, MW ∿6000, containing large amounts of tightly bound cadmium. In fact, metallothionein accounts for 1-2% of the total soluble protein in horse kidney cortex. In addition to small amounts of iron and copper, metallothionein contains up to 6% cadmium and 2.2% zinc by weight. Low pH and EDTA remove and both p-chloromercuribenzoate and silver ions instantaneously displace cadmium and zinc from the 20 SH groups of metallothionein. This unusual protein has been isolated from human and rabbit kidney and liver, and rat kidney, but as yet it has no known function.

In addition to forming stable protein complexes, cadmium interacts strongly with phospholipid monolayers disrupting them at 10^{-3} M, so it is not surprising that cadmium toxicity seems to result largely from disruption of membrane dependent processes. Thus, chronic cadmium intoxication in man and experimental animals causes kidney damage and proteinuria, where proteins of the size 10,000 to 200,000 MW are spilled in the urine due to kidney disfunction.

Long term feeding of cadmium to rats or rabbits has been reported to produce hypertension which can be prevented by simultaneous administration of zinc or chelating agents, but despite speculation, cadmium has not been demonstrated to cause hypertension in man. Recent studies have shown no correlation between urinary cadmium and blood pressure, and cadmium content of liver and kidney is not increased in patients succumbing to hypertension.

As mentioned, cadmium forms complexes with nucleic acids, and in some undetermined fashion has caused developmental abnormalities in hamster embryos.

LEAD IS WIDELY SPREAD

Lead is another heavy metal which has experienced recent publicity, particularly the health hazards posed by lead in paint and in exhaust fumes of gasoline engines. Aside from these unnatural sources, lead is widely distributed in the atmosphere, oceans, earth and groundwater. It can be absorbed and concentrated by various plant and animal tissues.

Table I shows lead concentrations encountered on a global scale, and a correlation between industrial development and blood lead concentrations is patently lacking. Lead can occur in significant concentrations in members of isolated or non-isolated societies who have no known source of elevated lead intake such as food, water, cooking utensils or pottery glazes.

By comparison, suburban males in Philadelphia were found to average 13 µg lead per 100 ml blood, commuters 19 µg and urban dwellers 24 µg -- the last being roughly equivalent to values for New Guinea aborigines, a group apparently devoid of any usual source of exogenous lead.

Like mercury and cadmium, lead complexes with numerous functional groups on proteins, nucleic acids and membrane structures. In particular, lead can exhibit broad spectrum toxicity through inhibition of sulfhydryl enzymes. However, lead concentrations of 10^{-3} or $1-^{-4}$ M are generally required to inhibit sulfhydryl enzymes in vitro -- a concentration greater than that found in tissues and fluids of individuals known to be suffering from lead intoxication.

However, much lower lead concentrations specifically inhibit certain enzymes, one of which is δ-aminolevulinic acid dehydratase, an enzyme involved in heme biosynthesis. 10^{-5} M lead causes nearly complete inhibition in vitro of human red cell ALA dehydratase. Its activity decreases markedly in patients and animals suffering acute or chronic lead intoxication, and an inverse correlation between blood lead concentrations and ALA dehydratase activity in experimental animals has been reported. In fact, a similar correlation has been reported for seemingly normal individuals living in an

TABLE I

LEAD CONTENT OF BLOOD FROM PERSONS OF DIFFERENT LOCALES

	Lead µg/100 ml blood
Finland	28
New Guinea	22
Egypt	28
England	23
Japan	21
Czechoslovakia	20
California	19
Ohio	18
Chile	18
New York City	17
Argentina	16
Israel	16
Yugoslavia	15
Holland	15
Italy	13
Poland	12
Sweden	9
Peru	7

urban environment, but biochemical and medical interpretations of these findings are problematic for the following reasons.

In the published assay procedures for the human enzyme, kinetic parameters such as V_{max}, K_M and pH optima are not available. Suitable kinetic standards are lacking. Also, the assay product for this enzyme is unknown making delineation of synthesis and breakdown steps impossible. Furthermore, anomalous temperature effects involving lead have been reported, but not accounted for. So previous conclusions derived from ALA-dehydratase kinetic considerations may be questionable.

QUESTION OF SUITABLE CONTROLS

More significantly, medical implications would require correlations of enzyme activity with the concentrations of lead present, a criterion which has not been fulfilled. Furthermore, effects of lead on ALA dehydratase activity in red blood cells vary with the maturity of the erythrocytes. Indeed, 10^{-7} M lead has been reported to stimulate heme biosynthesis in rabbit bone marrow.

Suitable controls pose yet another problem, for although the enzyme is inhibited by low concentrations of lead, this is not to say that the dehydratase activity is therefore an index of blood lead concentrations, especially when alcohol is known to inhibit this enzyme strongly.

Until normal values of this enzyme activity have been established for a normal population under suitably standardized assay conditions, and until the possible role of parameters such as other metals has been explored,

no definite conclusions can be reached, and one might question the necessity of removing lead from gasoline in view of the undisposable parameters which have not been ascertained -- not the least of which is reliable measurements of blood lead concentrations for a normal population.

Aside from its toxic properties, it should be kept in mind that lead has been reported to enhance at least 10 different enzyme activities.

Throughout this discussion I have tried to point out how heavy metals can play both beneficial and detrimental roles in biology, and to suggest that their ubiquitous occurrence in biology may signal an undiscovered, essential role in nutrition. Thus, the lack of success in establishing heavy metal deficiency states does not preclude the existence of a biologically essential function. In fact, control analyses at the requisite low levels for these and other metals may well preclude such studies temporarily.

Two heavy metals which have received more attention for their antipruritic capacity than for their possible roles in biology are silver and gold, both of which have been used therapeutically for many years. Silver has been used medicinally for thousands of years. However, increased use of silver followed the recommendation for its use in treating nervous disorders by Paracelsus, a 16th century Swiss alchemist and physician whose real name was Theophrastus Bombastus von Hohenheim.

Treatment was based on the believed relation between silver and the moon. Namely, lunatics were under the influence of the moon goddess, Luna, and accordingly, deranged people (lunatics) were treated with Lunar

Caustic (silver nitrate) to bring one's silver function back to normal. In fact, silver nitrate was used to treat epilepsy until the late 19th century. Today its medicinal use and toxicity are limited to its caustic action resulting from localized precipitation of proteins as silver salts.

Gold found sporadic use in the 1880's and early 1900's in the treatment of syphilis and tuberculosis, and even if these experiments were disappointing, they served to stimulate interest in the use of gold for the treatment of arthritis, because arthritis was thought to be a form of tuberculosis. Its use for treating syphilis and tuberculosis were short-lived, but in spite of ACTH and corticosteroids, gold therapy still plays an important role in the management of rheumatoid arthritis.

Experimentally in mice, gold salts protect against infection by a pleuropneumonia-like streptococcus which in mice and rats causes a disease similar to rheumatoid arthritis. Secondly, subcutaneous injection of rats with a particular gold salt inhibits transaminase and formation of glucosamine-6-phosphate in connective tissue, but not in liver. Lastly, for arthritic patients gold concentrations in painful joints reaches twice that in uninvolved joints, but its mechanism and therapeutically active form(s) in these phemomena is unknown.

A considerable problem with gold therapy is its toxicity which manifests at concentrations only slightly above the effective therapeutic dose. When toxic levels are reached gold can cause numerous membrane lesions, neuritis, peripheral encephalitis, and kidney damage, but still it finds widespread use in the treatment of rheumatoid arthritis.

That metals play a highly complex role in biological processes is obvious in the case of transition metals, which have been studied more extensively. For these, numerous metal ion deficiencies and diseases have been established including diseases which result from metal ion antagonisms or imbalances where deficiencies of one metal relate to abnormal levels of a second. For example, excess copper can displace zinc in the body, but administering zinc can normalize levels for both these essential elements. There is no reason to expect or predict that once understood, the roles of heavy metals in biology will be any less complex.

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Selenium in the Environment

Selenium has been described as one of the "most maddening frustrating nutritional minerals to examine in the entire table of elements" (1). At O.S.U. we concur with this statement as we have been working with selenium for the last 15 years and we still are unable to explain many of its functions and interrelationships. However, since its discovery by Berzelius in 1818 in the sediment of a sulfuric acid plant much has been learned about this element. Because of similar chemical properties it has often been linked with sulfur and tellurium. In fact, there is a Selenium-Tellurium Committee, primarily from industry, which publishes a monthly selenium abstract service.

Selenium even exhibits its paradox to glassmakers who use it either to make glass clear, or conversely, to make red glass redder and more opaque. Similarly, electrically it is a splendid insulator but, when exposed to light, it is an excellent conductor and is used as such in many copy machines.

To begin, let us examine where selenium is in the environment and what does it do. Where is selenium found? It is a relatively scarce

element and has a mean content in the crust of the earth about 0.09 ppm and is 66th in order of abundance (2).

Molybdenum is a trace element of biological importance and sparsely distributed in nature. Yet it is present in the crust of the earth in a concentration 25 times that of selenium. Sulfur, on the other hand, is related crystallo-chemically as well as geochemically with selenium. In the earth's crust the accepted content of S is 520 ppm. This concentration is rarely achieved by selenium in any material. Therefore the mean S:Se ratio is around 6,000. This same ratio for shale is around 4,000.

Selenium occurs especially in association with sulfur deposits and may also be found in combination with elements such as iron, lead, copper, and arsenic. In most soils not derived from sedimentary rocks the selenium concentration may be in the order of 0.05 ppm. In sea-bottom sediments and sedimentary rocks selenium values may be as high as 1.2 ppm. However, there are certain seleniferous regions in the world with much greater concentrations of selenium. In addition to South Dakota and Wyoming, seleniferous areas have been reported in Canada, Mexico, Ireland, Australia, Israel and China.

The areas involved vary from rather extensive ones to more or less isolated portions such as we have in the Rome valley in Oregon. Usually the rainfall is less than 20 inches which is insufficient to leach out the water soluble selenium compounds. Also many of the rock formations

which are high in selenium are resistant to weathering and seldom form soils for vegetation, a fact fortunate from the standpoint of selenium toxicity.

SELENIUM IN MANY LOCATIONS

Conversely, a selenium deficiency is found in the soils of both N.W. and N.E. United States, along the East Coast and in parts of S.W. Canada. In these locations the soil was formed before the selenization period of the area near the Rocky Mountains. The selenium content of most soils has been estimated to be 0.01 - 0.2 ppm (3).

The selenium concentration in the major oceans is estimated to be about 0.09 micrograms per liter. Samples from the Antarctic were lowest, 0.05, and Long Island Sound the highest, 0.11 micrograms per liter. Selenium is also found in small quantities in ground and surface waters ranging from 0.1 to 100 micrograms per liter. The safe upper limit for drinking water is stated to be 10 ppb by the U. S. Department of Health. The presence of selenium in surface waters is a function of both pH and drainage slope with the highest concentrations coming generally from specific spring waters (3).

The selenium content of coal ranges from 0.1 to 3.9 ppm and this when burned introduces selenium into the atmosphere for redistribution on the earth's surface. Some of this atmospheric selenium from coal undoubtedly ends up as atmospheric dust which can vary from 0.05 ppm in San Francisco to as high as 10 ppm in office buildings in St. Louis (3).

In general, plants may be divided into three groups: (1) Those which discriminate against selenium and absorb little (soy bean will have about 1/4 the soil concentration). (2) Those which concentrate selenium to limited degree (wheat and barley may have up to 10 times the soil concentration) and (3) Those which accumulate selenium (Astragulus spp. and Stanleya spp.) may have more than 1,000 times the soil concentration. In Oregon most plants do not contain enough selenium and lambs and calves die from the lack of it. Most crop plants are of the second type and concentrate the selenium only to a minor extent. Plants which grow on soils rich in selenium may concentrate the selenium and cause death to the animal consuming them. In Wyoming and North Dakota there are more than 24 species which may be classified as accumulators.

After the plant takes up the selenium, what happens? Let's first consider plants with abnormally high amounts as this part of the paradox was discovered first. Marco Polo noted in his journals in the thirteenth century that in parts of Western China, his horses hoofs would drop off if the animals grazed certain plants.

Slightly more than 100 years ago a similar observation was made by an Army surgeon at Ft. Randall in Nebraska concerning horses of the U. S. Cavalry. In 1907 and 1908 more than 15,000 sheep died in a region near Medicine Bow, Wyoming and their death was attributed to certain plants in the area now known to be selenium accumulators. Although these plants generally have a garlic-like smell and are offensive to livestock, under conditions of stress they will be consumed with disastrous results. This has been the case for sheep, cattle, horses and even pigs. The affected

animal staggers for a short distance, lowers its head and ears, develops diarrhea, abdominal pain and bloating which rapidly leads to coma and death. "The staggers", as it was called, is still seen on occasion where these plants grow. Selenium is the only element known to be absorbed by plants in sufficient amounts to cause the death from acute poisoning of animals which eat them.

CHRONIC POISONING

Chronic selenium poisoning is caused by consuming plants containing from 10 - 30 ppm selenium. That selenium was the culprit was discovered by the South Dakota Experiment Station workers who did extensive studies once they had implicated selenium as the cause of "alkali disease". They then proved their theory with poultry and animal experiments. They first had to disprove that the general symptoms were not due to ergot, nor fungus diseases, nor poison from yellow spiders according to the Sioux Indian legend, nor known poisonous plant glucosides or a peculiar ratio of mineral elements.

The first hint came in 1930 after a rat feeding experiment where rats fed toxic grain lost their hair, developed necrotic livers and died; and this led to the postulation of arsenic, thallium or some other less common element as a cause of the condition. The next year it was found that the toxic grain had several times the concentration of selenium of non-toxic grains. The toxic properties of selenium were determined experimentally and the cause of "alkali disease" and "blind staggers" was known. It was found that chronic symptoms started with lack of vitality,

emaciation, rough coat, anemia, erosion of the long bones causing stiffness, loss of hair, and soreness and sloughing of the hoofs (4). There was also atrophy of the heart and liver with symptoms varying between species.

In poultry, eggs from selenized hens failed to hatch or had deformities. Selenium in the milk could even affect the young. The same South Dakota workers performed numerous rat experiments and found that the relative toxicity of selenium from different sources was wheat > corn > barley > selenate > selenite. It was found that diets with as low as 5 ppm selenium prevented normal growth. They determined also that selenium is more toxic than arsenic, vanadium, tellurim or molybdenum. When fed to animals the greatest concentrations of selenium were found in the liver, kidney, heart and spleen with smaller amounts in practically every part of the body.

This work also identified the "converter" plants. These plant species have the ability to absorb selenium from the soil which is apparently nonavailable to other crops and grass plants. These "converter" plants, upon decaying, supply selenium in a form which is then available to other plants and which can play an important role in the selenium problem. The "converter" plants are usually the common "indicator" plants which tend to have a high concentration of selenium.

In humans one of the first signs of chronic poisoning is brittleness and malformation of fingernails. Urine concentrations of selenium from individuals living in seleniferous areas may be as high as 1.33 ppm selenium.

Acute toxicity from a single dose of selenium has ranged from 1 mg/k bodyweight in the rabbits to about 3 mg for rats and horses, 8 mg for sheep, 11 mg for cattle and 15 mg for pigs (2). Prolonged feeding of rats of 3 - 4 ppm dietary selenium produced evidence of selenium toxicity while only 1 ppm has produced detectable changes in hemoglobin, serum protein, red cell count, and in the albumin:globulin ratio in chicks.

Following long-time ingestion of sublethal amounts of selenium, all tissues tend to accumulate selenium to a varying degree. The selenium concentration in hair is found to vary from 1 - 4 ppm in cattle from non-alkali disease areas to 10 - 25 ppm from the seleniferous range. This would seem to indicate that hair analysis could be indicative of the selenium status of the animal.

ECONOMIC IMPORTANCE

The economic importance of the selenium problem has been felt in a number of ways. Seleniferous areas originally obtained for grazing land caused such large livestock losses that the land use was changed to grain production. Grain produced in these areas smelled like garlic and was often sold at a discount, sometimes amounting to 50%. This was also true for hay. As a result the government Resettlement Administration finally purchased about 100,000 acres because of its selenium content and reputation as a toxic area.

Selenium toxicity in man has not been as well studied. However, selenium has long been regarded a toxic substance with elemental selenium

relatively nontoxic and hydrogen selenide extremely toxic. It is fortunate that selenium is utilized commercially, principally in the elemental form. Only seldom have serious intoxications from the indistrial use of selenium and its compounds been reported. The selenium compounds have been mostly absorbed through the lungs via dust or fumes and through the skin.

Chronic symptoms include depression, nervousness, occasional dermatitis, gastrointestinal disturbances, giddiness, and a garlicy odor of breath and sweat. Selenium is excreted principally in the breath, urine and feces. The concentration in the urine varies considerably and strangely, it is not necessarily correlated with symptoms of selenium toxicity. The maximum allowable amount of selenium in urine is 100 micrograms per liter. This limit may be reached by inhaling air containing 100 micrograms of selenium per cubic meter for an eight hour day.

Interestingly, a person's diet may influence detoxification of selenium in the body. The protein content of food plays an important role. Generally a high protein diet may protect a human from chronic selenium intoxication.

Arsenic has also been found to counteract the toxic effect of selenium in rats, dogs, pigs and chicks. It has even been suggested that a tonic containing arsenic be used for the prevention of selenium intoxication in exposed workers. This is still a hypothesis as experimental evidence is lacking. Bromobenzene, also a rather toxic substance in itself, has been suggested to accelerate the excretion of selenium in urine but has proved to be of doubtful merit. Other suggested medicants are increased amounts

of ascorbic acid, vitamin K, and glutathione in the diet as these substances are found to be lowered significantly in the body after exposure to selenium.

Selenium compounds vary widely in their toxic effect on humans and care should be exercised in handling selenium and its compounds, particularly hydrogen selenide. Usually the industrial hazard is obliviated by adequate ventilation, and proper protection of the skin from certain of the salts which can vary from causing irritation and inflamation to being a severe desiccant(3).

Research leading to the fact that selenium is also an essential element started in 1939 when Klaus Schwarz found that para amino benzoic acid was not an essential nutrient for rats which were fed a highly purified casein diet. However, these rats died with liver necrosis within a month when fed this diet. They would grow normally with a supplementary feeding of wheat germ or whey. First, tocopherol was found to be one liver-protecting factor and cystine was a second. A third factor also was found to be present in ordinary casein and certain yeasts.

It became known as Factor 3 and was found to be concentrated in the kidney where it was bound to protein. It had a low molecular weight, was insoluble in lipid solvents, soluble in polar solvents and when concentrated Schwarz noted a garlic-like odor which caused him to consider selenium (5). Previously the Cornell workers (6) had found that Factor 3 prevented exudative diathesis in chicks and in conjunction with Schwarz proved that selenium as selenite or selenoamino acids were highly potent sources of Factor 3 activity for both chicks and rats. About the same time a group

from Lederle Laboratory independently confirmed this finding after ashing a crude Factor 3 preparation and finding considerable activity even in the ash.

VARIETY OF SYMPTOMS

That selenium prevented white muscle disease in lambs was reported by OSU workers in 1958 (7). Since that discovery some disorders prevented by selenium include microcirculatory disorders in chicks, rats and pigs; myopathies in lambs, calves, and turkeys; reproductive disorders in rats, sheep and cattle and other miscellaneous symptoms such as pancreatic lesions in mice and chicks, and loss of hair or feathers in rats and chicks. We have also found many of these selenium responsive disorders present in the squirrel monkey when fed a semisynthetic diet with torula yeast as the source of protein (8).

This was the first report on experimentally produced selenium deficiency in subhuman primate as a way of answering the question as to possible selenium deficiency symptoms which might be observed in humans. Selenium supplementation had previously been reported to be effective on growth and reticulocyte formation in certain kwashiorkor children who had failed to repond to the usual protein treatments, indicating the possibility of a definite role (3). That selenium deficiency exhibits such a variety of symptoms depending on the species would seem to indicate that it is essential for several rather major biochemical phenomena within the animal body.

Schwarz also developed a biological assay to study Factor 3 activity. Rats were fed a dietary composed mainly of torula yeast, sucrose, and vitamin E free lard plus the other usual vitamin and salts. This diet is low in the sulfur-containing amino acids, unusual in its unsaturated fat acids from the yeast, and lacking in vitamin E. Using certain animal handling procedures, weanling rats will either die of liver necrosis at 21 days or if supplemented on the 13th day of experiment will afford a biological assay which can determine the biological availability of selenium in concentrations as low as 5 ppb.

At this concentration all rats die. Using bioassay data Schwarz has concluded that one atom of Factor 3 selenium is equivalent in selenium response to 700 - 1,000 molecules of vitamin E or 0.4% l-cystine whose activity may really be due to selenium contamination. While Factor 3 is still unidentified it is only about three times as potent as the easily available inorganic selenite.

When one considers that usual normal dietary levels of selenium for most species range from 0.1 - 0.5 ppm to protect against selenium responsive disorders, it is not really surprising that the identity of Factor 3 is still unknown. And even when selenium deficient forages are fed we have found that white muscle disease may be prevented in the lamb by treating the pregnant ewe with oral or injected selenite (0.75 mg/10 KBW) a couple of times prior to parturition or by treating the lamb with oral doses or injections of small amounts of selenium at intervals of a week or two. Rats, chicks and monkeys also respond quickly to supplementary selenium, either dietary or injected.

Initially neutron activation analysis was the analytical tool of choice used to determine the extremely small smounts of selenium present in tissues and foodstuffs. Other less costly procedures have since been developed. We use a procedure developed by the Cornell workers (9) which combusts the sample in an oxygen flask. Following solution of the ash in dilute acid the fluorescence of 3,3'-diaminobenzidine (DAB) is determined; this is proportional to submicrogram amounts of selenium.

This procedure while accurate is extremely tedious, but it is the only practical analytical method that we have found suitable for determining the selenium concentration in certain forages. Our agreement with the selenium values for the National Bureau of Standards orchard leaf sample (.08 ppm) and liver sample (1.1 ppm) has been excellent.

The biochemical role of selenium in living organisms is still unknown although a number of hypothesis have been presented. Selenium seems to be acting similarly to iodine, cobalt and chromium which are of significance primarily to animals. Selenium has little influence on plants or microorganisms. Alfalfa and other forages may be grown on selenium deficient soil with both a high yield and excellent forage quality.

On the other hand, torula yeast, which causes selenium deficiency when fed to animals, is produced commercially from spent sulfite liquor from paper mills. It would seem, therefore, that the function of selenium is not concerned with "biochemical universals" like the citric acid cycle but is probably more vital to animals' physiological systems such as muscular, endocrine or circulatory.

NO CLEAR EXPLANATION

It is not surprising also that there has been an interrelationship suggested between selenium and its chemical neighbor, sulfur. Not only are selenium analogs of sulfur-containing amino acids found in both plant and animal tissues, but also when severe selenium deficiency is produced in the rat in the presence of adequate vitamin E, epithelial tissues rich in the sulfur-containing protein keratin are abnormal, indicating an altered sulfur metabolism (10). With sheep fed clover hay supplementary sulfur as Na_2SO_4 decreased the effectiveness of selenium in preventing WMD. When sulfur-containing fertilizers were applied to alfalfa increasing the total sulfur content the result was also an increase in the incidence of WMD (11).

To provide more controversy, later experiments with alfalfa and K_2SO_4 instead of Na_2SO_4 provided results that the sulfate did not increase the incidence of WMD (12). Neither did 0.5% DL methionine alter the incidence of WMD. It was of interest that K_2SO_4 significantly increased the number of lambs with degenerative lesions of the heart (12). In these experiments, with over 20 lambs per group, the incidence of WMD ranged from 70 - 81% in the sulfate, methionine and control groups with no histological lesions found in lambs whose dams had been injected with only 5 mg of selenium one month prior to lambing.

There is still no clear explanation concerning the various conflicting reports in the literature of sulfur-selenium interrelationships. Inorganic sulfur may alter the metabolism of inorganic selenium more than organic sulfur. However, one must be sure to designate the forms of sulfur

and selenium used to aid in correctly interpreting the results (12). The incidence of WMD is less in lambs fed a low selenium grass hay or grass pasture than those fed alfalfa hay of the same selenium concentration which inherently contains more sulfur. Even when grass hay was supplemented with sulfate and magnesium oxide to effect the same levels as found in alfalfa hay, there was only a slight, not significant, increase in WMD; but these elements were without effect when added to the diet of ewes on pasture grass.

Further experiments using cysteine (which had proved a beneficial supplement for chicks) as a supplement to lambs did not alter the incidence of WMD. Dimethyl sulfoxide (DMSO) either fed or injected increased the incidence of WMD in lambs from ewes fed a selenium deficient alfalfa diet but had no effect on those with selenium injections or when ewes were maintained on low selenium pasture grass (13). These results indicate that factors other than selenium deficiency alone can alter the incidence of WMD and tend to explain why selenium was described "as a maddening and frustrating element".

The enzyme picture in blood from humans with muscular dystrophy and also lambs and calves differ from the normal. Plasma levels of lactic-dehydragenase (LDH), glutamic oxalacetic transaminase (GOT) and creatine phosphokinase (CPK) are three enzyme systems which are significantly higher in WMD lambs as compared with the normal. In fact, a high degree of correlation has been found between the enzyme activity and the degree of tissue degeneration found by histopathological studies.

We now are of the opinion that we do not have to do histopathological studies with lamb tissues as we have done in the past. Instead we can

determine the WMD status of the animal by determining the enzyme activity in the blood. This has proved to be very beneficial as we are able to continue to use these animals for further experiments which we previously had to kill. They are invaluable in replenishing our flock of sheep with animals which have a low selenium dietary history. This is extremely important in interpreting the results of a selenium experiment, for we have found that both sheep and rats do have a significant carry-over effect of selenium. By carry-over effect, we mean, for example, ewes which have been fed a normal selenium content hay will store selenium so that they may produce normal lambs the first year after feeding a low selenium content hay but when continued on a low selenium regimen will have a WMD lamb the second year (12).

Similarly, selenium deficiency symptoms in rats are not necessarily seen in the first litter but are quite striking in the following litters. This indicates that trace amounts of selenium are retained tenaciously by the mother, and it may take multiple births to deplete the animal's selenium storage. So it is of paramount importance to know the selenium status of the animal prior to experimentation and undoubtedly some of the "maddening frustrating" evidence found in the literature is due to experimental animals of varying selenium status.

NORMAL VITAL ROLE

Many biochemical roles for selenium have been suggested from being a biological antioxidant to involvement in long chain fatty acid metabolism. At present we are interested in finding out more about a

10,000 molecular weight protein which is present in normal lamb muscle but not present in WMD lamb muscle. This protein has a rather high concentration of glutamic and aspartic acids and lysine. Strangely the sulfur containing amino acids and potential selenium analogs are of relatively low concentration.

Yet selenium must play a normal vital role in the formation of this low molecular weight protein. A rapid breakthrough in this exceedingly interesting problem is frustrated as lambs are born only in the spring. We think that glutathione in some form is involved. Glutathione has been implicated in the formation of dimethylselenide in selenium detoxification in animal and in "in vitro" liver slice experiments (6). It is postulated that selenodiglutathione (GSSeSG) is formed by a nonenzymatic reaction between glutathione and selenous acid. The reduction of selenodiglutathione is caused by glutathione reductase, a TPNH-linked enzyme. The resulting selenide is then methylated by S-adenosylmethionine and a methyl transferase to form dimethylselenide which is exhaled. The functions of selenium and glutathione in our studies is undoubtedly different than this detoxification mechanism.

To add to the confusion, selenium interrelationships have been found with regard to WMD or liver necrosis with various tocopherols, antioxidants, minerals such as cobalt, arsenic, silver and mercury, both inorganic and organic sulfur containing compounds. It is also possible that there might even be synergistic exerting effects between some of these factors making the interpretation of results even more illusive.

About 30 years ago Nelson et al (14) fed rats seleniferous grain at dietary levels to contain 5 and 10 ppm Se and found 11 hepatic neoplasms

of low malignancy and four microscopic hyperplasias in a group of 126 rats. Russian workers reported similar results using sodium selenate. This has caused selenium to be labelled a carcinogen. In an effort to get more information regarding selenium as a carcinogen the National Cancer Institute provided us with a grant to carry out an extensive study of the chronic toxicity of selenium in rats.

We used diets containing 12 and 22% casein, various levels of selenite and selenate, with or without added methionine and we even added a known hepatocarcinogenic agent, N-2-fluorenyl-acetamide (FAA), to determine its effect. Over 1,400 rats were used and over 1,100 autopsies were performed during this four year experiment. It was finally concluded that of the 63 neoplasms observed none could be attributed solely to the addition of selenium (3).

The minimum natural dietary selenium in these experiments was about 0.5 ppm selenium. This was inherent in the casein or other ration ingredients. Therefore we did not really know how selenium deficient rats fed adequate vitamin E would respond to supplementary selenite and the hepatocarcinogen FAA.

To answer this question we obtained 80 female weanling rats whose mothers had consumed only this low selenium torula yeast ration. These rats were selenium depleted. All of them were fed 150 ppm FAA which should produce hepatic neoplasia. One group of 20 rats were controls and had no additional supplement of selenium. The other three groups of 20 each

received 0.1, 0.5 and 2.5 ppm of added selenium. It was found that there was an inverse relationship of added selenite and cancer induction by FAA in rats.

That is, rats receiving 0.5 or 2.5 ppm selenium had less than 10% incidence of hepatic neoplasms at 240 days of age when the other two groups had a 40 and 75% incidence. Thus it would appear that selenium acts rather as an anticarcinogenic agent. We are presently continuing this study using different levels of two known carcinogens fed for a definite period.

CANCER MORTALITY IN PEOPLE

Recent publications have also suggested an inverse relationship of cancer mortality in people to levels of selenium found in crops in the different areas in the United States (16). However, criticism has been made of the way states were classified as to selenium status. For example Oregon was classified by these investigators as "moderate selenium" and yet Oregon is really known to be one of the lowest selenium locations in the world. Using Nebraska, Kansas, Oklahoma and South Dakota as selenium adequate states, the adjusted human cancer rate is about the same as Oregon, Washington, Ohio and Indiana which are low-Se states (17). It has also been reported that the selenium concentration of blood of gastrointestinal cancer patients is significantly lower than that of normal individuals; the difference, however, is not great, 0.15 vs 0.22 ppm selenium (18).

Conflicting evidence has also been presented in attempting to link an increased selenium intake with increased caries. However, it was pointed out that in an area in South Dakota where selenium toxicity has been observed the children had fewer dental caries than those at Bend, Oregon, a known selenium deficient area (17).

Another recent hypothesis regarding selenium is in its potential relationship to the so called "sudden infant death" (SID) syndrome (19). The hypothesis was based on the observation that there was a high mortality rate among apparently healthy baby pigs raised on a low vitamin E and selenium deficient ration. The mortality symptoms on autopsy were similar to the infant human syndrome SID. When SID blood samples were compared with normal infants, however, no significant difference could be found in the plasma vitamin E levels nor was there a significant difference between the mean level of whole blood selenium in SID and normal infants at least in the San Diego area. The disturbing feature in this study is the data that normal adults in the San Diego area averaged only 0.13 ppm selenium in their blood, a value which is 60 - 70% that of normal adults in Corvallis, a known selenium deficient area. I hope this study will be repeated in other areas.

With reference to the selenium content of food stuffs, it has been stated that a diet balanced in other nutrients will probably be nutritionally adequate with regard to selenium. If you want to be sure your selenium intake is adequate eat any kind of kidney, most sea foods, eggs and most cereals. Do not expect to find much selenium in vegetables,

fruits, dairy products and muscle meats (20). Of importance also is the fact that most ordinary cooking techniques - broiling, baking, frying or boiling does not result in major losses of selenium from most foods (21).

In conclusion while selenium is present in the environment in really minimum concentrations, it is an extremely toxic element and can exert its lethal effect on animals in the ppm range. In our own state the deficiency of selenium causes white muscle disease and other disorders in lambs, calves and possibly other animals. These myopathies will respond to minimal doses of selenium in the sub-microgram range. From all this discussion it is evident that selenium is indeed a wonderful but "maddening and frustrating element" both toxic and yet essential.

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