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Title: THE COMPARATIVE METABOLISM OF CADMIUM, MERCURY AND ZINC AS ENVIRONMENTAL CONTAMINANTS IN THE FRESHWATER MUSSEL, MARGARITIFERA MARGARITIFERA

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Dr. David L. Willis

The uptake, tissue distribution and retention patterns of cadmium, mercury and zinc were examined in the freshwater mussel, Margaritifera margaritifera. All three of these heavy metals are found in the environment. Both cadmium and mercury are toxic at low environmental concentrations. Zinc may also be toxic at low concentrations but is also a required microelement in plants and animals.

The mussels were maintained in separate solutions containing radioisotopes of these metals at 14.5°C. The uptake was followed for 39 to 80 days of chronic exposure, at which time a near equilibrium was reached in most experiments. At this point all animals were placed in uncontaminated water. Half of them were sampled at intervals to determine changes in tissue distribution. The remainder

were regularly counted to determine the pattern of retention. An Armac liquid scintillation whole-body counter was used for all determinations.

The tissue distribution was remarkably constant over separate studies lasting from 67 to 150 days. Multicomponent retention patterns were observed for each metal. The percent of original whole-body activity remaining after 81 days was 87% for methylmercuric chloride, 69% for mercuric nitrate, 76% for cadmium chloride and 57% for zinc chloride. Retention studies were terminated after approximately 81-150 days. The long term retention components for cadmium chloride, methylmercuric chloride, mercuric nitrate and zinc chloride revealed biological half-lives of 835, 860, 194 and 103 days, respectively.

The inhibition of zinc uptake in mussels was demonstrated when 2.0 ppm cadmium was added to the experimental solution. This level of cadmium also proved toxic to the mussels with a median survival time of 88 hours. When the cadmium concentration was doubled (4.0 ppm) the median survival time was reduced to 61 hours.

The Comparative Metabolism of Cadmium, Mercury and Zinc as Environmental Contaminants in the Freshwater Mussel, Margaritifera margaritifera

by

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INTRODUCTION

Heavy Metals and Aquatic Pollution

> . . . water pollution is any impairment of the suitability of water for any of its beneficial uses, actual or potential, by man-caused changes in the quality of the water.
> (Warren, 1971)

Concentration is the key word in determining what constitutes an aquatic pollutant. Neither foreign nor naturally occurring substances may be classified as pollutants until their concentration in the water is great enough to detract from its actual or potential beneficial uses. Water pollutants may be gaseous, solid or liquid. If liquid, they may contain dissolved or suspended material.

The term "heavy metals" is generally used in reference to metals with a density greater than 5, and includes about 40 elements in all. Heavy metals have been, and are being, released to the environment by two principal means, weathering and the activities of man. The natural levels of these metals in an aquatic environment are governed by the geological characteristics of the eroded rocks and soils of the region. Therefore, metal concentration varies naturally depending upon the region, rainfall, season, water temperature and the volume and rate of river flow.

Many domestic and industrial wastes are also released to the environment. Metals may be discharged directly to surface waters or to the atmosphere. These wastes may remain local contaminants or to the atmosphere. These wastes may remain local contaminants or they may be carried great distances. The atmospheric releases eventually either directly settle out or are carried back in rain or snow to reach the surface waters. Regardless of their source, most heavy metals eventually reach surface or subsurface waters. As a result of human activities, the flow of metallic contaminants into the aquatic environment has increased exponentially over the last several decades to such a degree that man may be no longer able to escape exposure to potentially hazardous concentrations of them.

Environmental Persistence and Ecological Modification

Perhaps the most important factor in the environmental hazard from heavy metals is their persistence. Free metals may be subject to biological and chemical alterations in the environment. They may be oxidized to ionic species followed by binding to form salts and/or biological conversion to methylated forms, subsequently being transformed back to inorganic compounds for recycling. Even though the chemical forms may rapidly change from one to another, accompanied by changes in toxicity, heavy metals generally remain toxic to some degree in most forms.

Heavy metals in aquatic systems may remain in solution or in suspension or may precipitate and settle to the bottom. Dilution and dispersion by rivers, oceans, winds and biological transport are natural means of reducing local pollutant concentrations. Simultaneously, many plants and animals possess the ability to accumulate and retain many elements including heavy metals. Concentrations of these metals in the upper trophic levels may be many orders of magnitude above the original water or air concentrations. Each metal tends to have a characteristic route and exhibits its own rate of movement through the many individual compartments of a biological system. Metallic wastes consequently tend to remain a continuous potential hazard to plants and animals in the environment for long periods of time. Some of the same heavy metals that are potentially toxic are required in trace concentrations for normal growth, metabolism and reproduction in all plants and animals.

Trace Metal Requirements

Essential trace metals function as a component of, or in association with, organic molecules--most commonly with enzymes. There are many quantitative and qualitative differences in trace metal requirements between major groups of organisms. Requirements also differ in individuals of the same species living under different conditions. There is an optimal range of concentrations, which is

sometimes quite narrow for each element in each organism.

Several heavy metals are needed in trace quantities by biological systems. Manganese and magnesium are responsible for activating a number of enzymes, such as phosphate transferases and decarboxylases and others associated with the tricarboxylic acid cycle. Magnesium also activates acyl transferases and at least one reductase (Bowen, 1966). Iron and copper are present in various respiratory pigments (e. g., hemoglobin, myoglobin, hemocyanin) and in oxidative enzymes which play a central role in metabolism (cytochromes, catalase, peroxidase and various metalloflavoproteins). Cobalt occurs in vitamin B_{12}, and zinc is an integral part of such enzymes as carbonic anhydrase and several dehydrogenases (Nilsson, 1970). The concentration of the essential metal is important. Most trace metals exhibit a concentration plateau of optimum utilization and any significant increase in the metallic concentration will initiate enzyme inhibition through a loss of the organism's homeostatic control mechanism.

Homeostatic Control Mechanism

Organisms are, in general, equipped with some sort of homeostatic mechanism to regulate the amount of essential trace metals that are incorporated into their tissues. This protective mechanism will compensate for minor environmental fluctuations of the metals, but

may fail when the organism is exposed to excessive concentrations. This failure could result in toxic effects. In the case of nonessential metals, plants and animals have apparently failed to develop similar homeostatic control mechanisms. Consequently, exposure to even small concentrations of such toxic metals as lead, mercury or cadmium, results in uncontrolled accumulation by the organism. Most toxic metal pollutants, therefore, are cumulative poisons.

Metal Toxicity

An element is said to be toxic if it has any deleterious effect on an organism when accumulated above a certain concentration. By this definition, all elements are toxic at very high concentrations compared to natural environmental levels. Some elements are even toxic at environmental concentrations. Poisonings in localized human populations will be discussed later.

This research deals with three heavy metals; cadmium, mercury and zinc. Both cadmium and mercury are toxic elements. By contrast, zinc is a required trace element.

Experimental Metals

Cadmium

Cadmium is a white metallic element, softer but denser than zinc, with which it is often associated. The metal is industrially

produced as a by-product of the refining of zinc, and to a lesser extent, lead and copper. In the past, cadmium was prepared exclusively from zinc-dust by a fractional distillation process, whereby the more volatile fractions are progressively enriched in cadmium. Due to the difficulty of condensing cadmium vapor, this process led to considerable losses of cadmium to the environment. More recently, cadmium has been produced by electrolytic methods.

Uses of Cadmium

During the last two decades the use of cadmium has increased considerably. The metal is most widely employed as a metallic coating to protect primarily ferrous metals against corrosion. A film with a thickness of 5 μ affords the same rust protection on iron as 12 μ of zinc, or 25 μ of nickel (Remy, 1956). Protective cadmium plating is used on numerous machine components. Alloys are extensively used in the electrical industry. Being a very efficient neutron absorber, cadmium is used in nuclear reactor control rods. Mercury-cadmium amalgams find use as dental fillings.

The uses of cadmium and its compounds are rapidly increasing, causing a sharp increase in environmental contamination. Cadmium is released to the environment during the manufacture, use and eventual disposal of these products. This cadmium will, in general, find its way into aquatic systems either directly from industrial liquid

effluents or by the meteorological phenomena discussed earlier.

Cadmium Toxicity

The severe toxicity caused by cadmium in man has been known for over a century. In spite of this and our increasing knowledge of cadmium intoxication, only 20 cases of cadmium poisoning via the oral route were recorded by Fairball during investigations prior to 1941. In the five-year period from 1941-1946, he found at least 689 documented cases of cadmium poisoning resulting from inadvertent oral ingestion (Flick, Kraybill and Dimitroff, 1971).

"Itai-Itai" Disease. An unusual chronic disease afflicting Japanese inhabitants living along the Jintsu River in northwestern Japan aroused considerable anxiety for 15 years prior to the discovery of its cause. It was termed "itai-itai" disease from the painful cries of its victims. It appeared most prevalent among women 50-60 years old. Between 1945 and 1960 there were 200 cases, half of whom died (Kobayashi, 1970). Decalcification of the skeleton resulting in hypercalciuria coupled with extreme loss of other skeletal minerals, left the bones as transparent to X-rays as soft tissue. Serious pain and multiple fractures were prevalent.

The first clue to the cause of the disease came in 1960, when Kobayashi (1970) analyzed bones and other tissues of patients and found a markedly high content of cadmium, zinc and lead. These

metals were traced to an operating nonferrous metal mine located on the upper reaches of the Jintsu River, a mine which produced zinc, lead and cadmium and discharged its wastewater to the river. Animal experiments conducted by Kobayashi (1970) demonstrated that symptoms of osteomalacia (bone decalcification) were induced by cadmium. Kobayashi (1971) stated that the increased production at the mine together with faulty treatment of the wastewater polluted the Jintsu River heavily during the Second World War. Downstream, where rice is cultivated, this water was used for irrigation and drinking water. Not only was the growth of the rice injured and the harvest decreased, but the concentration of cadmium in polished rice sometimes reached 125 parts per million (ppm) in the ash (Kobayashi, 1970). Other samples of polished rice ranged from 120-350 ppm in the ash compared with 21 ppm in a control area (Friberg, Piscator and Nordberg, 1971).

In 1955, and apparently unrelated to the disease occurrence, successful waste treatment was developed at the mine. As a result the agricultural damage was gradually corrected and the number of cases of this disease rapidly decreased.

Mercury

The problem of environmental mercury contamination in the United States is potentially grave. Only recently have efforts been made to define its magnitude. Although the toxic qualities of metallic

mercury and methylmercury have long been known, the current situation is the result of the extensive increase in the agricultural and industrial uses of mercury in recent decades. Environmental mercury contamination results from both natural processes and man's activities.

Natural Processes

Mercury may be introduced into surface and ground water by weathering and erosion of rocks and soils. The major natural source of the element is cinnabar (mercuric sulfide), which contains about 86% mercury by weight. Cinnabar is generally found in mineral veins or fractures near volcanic or hot-spring areas. Surface waters in the United States, except where influenced by manmade pollution, generally contain less than 0.1 part per billion (ppb) mercury. Normal groundwater is considered to range between 0.02-0.07 ppb mercury. Relatively high concentrations of mercury are likely to occur in underground waters because of their longer and more intimate contact with mercury-rich minerals. Hot springs and geothermal stream fields have also been associated with high mercury levels (0.5-3.0 ppb) (U.S. Geological Survey, 1970).

The vapor pressure of mercury is 0.001426 mm Hg at 22 ^{o}C and, thus, its vapor is found in the air over mercury, base and precious metal ore deposits in excess of normal atmospheric levels encountered

in air over nonmineralized areas. These elevated atmospheric levels ranged from 3-9 ng/m^3 in the areas investigated by the U.S. Geological Survey (Report no. 713, 1970). Rain and snow scrub mercury from the atmosphere and it then becomes associated with the upper several inches of the soil. This mercury can be carried to streams by runoff.

In summary, the weathering and erosion processes are estimated to transfer about 4,500 metric tons of mercury per year from the continents to the oceans via the rivers (Goldberg, 1970).

Man's Activities

There are two current major industrial uses for mercury. The agricultural industry employs mercury compounds as seed-grain coatings to combat mildew and disease of the cereal before germination. Manufacturing industries use many forms of mercury which will be discussed in detail below. Unfortunately, both of these uses are dissipative resulting in large annual releases to the environment. Worldwide mercury usage has increased greatly during the 20th century, more than doubling between the years of 1947-1966 alone.

Mercury compounds were first used as seed dressings to control seed-borne fungal diseases of cereals in Germany about 1914. Agricultural uses of mercury have increased many-fold since that time. In the 1940's liquid seed dressings containing methylmercuric

dicyandiamide (Panogen ^{R} and others) were introduced on the Swedish market. In a very short time Panogen ^{R} was the most widely used seed dressing. Less than ten years later conservationists charged that the methylmercury compound caused severe poisonings of seed-eating birds and their predators. It wasn't until September 1965 that "it was shown beyond any doubt that the use of methylmercury in agriculture was responsible for the poisoning and drastic decrease of wild bird populations" (Löfroth, 1970).

Chlor-alkali plants are generally considered to be the largest single source of manmade environmental mercury contamination. In this industrial operation mercury is employed as a flowing cathode in the electrolytic production of chlorine and caustic soda. The total chlorine capacity of mercury cells in the U.S. is about 7,300 metric tons per day. In 1969 the average loss of mercury to the environment from these plants was about 225 g of mercury for each ton of chlorine produced. This resulted in an estimated release of 1,800 kg of mercury into U.S. waters each day (Wallace et al., 1971). On a world-wide basis this industry requires about 400 metric tons annually for replacement of these mercury losses (Goldberg, 1970).

Environmental mercury contamination also results from the use of mercuric sulfate, containing 1% methylmercury, as a catalyst for the production of urethane and vinyl chloride plastics and acetaldehyde.

Phenylmercuric acetate was long used as a slimicide in the pulp

and paper industry. It consequently found its way to adjacent surface waters in large quantities resulting in polluted lakes and rivers in a number of areas around the world. This practice has been banned, but large deposits of mercury still exist in the sediments downstream from these plants.

The burning of fossil fuels is another dissipative source of mercury. The actual amounts of mercury in such fuels are quite variable. Extraordinarily high levels (300 ppm) are found in coal interbedded with mercury deposits in Russia (Fleischer, 1970). Coal mined in the U.S. contains between 0.5-3.3 ppm mercury. Since 500 million metric tons of coal were burned in the U.S. in 1968, an estimated 250-1600 metric tons of mercury were released to the atmosphere (Wallace et al., 1971).

The second largest consumptive use of mercury is the manufacturing of electrical apparatus. The electrical industry uses over 450 metric tons of mercury each year in batteries, silent switches, high-intensity street lamps, photocopying machines and fluorescent lights. A large part of this mercury eventually finds its way into the environment. Other dissipative sources include the manufacture and use of pharmaceuticals, cosmetics and paints.

Goldberg (1970) concluded that, in addition to the 4,500 metric tons of mercury entering the oceans from continental weathering processes each year, about 8,350 metric tons (half of the world

production of mercury) is annually released to the environment in an uncontrolled way. Most of this eventually reaches the oceans.

Mercury Toxicity

Available human data indicate an intestinal absorption of more than 90% for a single ingestion of methylmercury (Bergland and Berlin, 1969) and a biological half-life of between 70-74 days (Selikoff, 1971). However, repeated minute intakes of mercury over a prolonged period could increase the body burden of this metal with time leading to permanent disability and even death.

The medical literature contains many examples of both acute and chronic human exposures to mercury. Industrial and epidemiological incidents make up the majority of unintentional exposures.

Industrial Exposures. Industrial exposures to mercury are usually through inhalation. Exposure to alkylmercurials may occur during the application of mercury to seed grain, but is relatively infrequent. Inhalation of metallic mercury vapors may occur in the chlor-alkali and electrical industries. Inhaled concentrations in the range 10-30 $\mu g/m^3$ tend to produce borderline cases of intoxication, while clear examples of toxicity occur in the neighborhood of 100 $\mu g/m^3$. Human exposure for six years to 250-3,200 $\mu g/m^3$ air concentration of phenyl- and inorganic mercurials as used in the pulp and paper industry, produced no evidence of poisoning. Thus, these compounds

must be considerably less toxic than vapors of metallic mercury (Wallace et al., 1971).

Epidemiological Exposures. Community poisonings in which mercury has been found as the causative agent have occurred in several countries. Only intoxications resulting from contaminated estuarine and freshwater ecosystems will be emphasized here.

When mercury contaminated fish and shellfish are ingested by man, methylmercury is assimilated. Methylmercury passes the "blood-brain barrier" with ease, which explains the severe neurologic manifestations and even death, which may follow the accumulation of small amounts of this compound. The slow rate of elimination of methylmercury by fish, shellfish and man potentiates this effect.

In 1953 severe neurologic disorders erupted among rats, cats, fish-eating birds and humans living in 11 small villages situated along the shores of Minamata Bay, southwestern Kyushu, Japan. In 1956 a common link was established between these cases--all had eaten fish and shellfish caught in the area. Fishing was banned in the bay as a result of the 42 human cases reported during the first 11 months of that year (Lofroth, 1970) and no new cases occurred in the following 19 months (Kurland, 1960). A heavy metal was suspected as the cause of the syndrome, by then called "Minamata Bay Disease."

A major research effort, centered around heavy metals, was initiated by a local university. Among other metals, their findings

included high concentrations of mercury in fish and shellfish from the bay. Matida and Kumada (1969) reported that the distribution of mercury in the sediments of Minamata Bay indicated that the source of the mercury was the vinyl chloride plant of Shin-Nippon-Chisso Co. Mercury concentrations in the sediments close to the plant were reported by Kurland (1960) to be 2010 ppm. Shellfish caught in the bay in 1958 were variously reported to contain between 11-41 ppm mercury (wet weight) (Löfroth, 1970) and from 27-100 ppm (Kurland, 1960).

In 1958 the vinyl chloride plant closed its effluent to the Minamata Bay and opened a new one to the Minamata River which was in a different water shed, not emptying into the bay. The following year shellfish containing 9-24 ppm mercury and new human poisonings were reported in that area. Waste treatment facilities were installed and later improved, resulting in drastic reductions in the mercury content of fish and shellfish.

By 1970, a total of 121 cases resulting in 46 deaths had been attributed to methylmercury in the Minamata area. Of these 121 cases, 23 were fetal intoxications, a cerebral palsy-like disease. In each case the mothers had eaten contaminated fish and shellfish but were themselves apparently free of the symptoms of the disease. Selikoff (1971) showed that, unlike inorganic mercury which passes the placenta poorly, methylmercury selectively crosses the placental

"barrier" and achieves a 30% higher concentration in fetal red blood cells than in maternal red blood cells.

A second area of Japan was stricken in 1965. Villages on the Agano River in Niigata prefecture had reported 47 cases, resulting in six deaths and one fetal intoxication by 1970 (Takeuchi, 1970). The chemical plant responsible for this mercury release closed down and has never reopened.

Allowable Daily Intake (ADI). Based on observed blood and brain mercury levels, a biological half-life of 70 days and distribution characteristics, it was estimated that 0.3 mg methylmercury/day for a 70 kg man would be toxic over an extended exposure. Implementing a ten-fold safety factor, a Swedish commission recently recommended an ADI for methylmercury of 0.03 mg/day or 0.21 mg/week for a 70 kg man (Berglund et al., 1971).

Zinc

The first U.S. production of zinc was in 1835. Zinc ores are commonly accompanied by lead ores and the two are mined together. The principal ores of zinc are sphalerite (sulfide), smithsonite (carbonate), calamine (silicate) and franklinite (zinc-manganese-iron oxide). The metal is employed to form numerous alloys with other metals. Brass, bronze and both soft and aluminum solders are some of the more important alloys. Large quantities of zinc are used to

produce die castings, used extensively by the automotive, electrical and hardware industries, and in the galvanization of ferrous metals. Zinc oxide is used in the manufacture of paints, rubber products, cosmetics, pharmaceuticals, plastics, batteries, and electrical equipment. Zinc sulfide is used in making luminous dials, X-ray and TV screens and fluorescent lights.

Many of these zinc products are associated with chronic releases through their normal use and disposal to sewers, dumps and sanitary landfills. Industrial processes themselves during the manufacture of these products may release zinc directly to surface waters.

Local aquatic conditions downstream from zinc mining operations are usually devoid of aquatic life. The toxic conditions in aquatic environments are attributed mainly to the lead and copper ores accompanying the zinc. Lead and copper are more toxic than zinc in much lower concentrations.

Zinc occurs in the same group of elements in the Periodic Chart as do the elements cadmium and mercury. Both cadmium and mercury are highly toxic elements. By contrast, zinc is relatively nontoxic and, in fact, is required in trace amounts for the normal growth and function of all plants and animals.

Zinc as an Essential Element

It was demonstrated over a century ago by the Frenchman

Raulin (as cited by Parisi and Vallee, 1969) that zinc was an essential element in the fungus Aspergillus. Subsequently, many investigators have found it to be universally present in organisms and usually in greater amounts than those of most other trace elements. Significant to the research reported here is that particularly high concentrations of zinc are found in shellfish, especially oysters (Underwood, 1971).

Keilin and Mann (1939) showed that zinc is a constituent of the enzyme carbonic anhydrase. Many other zinc-containing metalloenzymes were subsequently discovered and include pancreatic carboxypeptidase, alkaline phosphatase, tryptophan desmolase, and alcohol, malic, lactic and glutamic dehydrogenases. In addition, zinc was found to act as a cofactor in a variety of enzyme systems, including arginase, enolase, several peptidases, oxaloacetic decarboxylase and carnosinase (Parisi and Vallee, 1969).

It is now apparent that zinc is involved in a wide range of cellular activities and is vitally concerned with the fundamental process of RNA and protein synthesis and metabolism in plants, microorganisms and all multicellular animals.

Cadmium is an environmental contaminant that has long been known to be an antagonist of zinc. A partial replacement of zinc by cadmium in various tissues is in accord with many other studies which imply a competition between cadmium and zinc for protein-binding

sites, presumably including those of the zinc metalloenzymes. This phenomenon will be explored on a whole-animal basis in this study.

Zinc-65 as an Environmental Pollutant

Zinc-65 is associated with all nuclear weapons tests. It is not a fission product, but is produced by neutron activation of stable zinc components making up the weapons case and supporting structure. Columbia River water, containing trace amounts of zinc, was used for cooling the Hanford plutonium production reactors located in the state of Washington. As the water passed through the high neutron flux of the reactor core, small quantities of this zinc were converted to radioactive zinc. A considerable quantity of ^{65}Zn has been released into the Columbia River during the last 25 years. Effluent concentrations were, however, below the limits set by the U.S. Atomic Energy Commission. This small concentration of the radioisotope did provide scientists with a vast environmental radiotracer laboratory extending down the river, through the estuary, and into the northeastern Pacific Ocean. All of these production reactors have now been phased out.

Bivalve Molluscs as Indicators of Pollution

Organisms that take up large amounts of a pollutant and excrete it slowly will accumulate high levels of the pollutant in comparison to

the environmental concentration in which they live. These organisms are called biological indicators and are quite valuable for detecting the presence of trace amounts of pollutants in water.

Many invertebrates have the ability to concentrate various elements present in freshwater at very low levels. Such elements may be incorporated into tissues of the organism and subsequently be transmitted to other organisms through the food web, or excreted and made available for recycling. Additional criteria which must be satisfied by ideal indicator organisms are:

1) they must not be migratory, i.e., they must relate conditions at the site of collection;
2) they must be abundant and easily obtained;
3) they must be distributed over a wide geographical range, so fewer indicator species are required for site comparisons;
4) they should be an indicator for several different pollutants.

Bivalves meet these criteria as exceptionally fine biological indicators of metallic pollutants. Of particular interest here is the ability of bivalves to accumulate cadmium, mercury and zinc from their environment.

Heavy metals are concentrated in bivalve tissue by several pathways: direct adsorption and absorption of metallic ions from the water to exposed tissue surfaces, ingestion in the intestinal tract

from particulate food for distribution throughout the visceral mass (Galtsoff, 1964). Through filter feeding activities, bivalves ingest particulates of various sizes, and a variety of planktonic life. Metallic ions may adhere tenaciously to these suspended particulates whether inorganic clay particles, diatoms or algal cells thereby being taken up by the mollusc. Electrical properties of both food particles and mucus sheets may also determine whether or not particles are readily absorbed. Korringa (1952) writes,

> . . . positive polyvalent ions like Al^{+3}, Cu^{+2}, Fe^{+2}, Zn^{+2}, Hg^{+2} and Mn^{+2} are very easily caught and accumulated by the oyster, but not positive monovalent ions like Na^{+} and K^{+} (though present in greater quantities), or negatively charged ions (p. 278).

The magnitude of this accumulation (concentration factor) depends upon many factors which will be discussed later.

Experimental Animal

The freshwater pearl mussel, Margaritifera margaritifera (Figure 1), is a ubiquitous species in North America, western Siberia, northeastern parts of Asia, and central and northern Europe. Its southern range extends across the Japanese Islands to slightly below the 40th parallel in the western United States.

> The species, M. margaritifera (Linnaeus), in the strict sense has the widest distribution of any Naiad in the world. The Pacific coast M. margaritifera falcata is largely to be distinguished from the species by the color of the nacre. The color of the nacre of falcata has been variously described as peach-blossom, salmon red and dull purple (Ingram, 1948, p. 84).

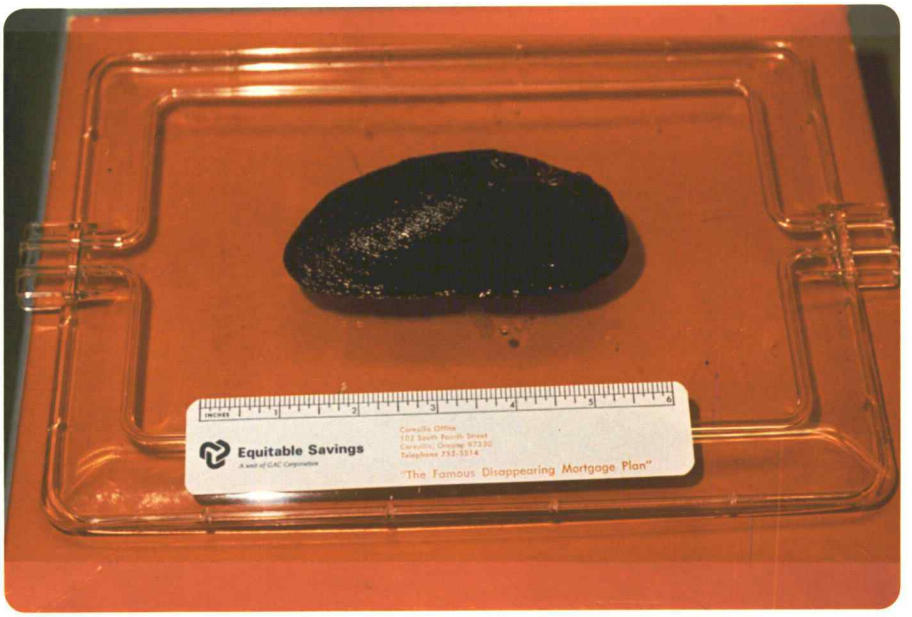

Figure 1. The freshwater mussel, Margaritifera margaritifera.

Habitat

M. margaritifera is usually, although not exclusively, a river inhabitant. Boycott (1936) described the environment as "a well-defined river habitat, though it is a little difficult to specify the essential qualities. . . . Most of its habitats are places in which fishermen would expect to get trout and hope for salmon. . . ." These mussels are found either on top of or burrowed into (as much as 60%) a variety of granular bottom materials ranging from sand to large cobbles and to be generally absent from silted bottoms. They inhabit waters up to 3 meters in depth, but in general range from 0.5-1.5 m.

M. margaritifera is quite tolerant of cold temperatures, as indicated by its extensive distribution in northern latitudes. It has been reported in European mountain streams where the summer water temperature reaches only about 13°C (Roscoe and Redelings, 1964).

Larval Stages

Unlike Anodonta, the young M. margaritifera stages (glochidia) are not retained in the gills of the parent over the winter, but are expelled during the late summer. These larvae must attach themselves to the gills of fish as ectoparasites. The parasite becomes encysted for about 14 days depending upon the temperature of the water (Roscoe and Redelings, 1964). Once the adult organs are formed, the

young mussel breaks out of the cyst and falls to the bottom to grow to adult size (Hunter, 1964). Serious losses of young trout have been reported (Murphy, 1942) in rearing ponds along the Truckee River, California from such paracitism.

Age

The common idea concerning the great age reached by these mussels is an old one expressed first in the 18th century (Hendelberg, 1960). In 1926, Altnöder (as cited by Hendelberg, 1960) first demonstrated that the lines of growth on the shell were really annual features. Hendelberg (1960) established that the number of layers in the ligament actually coincided with the number of annual lines on the shell. After counting these layers and adding the number of years corresponding to a measured distance along the dorsal margin, an estimate of the mussel's age is obtained. Using this method Hendelberg randomly collected 30 animals from one bed. The youngest animal was estimated to be 13 years old and the oldest animal was estimated to be 116 years of age. Roscoe and Redelings (1964) reported that, if Israel's 100-year estimate for M. margaritifera is correct, it is the longest-lived invertebrate known. (A similar age has been established for the giant clam Tridacna.)

The ecological importance of the great age reached by these mussels is two-fold. As a biological indicator these mussels may

reveal the past history of the quality of the water. The lack of homeostatic control renders these animals susceptible to accumulation of toxic levels of metallic pollutants. These animals then could be considered unfit for human consumption after a certain age is reached in a specific area.

Purpose of the Study

The general purpose of the study was to examine the effects of simulated cadmium, mercury and zinc contamination on the freshwater mussel, Margaritifera margaritifera. The specific objectives were to:

1) determine the uptake pattern of cadmium, mercury and zinc during chronic exposure to radioisotopes of these metals in water;
2) compare the retention patterns following chronic uptake for each metal;
3) determine changes in the tissue distribution of these metals during long retention times;
4) determine if there were inhibitory effects of different concentrations of cadmium on the uptake of several concentrations of zinc;
5) identify an acute toxic level of cadmium for the freshwater mussel.

This study could indicate how much of the metallic pollutant might be taken up into the edible soft parts of shellfish and how long these metals would remain in the organism after its removal from a contaminated area. This one component of an aquatic food chain may shed light on the length of time an aquatic system may remain polluted after all metallic effluents have ceased.

METHODS AND MATERIALS

Collection and Maintenance of Animals

Collection

Freshwater mussels were collected from a channel found on the shoreside of a small island near the east bank of the Willamette River approximately 3.7 km north (downstream) of the Harrison Street Bridge in Corvallis, Oregon. During the summer, mussels were collected by hand in water ranging in depth from 40-60 cm. The river rises considerably in the fall and the current passing through this area exceeds 9 km/hr. Consequently SCUBA divers were used to collect the animals at that time. During periods of winter flooding, collecting at this site was not possible and mussels were purchased from Carolina Biological Supply Co. (Gladstone, Oregon). These mussels had been collected from other portions of the Willamette River drainage basin.

Mussels to be used immediately in laboratory experiments were transported to the laboratory in plastic buckets. Excess animals were placed in a plastic clothesbasket and suspended in the Willamette River from the city water intake structure. These could easily be recovered when needed.

The mussels were mature forms of both sexes ranging in weight

from about 70-160 g. Animals never varied more than 1.5 times the mean weight of the other animals in any one experiment.

Maintenance

Upon return to the laboratory, the animals were acclimatized for one week in large aquaria containing approximately 20 liters of aerated artificial stream water (Doudoroff, 1956) (Appendix Table 1) and fed powdered wheat starch (about 800 mg) every other day. The experimental trays used for the individual experiments were constructed of polystyrene and measured 31 x 26 x 10 cm. To avoid excessive radioactive contamination and still maintain refrigerated experimental solutions, the test trays were placed in a 183 x 56 x 15 cm fiberglass-lined wooden trough containing non-contaminated circulating refrigerated water. The temperature of the experimental water was maintained at $14.5 \pm 0.5^{\circ}C$ by a Westinghouse remote cooler.

The water in the test containers was continuously aerated. The frequency of complete water renewal and the number of animals involved depended upon the experiment to be conducted. The animals were initially scrubbed with a vegetable brush to remove attached algae and sediment. The shells were air dried, numbered with nail polish and covered with four coats of Krylon plastic ignition spray. The Krylon was applied to reduce the physical sorption of metallic

ions to the shells. The Krylon spray dried immediately, but the animals were left out of water for about 15 minutes following the last coat. Small amounts of the Krylon normally sloughed off during the first few hours after the animals were placed in their test trays. To avoid any possible toxic effects from this material the water was changed at 4, 16 and 36 hours. At this point the appropriate radioisotope was added and the experiment begun.

Radioisotopes Used as Metal Tracers

The radioisotopes of the elements under investigation were selected on the basis of commercial availability, radioactive half-life and type of energy of emission.

The radioisotopes chosen for these studies were ^{115m}Cd (specific activity = 0.396 mCi/mg Cd^{+2}), ^{203}Hg (specific activity = 6.21 mCi /mg Hg^{+2}) and ^{65}Zn (specific activity = 5.90 mCi/mg Zn^{+2}). The radioactive half-lives of these three radioisotopes are 43, 47 and 245 days, respectively. The ^{65}Zn and ^{115m}Cd were administered in the chloride form, whereas ^{203}Hg was given both as mercuric nitrate and methylmercuric chloride. Radioactive solutions were prepared in clean volumetric flasks and diluted to required concentrations of the metal ion with 0.5 N HCl. The pH of the test water was maintained at approximately 7.6.

The above radionuclides are all gamma emitters, thus enabling

the use of a whole-body liquid scintillation detector. Cadmium-115m emits three gamma rays of interest, 0.934 MeV (1.9%), 1.289 MeV (0.9%) and 0.485 MeV (0.31%). Mercury-203 and ^{65}Zn each emit single gamma rays of 0.279 MeV (77%) and 1.115 MeV (49%), respectively.

The required initial activity to be purchased was determined for the total experiment by back calculating from the minimum acceptable radioactivity (for counting statistics) in the least radioactive sample anticipated. An activity of 3000 cpm (counts per minute)/5 ml of test water was used as the basis for the calculation. Additional points which must be considered in this calculation are the count rate of the sample, its size, counting system efficiency, physical and biological half-life of the tracer, scale of the experiment (volume of maintenance water required) and activity needed.

Since these experiments were designed for a specific metal ion concentration, specific activity had to be determined based on the following: quantity of metal to be administered to test water and specific activity of metal needed from commercial supplier.

The Armac Counting System

The Armac liquid scintillation counting system consists of a large volume detector, amplification electronics and spectrometer for detecting and recording photons given off by gamma-emitting

radionuclides (Figure 2). This type of system is especially designed to assay the whole body radioactivity content of small animals without necessitating the sacrifice of the animal.

The animal chamber (1800 cm^3) is constructed of thin stainless steel and is located in the center of an elongated doughnut-shaped cylinder containing the liquid scintillator. Six photomultiplier (PM) tubes mounted at the rear of the scintillator cylinder monitor the interactions of the gamma rays with the solution. The scintillator solution consists of 5 g/l PPO (2, 5-diphenyloxazole) as the primary fluor and 0.5 g/l of a secondary solute (wave shifter) dimethyl-POPOP (1, 4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene) in a toluene solvent. The entire detector is surrounded by 5 cm of lead shielding (Figure 3).

Because of the low atomic number and finite volume of the scintillator, Compton-scatter is the dominant form of interaction between gamma rays and organic scintillators--liquid or plastic--in the energy range of 20 keV-30 MeV. The scintillation process begins with a penetrating gamma ray interacting with the detector solution transferring some of its energy to a solvent molecule. The electronic excitation energy of the solvent molecule is then transferred to the molecules of the primary solute which is a fluor. With a maximum emission energy of 3800 Å the excitation energy of the primary fluor is absorbed by the secondary solute (wave shifter) and re-emitted at about 4300 Å. The purpose of introducing the wave shifter is to

Figure 2. The Armac liquid scintillation counting system.

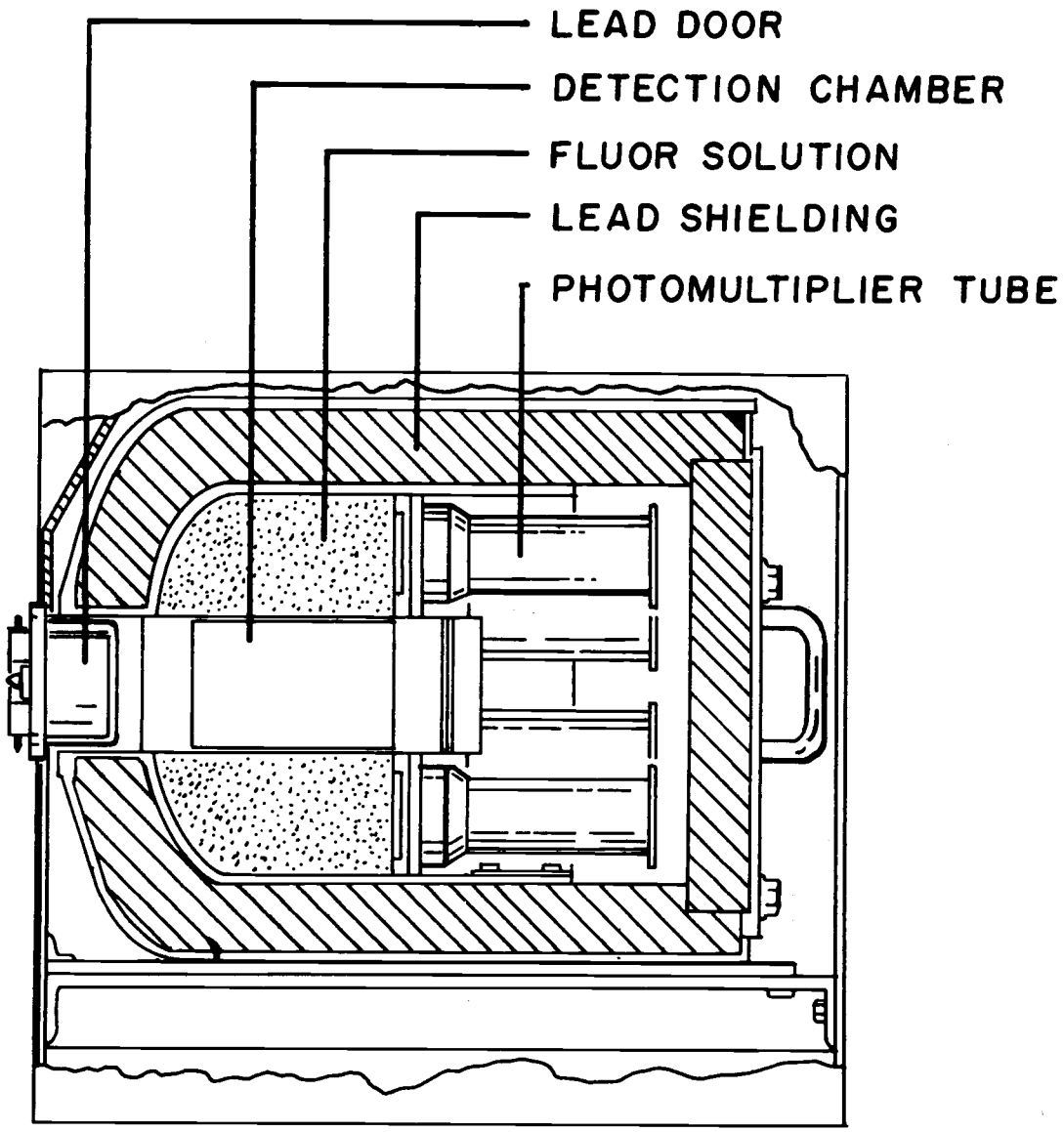

Figure 3. Longitudinal section of the Armac scintillation detector.

achieve a better match between the emission spectrum of the scintillator and the spectral response of the photocathodes of the PM tubes. When a photon interacts with the photocathode of a PM tube it ejects one or more photoelectrons which undergo multiplication about 10^6 times while passing through the dynode stages of the PM tube. The resulting electrical pulse is amplified for input into a spectrometer which sorts it according to pulse height and records the event.

Sample Position and Standards

The detection efficiency within the animal chamber varies considerably depending on the placement of the sample. Thus, the position of the sample must be identical each time it is assayed. In order to maintain this position for repeated analyses, a specially constructed polystyrene sample holder was employed (Figure 4).

In order to correct animal assays for the physical decay of the radiotracer and variations in both detector and spectrometer efficiency with time, radioactive standards were prepared and sealed in glass tubes. These standards were counted before and after each sampling period.

Efficiencies for the various radioisotopes in the Armac detection system were determined by doubly encapsulating radioactive solutions in heavy plastic bags embedded in a pool of silicone rubber surrounded by an empty shell of the test species. This assured the same counting

Figure 4. Polystyrene animal holder designed to maintain constant positioning during repeated assays.

"geometry" as for the test animals themselves. These efficiencies are given in Table 1.

Table 1. Counting efficiencies in the Armac Detector System.

Isotope	"Window" setting (arbitrary units)	Gain (%)	% Efficiency
^{115m}Cd	100-700	5.5	0.422
^{203}Hg	80-600	20.0	32.30
^{65}Zn	240-600	4.7	9.072

Uptake Studies

The uptake and accumulation of cadmium, mercury and zinc in the freshwater mussel were examined in five experiments each utilizing 11-15 animals. Experimental animals were maintained in four liters of aerated stream water held at $14.5 \pm 0.5^{\circ}C$ to which was added a radioactive solution containing one of the metals. The following metal concentrations were chosen which could realistically be encountered in the environment: 10 ppb cadmium (as chloride), 0.8 ppb mercury (as nitrate), 0.9 ppb mercury (as methylmercuric chloride), 624 and 64 ppb zinc (as chloride). Since a slurry of food (wheat starch) was dripped into the trays every other day providing a surface for metal ion adsorption, uptake and accumulation were actually from a combination of water and food. The mussels were periodically removed from the radioactive solution, allowed to purge themselves of

most of the test solution contained within their shells, then placed in fresh nonradioactive stream water for ten minutes to further cleanse themselves of nonbound metal. The animals were taken from the wash water three at a time and allowed to purge themselves for three minutes before being rubbed and rinsed in tap water to remove any mucus accumulated on the shell. Next they were placed in Baggies®, weighed and counted in the whole-body detector. After each animal was assayed it was returned to the radioactive solution to continue its accumulation.

The radioactive solution was assayed each day and additional radioactive stock solution was added as needed to maintain the desired concentration of metal ion. The experimental containers were emptied and scrubbed to remove accumulated starch and pseudofeces and refilled with freshwater containing the original metal ion concentration every four days throughout the uptake experiments. A radioactive standard was counted each time a group of animals were assayed enabling the data to be corrected for decay.

Retention Studies

The retention studies for cadmium and zinc were initiated after near equilibrium had been reached in chronically exposed freshwater mussels. Near equilibrium was not reached in the methylmercuric chloride uptake study, but after 57 days the slope of the curve

appeared constant, so uptake was terminated and retention begun. The uptake of mercuric nitrate was terminated before equilibrium was reached and retention started because of an abnormal mortality rate in both experimental and control animals.

Upon ending the chronic uptake experiments, each animal was prepared for counting as described earlier and counted to determine its initial whole-body radioactivity. The animals were then placed in nonradioactive water maintained at $14.5 \pm 0.5^{\circ}C$ to commence the retention study. Each animal was reassayed regularly at first, with the frequency of analysis decreasing as the change in slope of the retention curve decreased. The water was changed daily throughout the experiment to avoid any reabsorption of excreted metallic ions. A radioactive standard was counted each time the animals were assayed to correct for the physical decay of the radioisotope.

Tissue Distribution Studies

The distribution of cadmium, mercury and zinc in the tissues of the freshwater mussel was determined for specific areas of interest on the retention curve, i.e., after major changes in the slope of the curve. The number of animals used for each value was 4-6.

Prior to sacrifice, the whole-body radioactivity was determined in the Armac liquid scintillation system. The animals were then shucked, and shells and total soft parts were individually washed

thoroughly, placed in Baggies ^{R}, weighed and counted. The soft parts were then dissected into five components: the gills, adductor muscles, foot, visceral mass and mantle and labial palps. These tissues were individually weighed, packed into counting tubes and assayed in the Armac system. A radioactive standard was counted each time a tissue distribution study was performed to enable correction for decay of the radioisotope used as the tracer.

Inhibition Studies

Inhibition of zinc uptake in the freshwater mussel by cadmium would be demonstrated if the rate of zinc uptake could be decreased by the presence of cadmium in the water. Three separate experiments were carried out to investigate this possible effect.

In the control experiment, seven groups of six mussels each were placed in separate trays containing 2.5 liters of stream water maintained at 14.5 $\pm$ 0.5°C. Each group of mussels was exposed for four hours to a different concentration of $^{65}ZnCl_2$ (0.2, 0.4, 0.8, 1.2, 1.4, 1.6 and 2.0 ppm). At the conclusion of the experiment the intact mussels were weighed, assayed for radioactivity and shucked. The shells and soft parts were then individually weighed and counted in the Armac counting system.

The first inhibition experiment consisted of six groups of six mussels each maintained in stream water held at 14.5 $\pm$ 0.5°C. Zinc was

introduced into the water at concentrations from 0.2-1.6 ppm. Cadmium at 0.6 ppm was then added to each container and the four-hour exposure period begun. Upon completion of the experiment the animals were analyzed as stated previously.

The second inhibition experiment also at 14.5 $\pm$ 0.5°C consisted of seven groups of six animals each. Zinc in water concentrations of 0.2-2.0 ppm was utilized in the presence of 2.0 ppm cadmium. After four hours the animals were analyzed as before.

Toxicity Studies

The median survival (mortality) times for M. margaritifera were examined for two concentrations of $CdCl_2$. Prior to starting a meaningful toxicity experiment, two interrelated parameters had to be determined to avoid significant detoxification of the experimental water during the experiment. The volume of water needed per animal, and the frequency of complete renewal must be determined. Shellfish tend to detoxify experimental solutions very quickly. Metal ions may be taken out of solution by physical adsorption to walls of the test container or shells of the bivalves and by metabolic incorporation by the shellfish. It was evident that a flowing constant concentration system would be ideal, however one was not available, so a semi-static system was adopted.

Doudoroff (personal communication) recommended the following

procedure:

1) Place a group of animals in container "A" and fill with a precise amount of contaminated solution,
2) after 12 hours empty the "used" water into a new container "B" containing a second set of animals.
3) Refill container "A" with a fresh supply of contaminated solution.
4) Repeat this procedure every 12 hours.

If the resulting survival times of the two groups of animals differ by more than 20%, then the test water has been significantly detoxified. The two parameters mentioned earlier may be adjusted to compensate for this detoxification.

In a series of preliminary experiments of six days duration each, both the optimal water volume per animal and the number of complete water changes per day were determined. As a result, it was found that 1.4 liters of experimental water per animal and changing the test water three times a day produced no significant differences in the toxicity patterns of the two groups of freshwater mussels.

Subsequently then, ten animals were exposed to 2.0 ppm cadmium in a first experiment under the above conditions, and six animals were exposed to 4.0 ppm cadmium in a second. Observations for dead animals were made at each water change. A mussel was classified dead if it did not respond to a stimulus applied to its foot after it began to gape.

The cumulative percent mortality was recorded against exposure time leading to a mean survival time determination.

RESULTS

Uptake Studies

The uptake and accumulation of $^{115m}CdCl_2$, $^{203}Hg(NO_3)_2$, $CH_3{}^{203}HgCl$ and two levels of $^{65}ZnCl_2$ were followed during chronic exposure for 45, 39, 57, 78 and 80 days, respectively. The magnitude of this accumulation is termed the concentration factor (C_f) and is defined as follows:

$$C_f = \frac{\text{concentration of an element in the organism}}{\text{concentration of an element in the water}}$$

The uptake data were expressed in dpm /g of animal weight and plotted as mean dpm /g of the number of animals exposed against time on a semi-logarithmic scale. The original data are presented as Appendix Tables 2-6.

Cadmium

The uptake and accumulation of cadmium by the freshwater mussel during chronic exposure is shown in Figure 5. Uptake was very rapid for the first week. The rate of uptake steadily decreased during the entire experiment. A near equilibrium was reached in the 15 animals during the seventh week of the experiment.

The concentration of Cd^{+2} in the test water was nominally 10 ppb. At near equilibrium, the population of mussels reached a

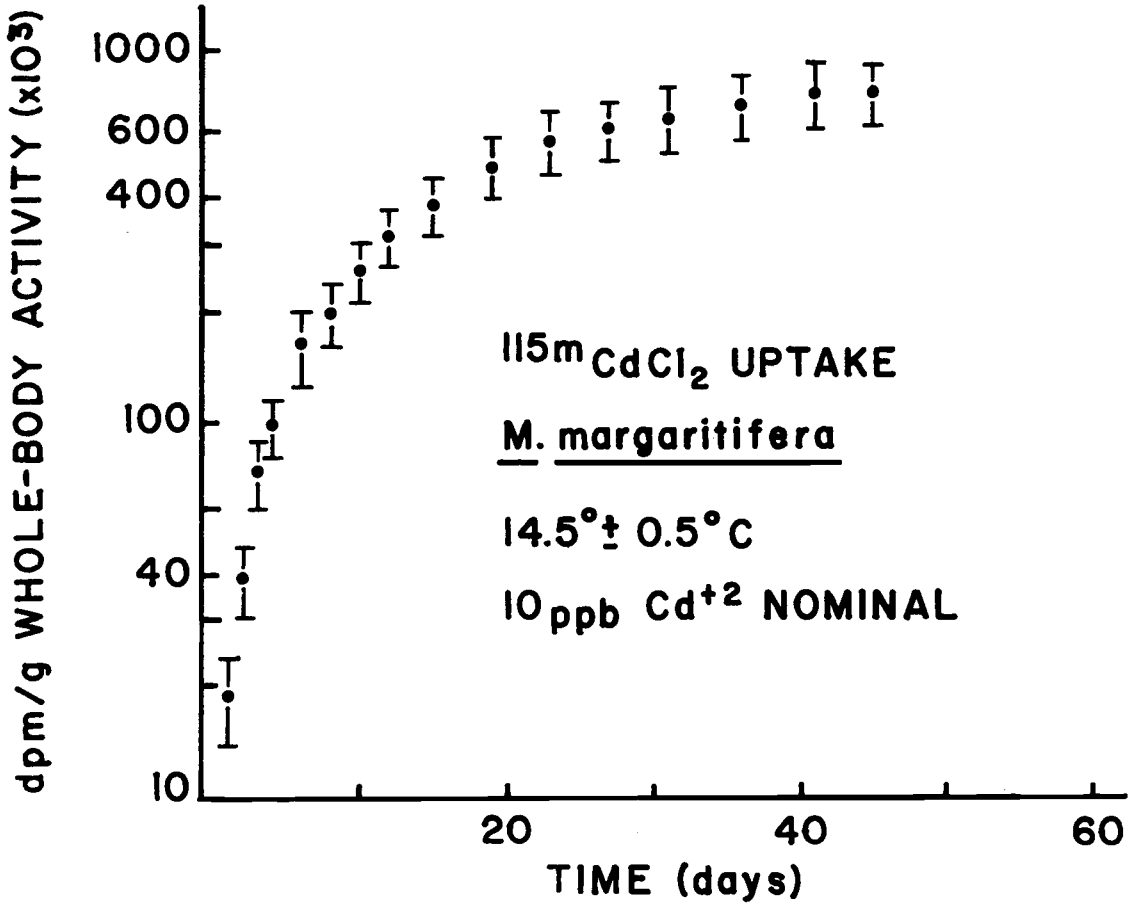

Figure 5. Whole-body uptake of $^{115m}CdCl_2$ by the freshwater mussel during chronic exposure. Each point represents the mean of 15 animals ±

whole-body C_f of about 150. However, the majority of the ^{115m}Cd (78.8 $\pm$ 5.3%) was associated with the shell. Thus, the concentration of cadmium in the edible soft parts was only about 20 times that of the water concentration. The natural cadmium concentration found in my animals prior to experimentation was 0.60 ppm (wet wt).

Mercury

The uptake of mercuric nitrate by M. margaritifera during chronic exposure to a nominal 0.8 ppb mercury is plotted in Figure 6. The accumulation of this compound in 11 animals was initially very rapid and appeared to be exponential. The rate decreased throughout the exposure period. Due to an excessive mortality of both experimental and control animals, uptake was terminated prior to near equilibrium in hopes of obtaining some retention data. After 39 days of exposure, the C_f for the edible soft parts was about 30.

The uptake of methylmercuric chloride by 11 freshwater mussels during chronic exposure to a nominal 0.9 ppb mercury is graphically shown in Figure 7. The uptake was very rapid for the first 18 days. At that time an abrupt change in the rate of uptake occurred. The slope of the uptake appeared nearly constant during the next 40 days, so uptake was terminated and a retention study begun. At the termination of this experiment the C_f for the edible soft parts was about 75.

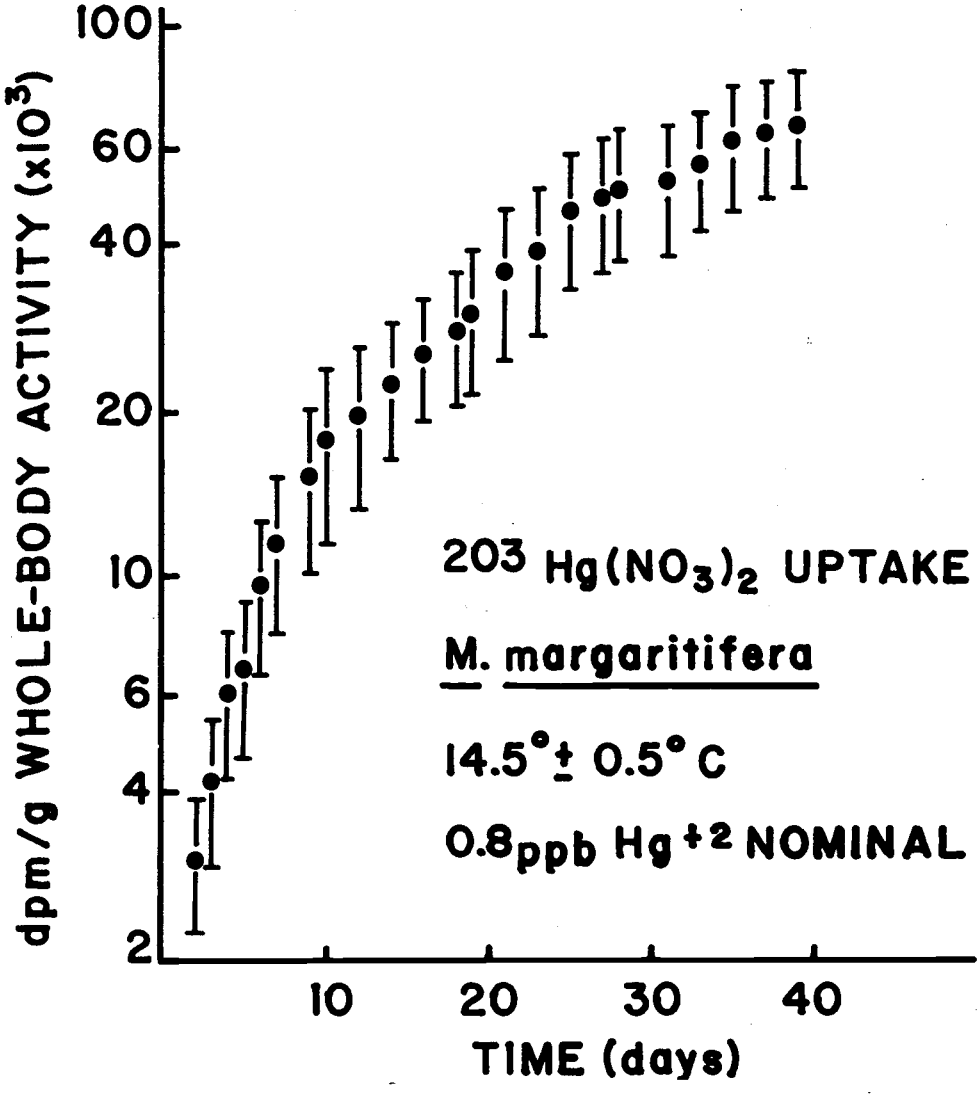

Figure 6. Whole-body uptake of $^{203}Hg(NO_3)_2$ by the freshwater mussel during chronic exposure. Each point represents the mean of 15 animals $\pm$ one standard deviation.

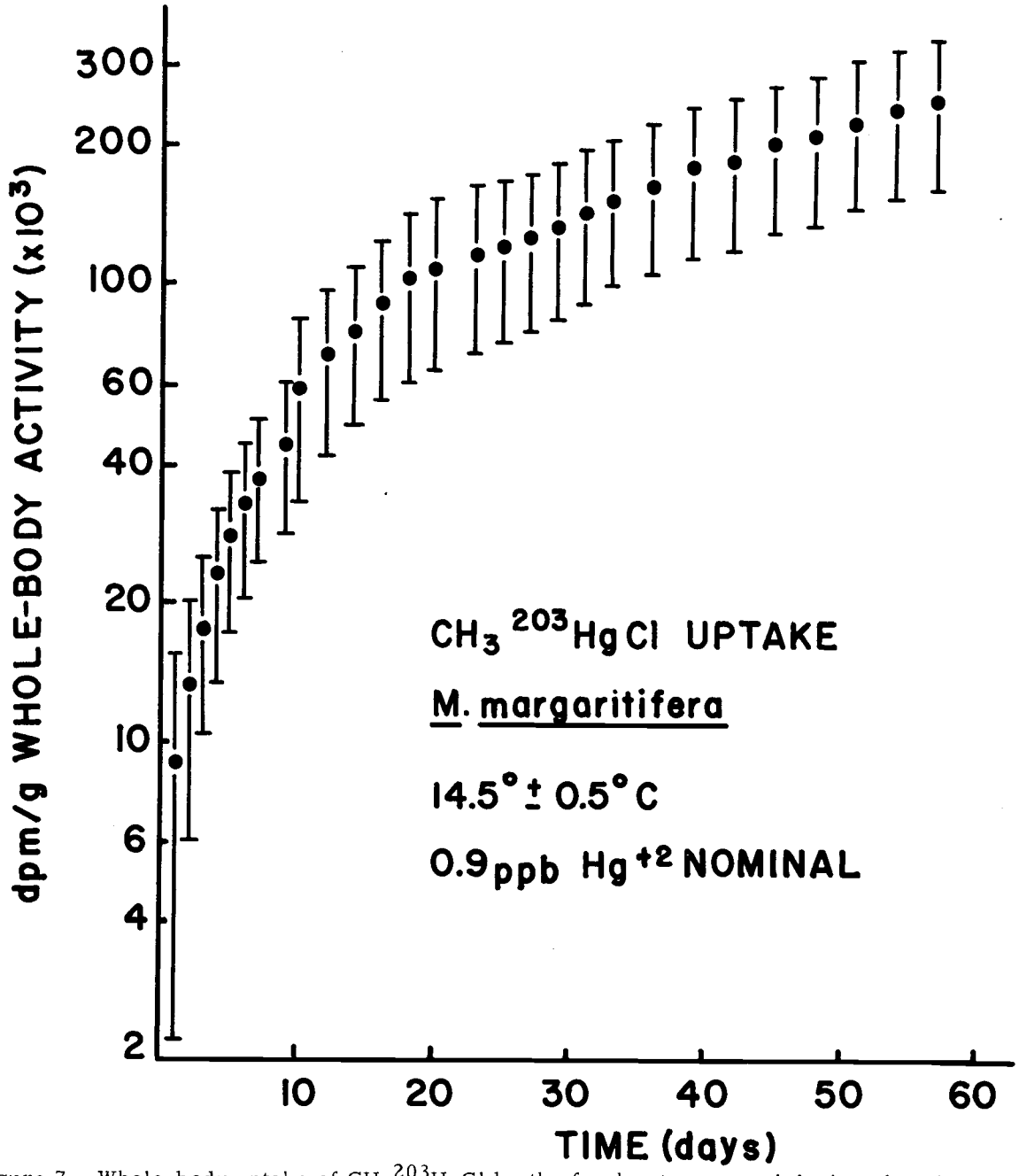

Figure 7. Whole-body uptake of $CH_3{}^{203}HgCl$ by the freshwater mussel during chronic exposure. Each point represents the mean of 11 animals $\pm$ one standard deviation.

Zinc

The uptake of two levels of Zn^{+2} (624 and 64 ppb) by these animals was followed during chronic exposure for 80 and 78 days and is depicted in Figure 8. Each point on the upper curve represents the mean of 15 animals, and on the lower curve, the mean of 12 animals. As expected, the rate of uptake for both concentrations of zinc was rapid at first and again showed the characteristic decreasing rate of accumulation. Near equilibrium was reached in both cases prior to termination of the experiment.

The C_f for the edible portions of these mussels were 16 and 26 for zinc concentrations of 624 and 64 ppb, respectively. The natural zinc concentration found in my animals prior to experimentation was about 67 ppm (wet wt).

Tissue Distribution

The distribution of cadmium, mercury and zinc in the gills, adductor muscles, foot, visceral mass and mantle and labial palps of the freshwater mussel was determined over time periods of up to 150 days after termination of chronic exposure. The tissue distributions of $^{115m}CdCl_2$, $^{203}Hg(NO_3)_2$, $CH_3{}^{203}HgCl$ and $^{65}ZnCl_2$ are shown in Tables 2, 3, 4 and 5, respectively. There appear to be no major shifts in the percent of these metals from one compartment to another within the soft parts observed over the length of the experiment. A few general trends in two of the metals are mentioned below.

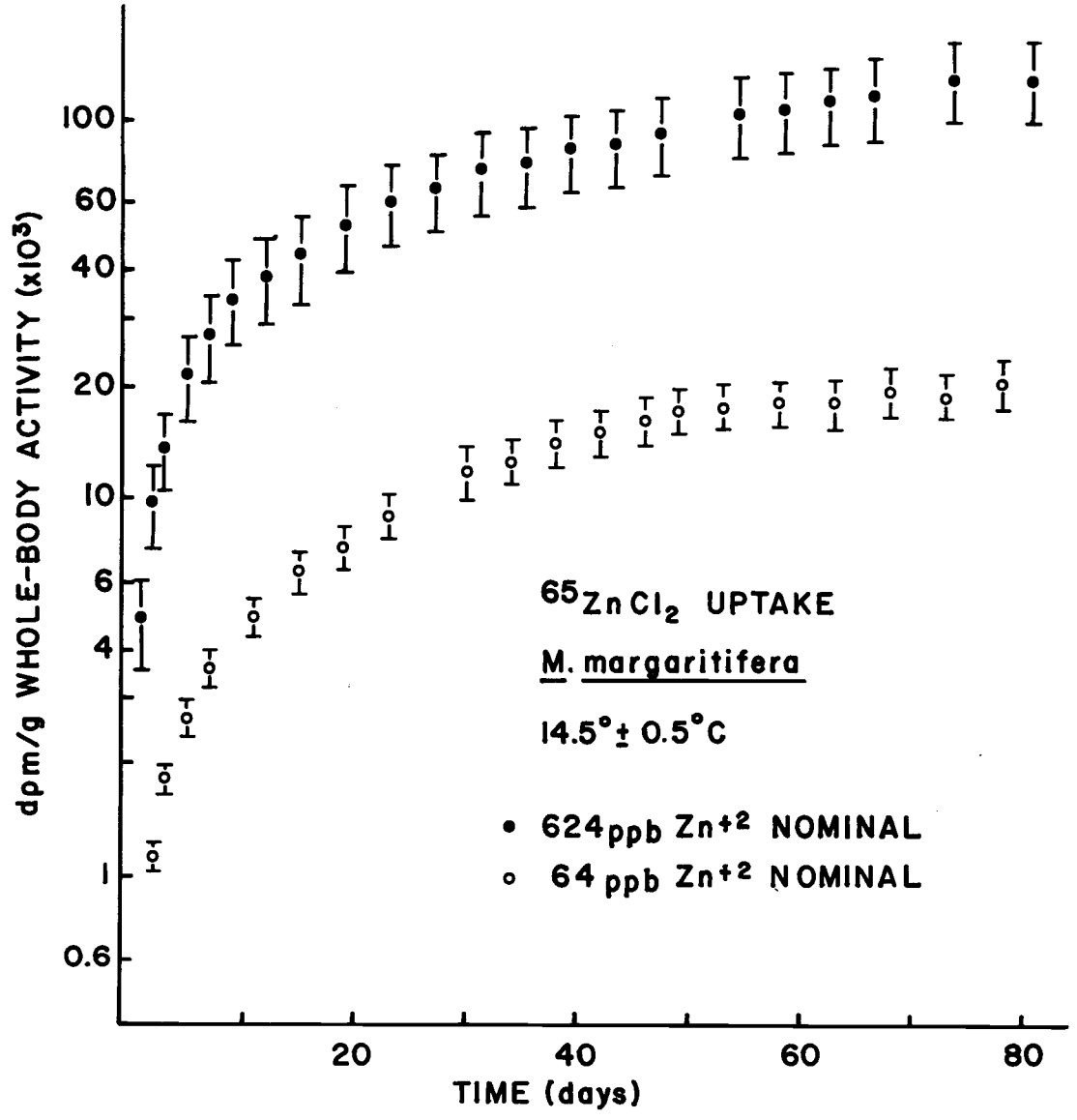

Figure 8. Whole-body uptake of $^{65}ZnCl_2$ by the freshwater mussel during chronic exposure. The top curve represents the mean ± one standard deviation of 15 animals. The bottom curve represents the mean ± one standard deviation of 12 animals.

Table 2. Tissue distribution of $^{115m}CdCl_2$ in edible tissue (% of total activity in soft parts ± one standard deviation) and partitioning between shell and soft parts (% of whole-body activity ± one standard deviation) in the freshwater mussel.

Tissue	Time (days)				
	0	29	62	80	125
gills	17.4 ±2.4	16.5 ±2.0	15.5 ±1.2	14.5 ±1.3	22.0 ±4.9
mantle and labial palps	17.0 ±5.7	16.6 ±7.7	12.5 ±3.2	21.1 ±8.9	22.1 ±4.3
adductor muscles	4.0 ±1.8	1.5 ±1.0	1.9 ±0.3	3.1 ±0.9	2.1 ±0.6
foot	4.5 ±1.3	3.4 ±1.1	3.0 ±0.9	4.6 ±0.9	2.9 ±0.6
visceral mass	52.1 ±13.3	49.1 ±10.0	59.0 ±4.2	48.7 ±11.4	41.8 ±10.0
% in shell of whole-body	78.8 ±5.3	70.2 ±3.7	58.4 ±5.8	56.1 ±8.4	57.4 ±7.6
% in soft parts of whole-body	13.0 ±4.6	25.8 ±5.9	39.7 ±5.8	41.8 ±9.2	39.4 ±8.5

Table 3. Tissue distribution of $^{203}Hg(NO_3)_2$ in edible tissue (% of total activity in soft parts ± one standard deviation) and partitioning between shell and soft parts (% of whole-body activity ± one standard deviation) in the freshwater mussel.

Tissue	Time (days)			
	0	72	97	150
gills	13.0 ±2.1	11.3 ±2.0	10.6 ±1.7	13.2 ±4.0
mantle and labial palps	8.4 ±2.9	7.9 ±1.7	8.3 ±2.1	11.4 ±4.0
adductor muscles	--	2.2 ±0.6	2.9 ±1.4	2.7 ±0.8
foot	3.7 ±1.1	3.7 ±0.7	4.2 ±1.3	5.6 ±0.9
visceral mass	55.8 ±7.5	66.7 ±1.7	65.2 ±7.2	71.7 ±6.0
% in shell of whole-body				13.7 ±5.8
% of soft parts of whole-body				85.1 ±6.8

Table 4. Tissue distribution of $CH_3{}^{203}HgCl$ in edible tissue (% of total activity in soft parts ± one standard deviation) and partitioning between shell and soft parts (% of whole-body activity ± one standard deviation) in the freshwater mussel.

Tissue	Time (days)				
	0	28	66	80	148
gills	15.4 ±2.4	11.3 ±1.3	10.9 ±1.7	9.3 ±1.8	9.5 ±1.1
mantle and labial palps	14.6 ±2.9	13.3 ±1.0	14.0 ±1.2	15.2 ±1.7	13.0 ±1.5
adductor muscles	15.1 ±2.2	18.4 ±2.4	20.7 ±4.6	17.0 ±3.5	12.9 ±2.6
foot	9.6 ±1.5	13.6 ±4.6	12.3 ±3.5	14.2 ±4.1	14.8 ±2.2
viscera	38.6 ±5.0	36.8 ±4.5	33.6 ±4.9	36.4 ±2.9	45.2 ±5.3
% in shell of whole-body	27.9 ±6.4	15.3 ±2.8	12.7 ±2.9	10.3 ±2.8	10.6 ±3.6
% in soft parts of whole-body	69.0 ±7.3	82.1 ±3.2	85.8 ±4.1	88.5 ±3.8	89.8 ±5.4

Table 5. Tissue distribution of $^{65}ZnCl_2$ in edible tissue (% of total activity in soft parts ± one standard deviation) and partitioning between shell and soft parts (% of whole-body activity ± one standard deviation) in the freshwater mussel.

Tissue	Time (days)		
	0	21	67
gills	12.3 ± 4.5	11.8 ± 2.3	14.2 ± 4.3
mantle and labial palps	43.8 ±10.0	52.3 ± 5.6	42.4 ±11.4
adductor muscles	3.0 ± 0.2	2.1 ± 0.4	3.2 ± 0.9
foot	2.3 ± 0.5	2.3 ± 0.7	4.5 ± 1.8
viscera	31.2 ± 5.4	24.2 ± 3.1	27.2 ± 3.9
% in shell of whole-body	66.6 ± 8.0	66.4 ± 4.0	68.0 ±13.6
% in soft parts of whole-body	34.3 ± 8.5	34.1 ± 4.5	32.9 ±15.5

Cadmium

The percent of cadmium in the total soft parts tended to increase from about 13% at the termination of chronic exposure to about 40% at day 62 of retention, and then leveled off through day 125. The amount of cadmium adsorbed to the shell declined in a corresponding manner.

Mercury

The visceral mass increased from about 56% of the mercuric nitrate associated with the total soft parts on day 0 to about 72% on day 150. The gills, containing about 15% of the methylmercury at the end of chronic exposure lost about 6% by day 148 of retention. The foot tended to increase in both mercury experiments, although not significantly. The percentage of methylmercury in the shell declined about 17% during the 148 days of exposure to uncontaminated water and in accord, the total soft parts increased about the same amount.

Retention Studies

The retention of $^{115m}CdCl_2$, $^{203}Hg(NO_3)_2$, $CH_3{}^{203}HgCl$ and $^{65}ZnCl_2$ was followed for 125, 150, 147 and 81 days, respectively. The data were expressed as percent of original whole-body activity and plotted against time on a semi-logarithmic scale. The original data are presented in Tables 7-10 in the Appendix.

Cadmium

The retention curve for cadmium is composed of two distinct components as shown in Figure 9. The short term component had a biological half-life (T_b) of 17.5 days and represented only 19% of the initial whole-body activity. The long term component had a T_b of 835 days and contained 80% of the initial whole-body activity. After 125 days of retention the mussels still contained 77% of the cadmium they had accumulated during chronic exposure.

Mercury

Mercuric nitrate retention involved a two-component curve, also. Figure 10 reveals an extremely fast first component containing 9% of the original whole-body activity. The T_b was not measured. The second component had a T_b of 194 days and involved 91% of the initial whole-body activity. At the end of the retention experiment (150 days) about 58% of the original mercury remained in the mussel tissue and shell.

The data from methylmercuric chloride showed a two-component curve plotted in Figure 11. The first component displayed a very short half-life (T_b = 5.9 days) involving 6.6% of the total body activity at the termination of chronic exposure. The long term component contained 93% of the initial whole-body activity and showed a T_b of 860 days. After 147 days about 82% of the original mercury remained.

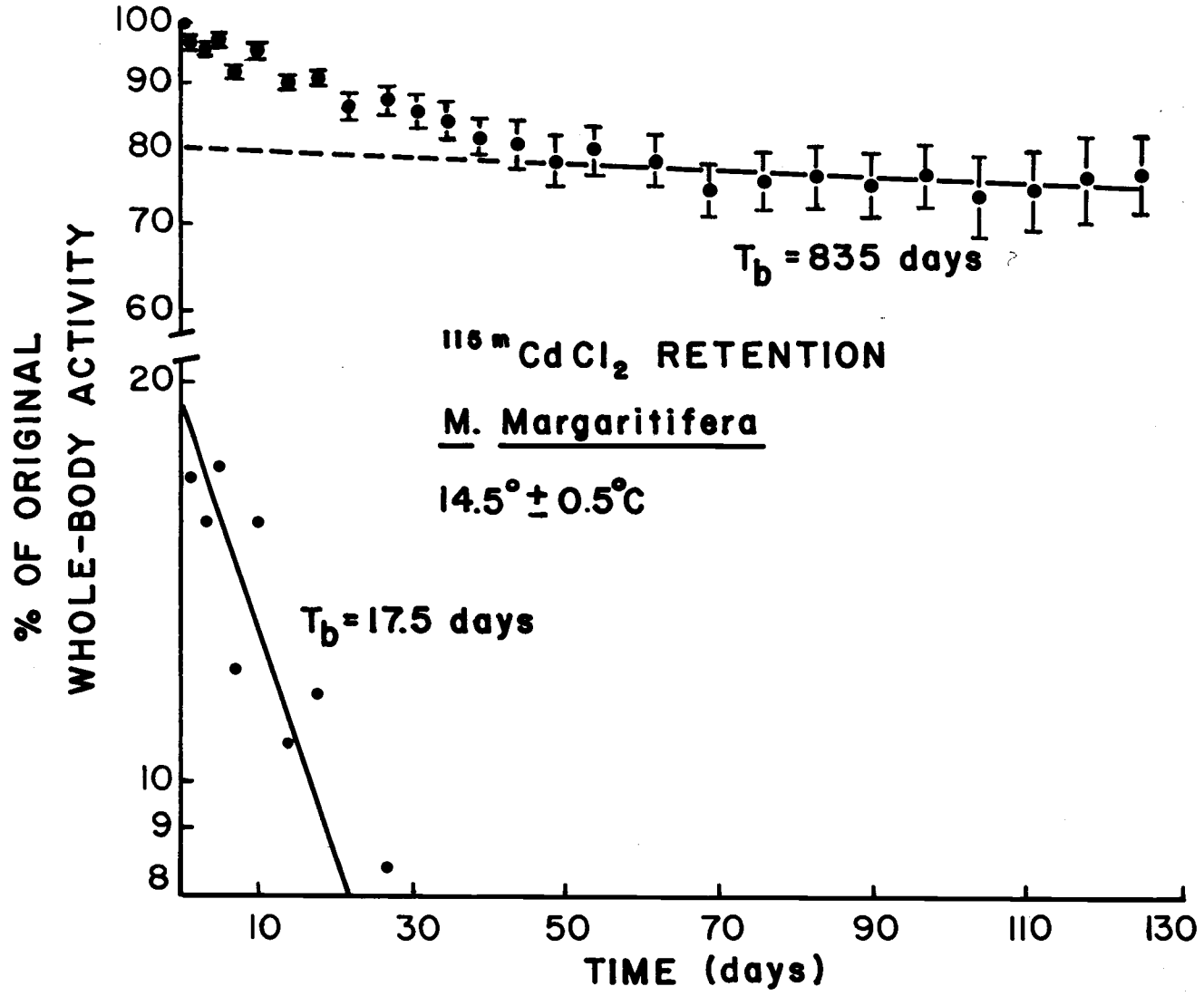

Figure 9. Whole-body retention of $^{115m}CdCl_2$ by Margaritifera margaritifera following chronic exposure.

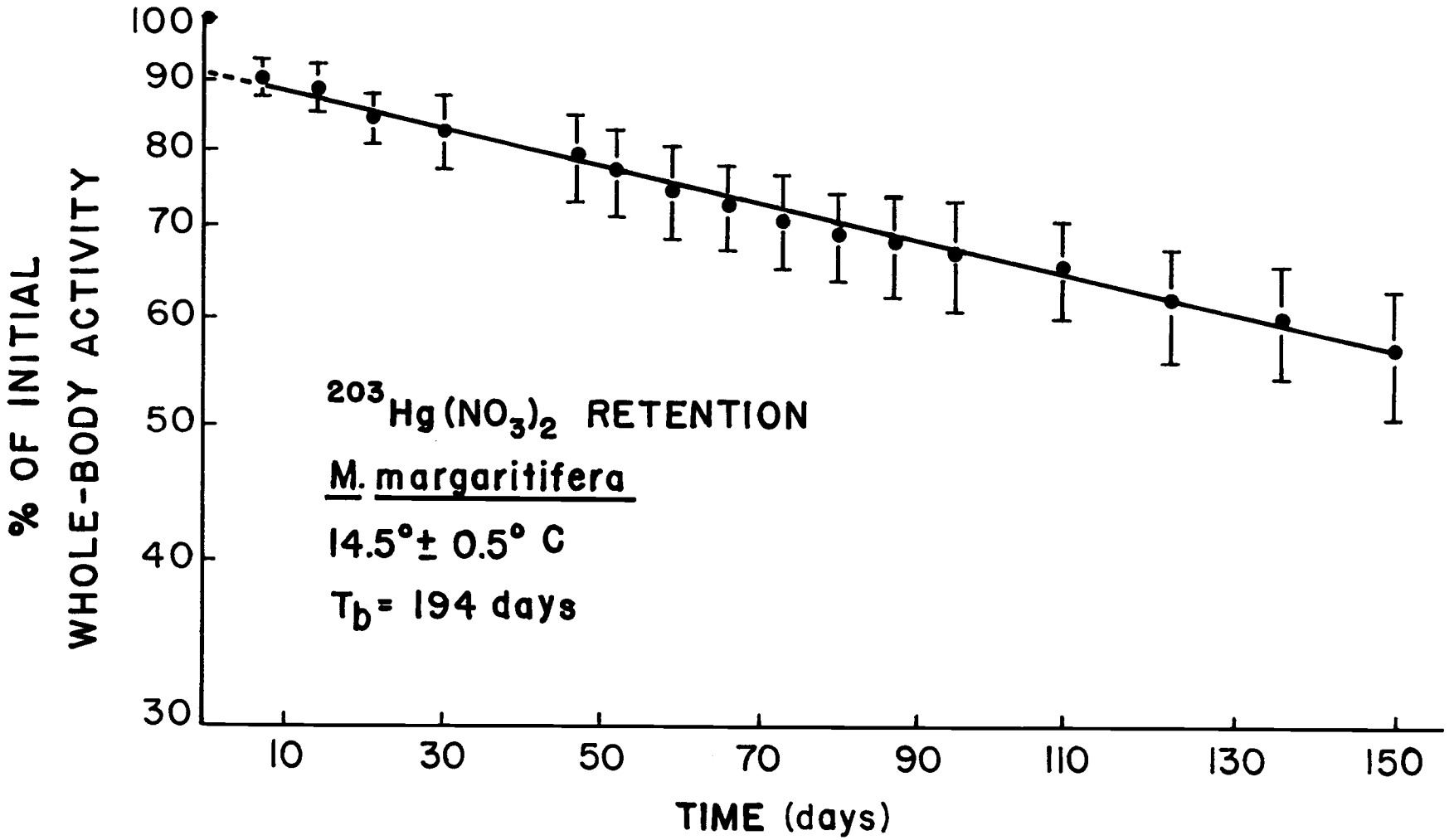

Figure 10. Whole-body retention of $^{203}Hg(NO_3)_2$ by Margaritifera margaritifera following chronic exposure.

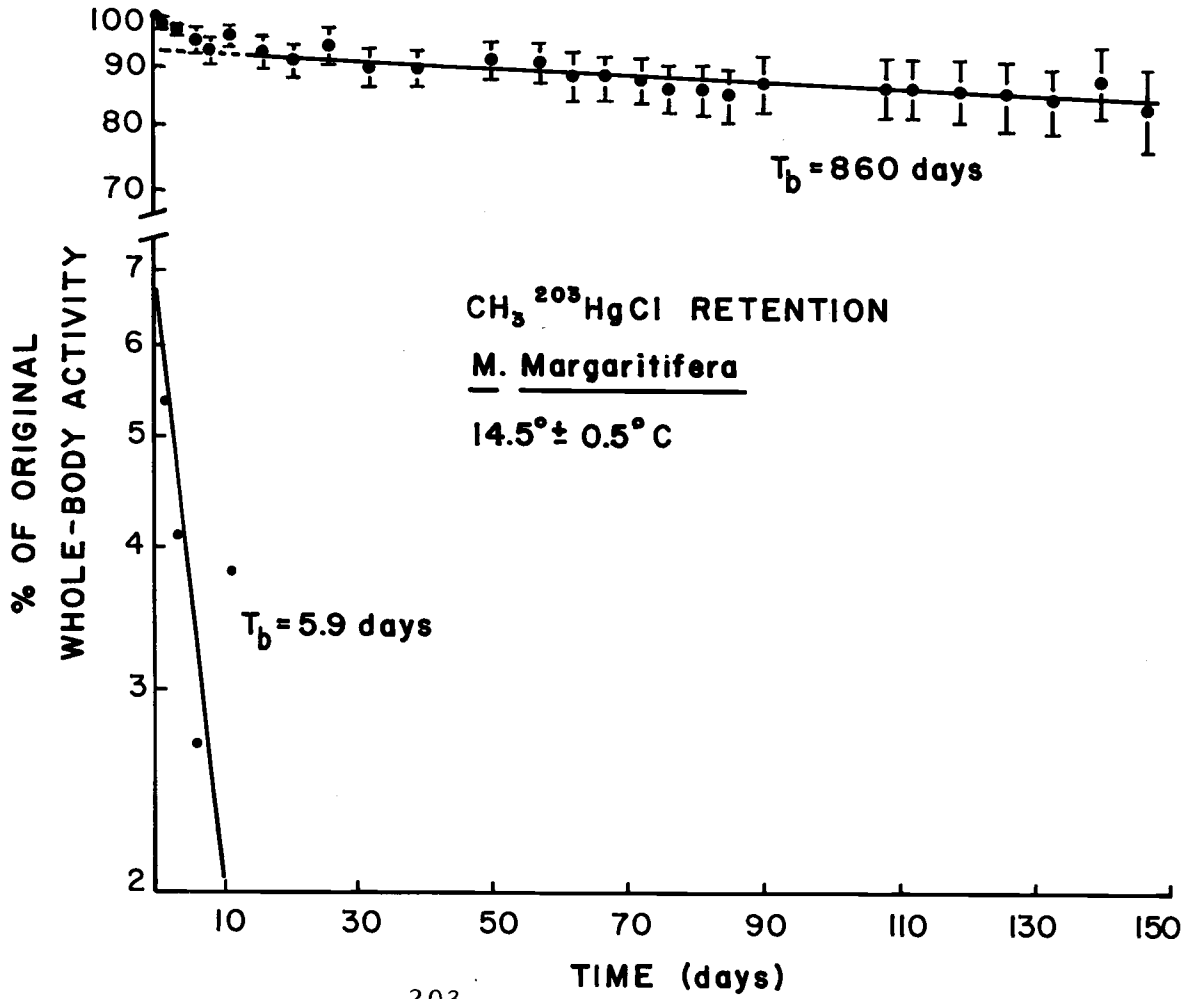

Figure 11. Whole-body retention of $CH_3{}^{203}HgCl$ by Margaritifera margaritifera following chronic exposure.

Zinc

A two-component whole-body retention curve for zinc is plotted in Figure 12. The short term component had a very short half-life (4.6 days) containing 13% of the initial whole-body activity. The T_b for the long term component was 103 days involving almost 87% of the whole-body activity recorded in the mussels at the termination of chronic uptake. After 81 days these mussels still contained about 56% of their initial body burden of zinc.

Inhibition Studies

The inhibition of zinc uptake by different concentrations of cadmium are shown in Figure 13. The data is expressed as dpm/g soft parts for four-hour exposure periods (rate of zinc uptake) against zinc concentration. The resulting data are not as clear as desired, but general trends may be seen. The rate of zinc uptake generally appeared to increase with an increase in zinc concentration. Upon the addition of 0.6 ppm cadmium to each group of animals followed by the four-hour exposure, there was no statistically significant inhibition of the zinc uptake. However, when 2.0 ppm cadmium was added to each concentration of zinc, very significant reductions occurred in every zinc concentration but 0.8 ppm zinc. The general trend showed that the greater inhibition occurred (82%) when the concentration of cadmium was 10 times higher than the zinc concentration, which was the largest difference studied. When the concentrations of zinc and cadmium were both 2.0 ppm, zinc uptake was inhibited about 22%.

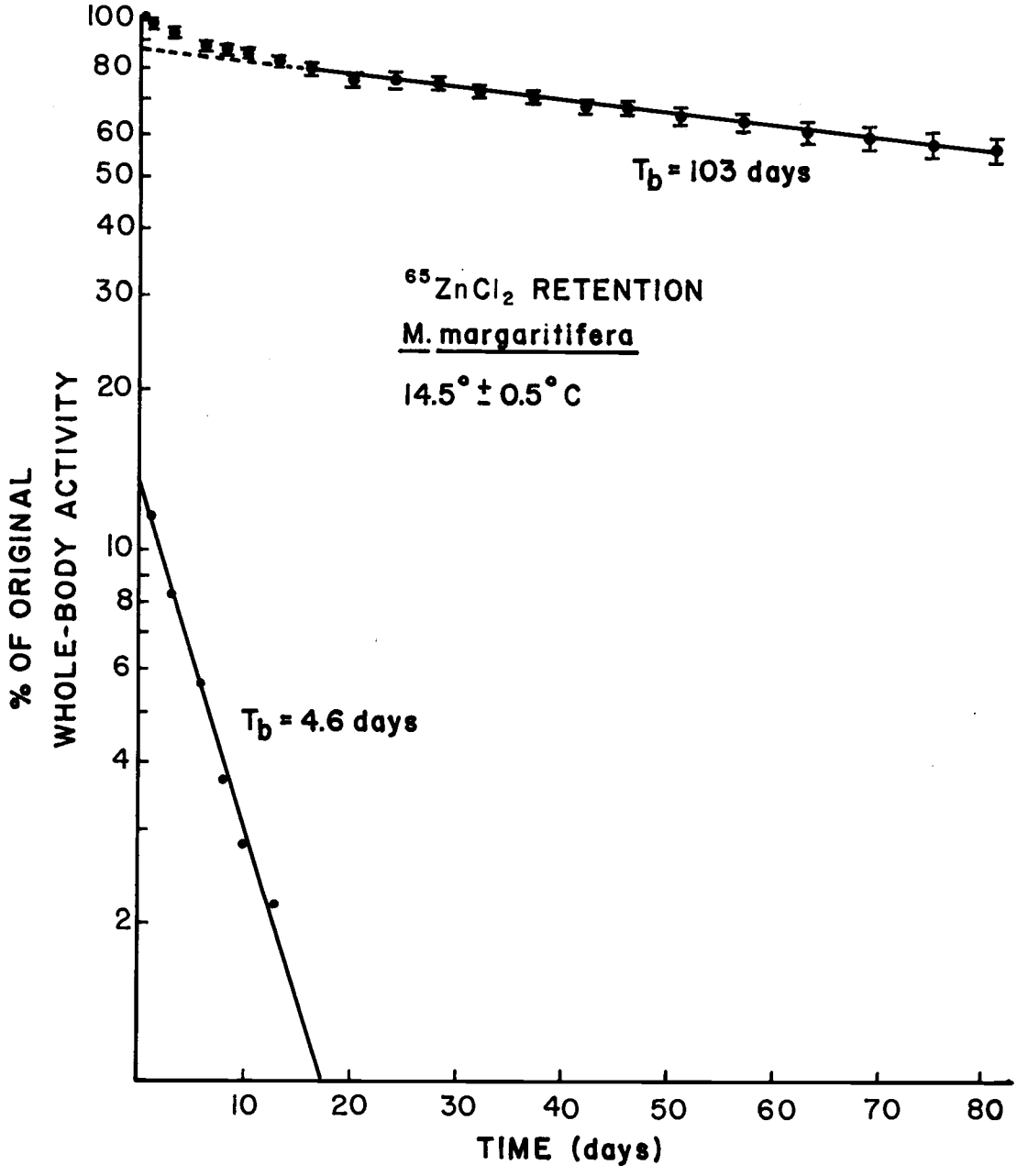

Figure 12. Whole-body retention of $^{65}ZnCl_2$ by Margaritifera margaritifera following chronic exposure.

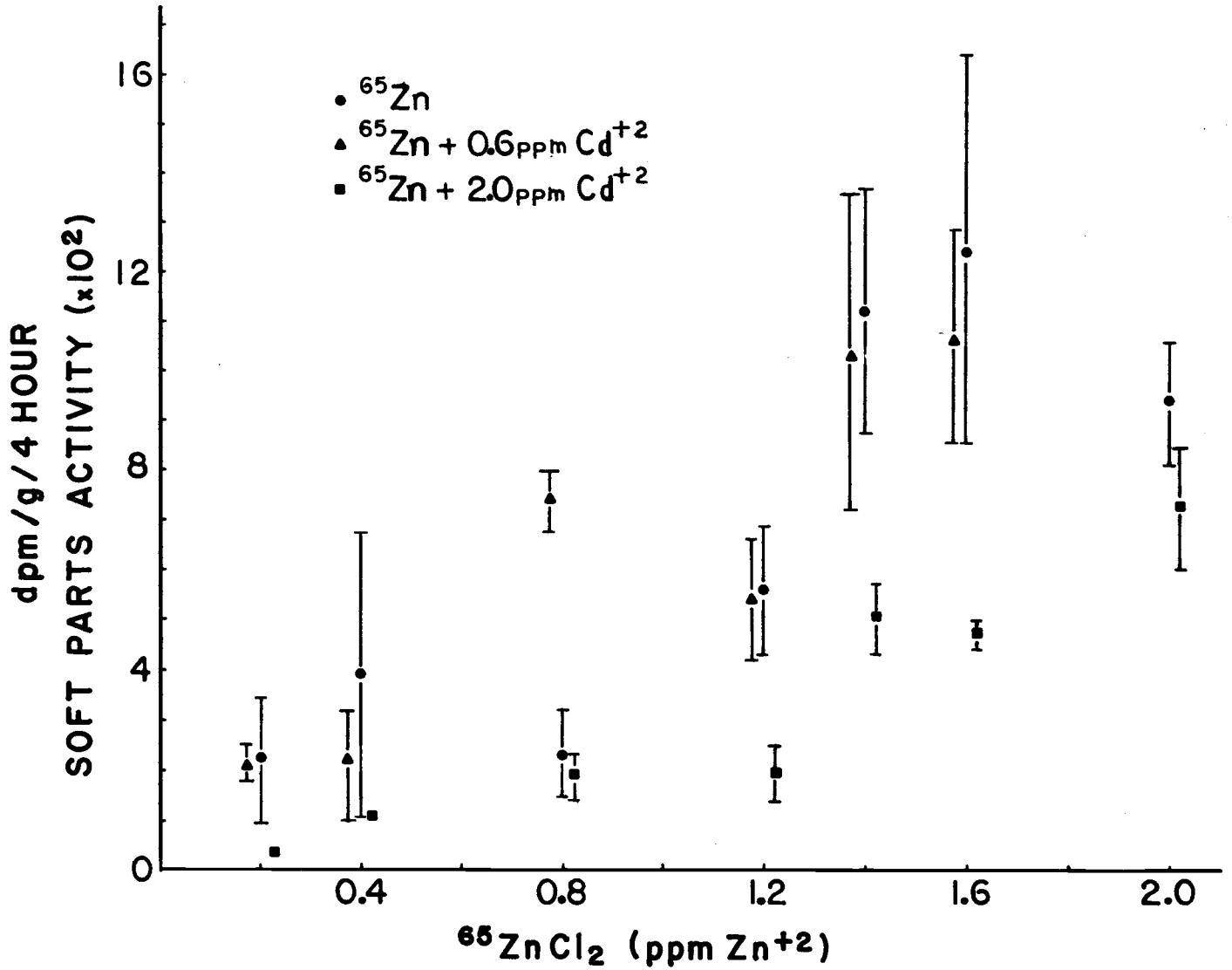

Figure 13. Inhibition of zinc metabolism by cadmium in Margaritifera margaritifera.

Toxicity Studies

Tolerance measured in terms of survival (mortality) rate in the laboratory will only indicate whether or not survival in nature is likely to be unfavorably influenced by a potentially lethal agent (Warren, 1971).

The median survival times for M. margaritifera were determined for two levels of $CdCl_2$ from the data plotted in Figure 14. When cumulative percentage mortality is plotted against exposure time, a skewed sigmoid curve usually results. It is desirable to straighten out this curve. Plotting percent response on a probability or probit scale instead of an arithmetic scale corrects the sigmoid nature of the graph. The skew apparently results from the logarithmic nature of biological time (Sprague, 1969). It is usually eliminated by using a logarithmic scale on the time axis. Therefore, the log-probit graph (Figure 14) results in a straight line plot of percent mortality against exposure time. It is very easy now to determine the median survival times. This type of graph also makes it apparent whether there are two or more different modes of toxic action. Changes in slope or grouping of lines provide the clues.

When mussels were maintained in 2.0 ppm cadmium, their median survival time was about 88 hours. Upon doubling the cadmium concentration to 4.0 ppm the median survival time of a different group of mussels was 61 hours.

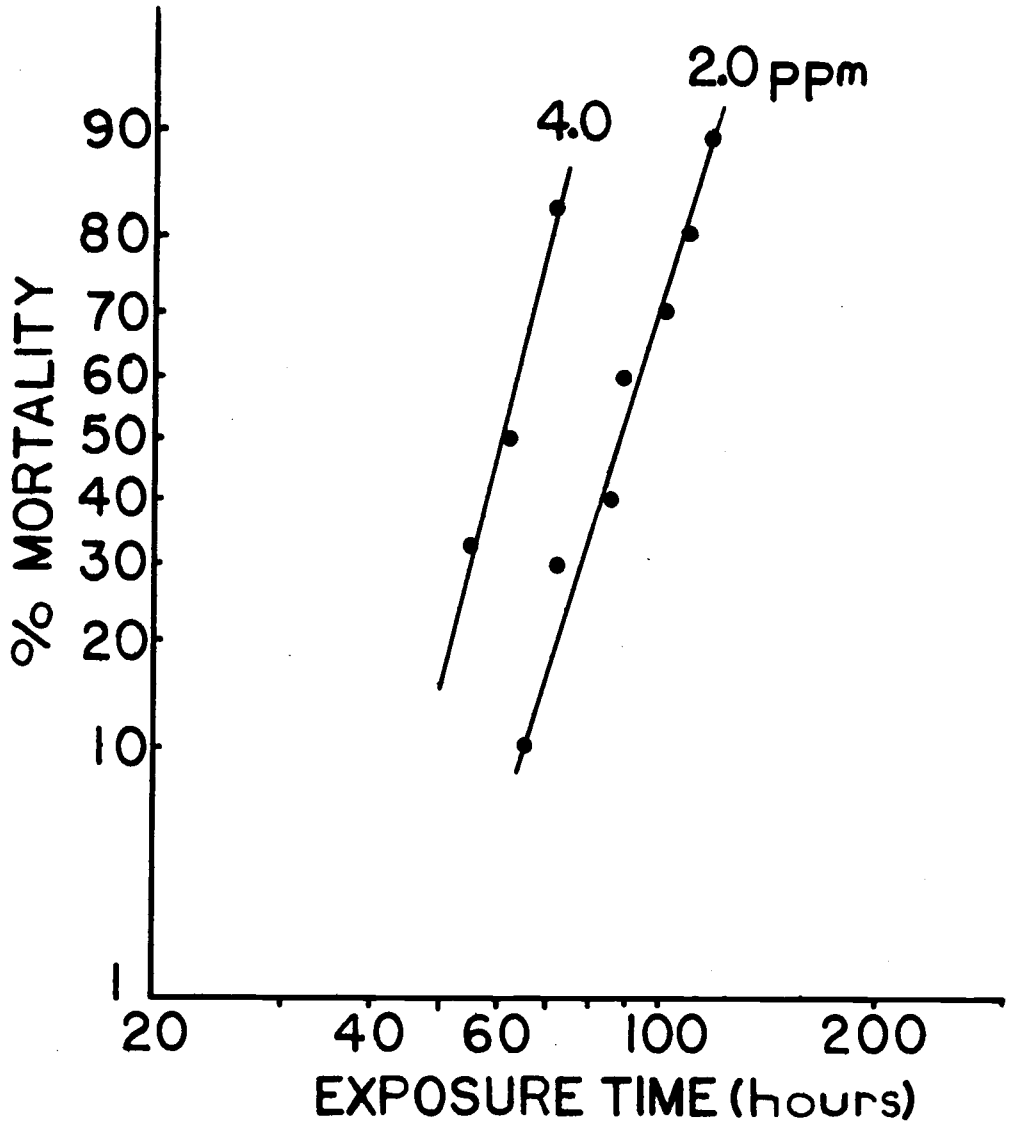

Figure 14. Median survival times of Margaritifera margaritifera for two concentrations of $CdCl_2$.

DISCUSSION

The maintenance of a healthy reproducing bivalve population which can serve as an excellent food source is important from both an economic and public health point of view. Bivalves will selectively take up certain materials, which may result in elevated concentrations in the edible tissues. These concentrations may lead to toxicity in the shellfish themselves and to human consumers. When these animals are exposed to a polluted environment, it becomes necessary to be able to predict toxic concentrations which might accumulate in bivalves and the length of time they might remain contaminated.

Uptake is the process of entry of materials into organisms and when uptake exceeds excretion, accumulation is its result. In the aquatic environment loss usually occurs simultaneously with the uptake process, the true intake rate is not directly observed, but only the net difference between intake and loss (Polikarpov, 1966b). When dealing with heavy metal pollution, the rate of uptake of these pollutants may be influenced by many environmental and biological factors. The concentration of the individual metal in the water is the most obvious environmental factor followed by the physical and chemical form of the element, the concentration of other metabolically similar elements, pH, and temperature. Some biological differences such as type and age of the organism involved, its physical activity, its food

habits and physiological demand for the metal (if any) will influence the rate of uptake. When accumulation occurs, its magnitude is termed the concentration factor (C_f).

It is impossible to measure the biological half-life of a stable metal in an organism. However, a radioactive tracer may be used to study this feature. The time required for an organism to excrete half of the initial concentration of a radioelement from its body is called the biological half-life (T_b). It is an indication of the turnover rate of an element in the animal. A factor commonly influencing this turnover is, in addition to the above for uptake, the metabolic rate of the animal. In invertebrates (and poikilotherms) the metabolic rate and the environmental temperature may be closely related.

In oysters, clams and mussels, all the soft parts are eaten by human consumers. This is not so for scallops, where only the large adductor muscle is eaten. Therefore the differential tissue distribution in bivalves is of public health concern. In marine bivalves cited here, the soft parts accumulate much more of the toxic metals than does the shell. By contrast, these laboratory experiments have shown that freshwater mussels concentrate large amounts of the metals in their shells, to be discussed later. Therefore, the shell of the freshwater bivalves might serve as sensitive indicators of heavy metal pollution in the aquatic environment.

Metals may exist in water as dissolved ions, in organic

complexes, adsorbed on clay particles, and as suspended precipitates contained in decaying organisms and other forms (Lisk, 1971). The most common form of cadmium in seawater appears to be Cd^{+2}, mercury may exist as the divalent mercury tetrachloride anion ($HgCl_4^{-2}$) and zinc probably occurs as Zn^{+2} (Bowen, 1966).

Cadmium

To my knowledge this is the first report on cadmium metabolism in freshwater bivalves. Therefore, comparisons and contrasts can only by made with other freshwater phyla or with marine bivalves.

Many significant differences exist between the freshwater and marine environments. Seawater contains most of the elements known to man in generally higher concentrations than found in freshwater. It has an average salinity of about 33‰ and seawater temperatures are more constant than in the freshwater environment. The chemical form of the elements may be different in the two environments. However, cadmium occurs as Cd^{+2} in seawater (Bowen, 1966) and predominantly as Cd^{+1} and Cd^{+2} in freshwater (McKee and Wolf, 1963). The oceans generally contain a higher percentage of suspended material. Thus, the binding properties of metallic ions would tend to increase the percentage of metals bound to suspended sediments in the marine environment. These complexes may move toward the bottom of the water column thereby increasing their availability to bivalves.

Uptake

The uptake pattern of cadmium has been observed in one species of marine bivalve. The east coast oyster, (C. virginica) exhibited an initial rapid uptake of cadmium but did not show any indication of approaching equilibrium after 100 days of chronic uptake under a controlled laboratory temperature of 20°C and 31‰ salinity (Shuster and Pringle, 1969). The uptake and accumulation of cadmium in the freshwater mussel, M. margaritifera, reported in this thesis reached a near equilibrium in about 45 days under controlled laboratory conditions.

Several factors could account for this difference. The most important are the differences between the marine and freshwater environments. The ionic concentration of the water influences uptake of metals from the water. The temperature used by Shuster and Pringle was 5.5°C warmer than the freshwater in my experiment. A direct correlation has been shown between increased temperature and the rate of accumulation of radioactive materials through metabolic processes in invertebrates from the Columbia River (Foster and Davis, 1955). Also the chemical form of the element was different in the two experiments. In my experiment the metal was in the chloride form when supplied to the animals, whereas Shuster and Pringle used the nitrate salt of cadmium.

Differences in the concentrations of the cadmium to which the shellfish were exposed in the two experiments also may explain the different results. Shuster and Pringle exposed two different groups of oysters to 100 and 200 ppb cadmium. While freshwater mussels in this study were exposed to only 10 ppb of the metal. It is generally recognized that an increase in the concentration of a metal in water will result in its accumulation in bivalves (Krumholz, Goldberg and Boroughs, 1957; Pringle et al., 1968) and in the fish (Boroughs, Chipman and Rice, 1957). This was demonstrated experimentally by exposing soft-shell clams (Mya arenaria) to 50 and 100 ppb cadmium (Pringle et al., 1968). These authors concluded that Mya was able to concentrate approximately twice the amount of cadmium in its tissues during a given period when the environmental level was doubled.

The initial concentration of cadmium in the tissues of the animal may contribute significantly to differences in rates of uptake or accumulation. Shuster and Pringle (1969) reported an average of 2.40 ppm cadmium in the wet tissue of these oysters prior to their experiment. This value is four times that found in the mussels taken from the Willamette River in my work. After 45 days of exposure to 100 ppb cadmium, the east coast oysters showed tissue concentrations of about 48 ppm, or a C_f of 480. By contrast, after 45 days of exposure to 10 ppb cadmium, the freshwater mussels showed tissue concentrations of about 0.20 ppm for a C_f of only about 20.

There are many reports on the levels of cadmium in marine bivalves taken directly from the oceans for analysis. Concentration factors, the ratio of the metal concentration in the animal to the metal concentration in the water, in general, have been shown to be much greater for stable elements in animals taken from the natural environment than found in laboratory experiments using chronic exposures to radiotracers (Polikarpov, 1966; Patel and Ganguly, 1969). This difference may be partially due to environmental variables and to the fact that animals taken from the natural environment are mature and have been exposed to a given level of trace metals since embryonic development, likely many years.

Interestingly, the animal in which cadmium was first measured was the scallop (Fox and Ramage, 1930). They found cadmium in the livers of all of the 11 individuals of Pecten maximus examined. Many years later, Pringle et al. (1968) analyzed four species of marine shellfish for cadmium and eight other metals. They found that the cadmium concentration in the soft-shell clam (Mya arenaria) and the northern quahaug (Mercenaria mercenaria) ranged from 0.10-0.90 ppm and 0.10-0.73 ppm cadmium (wet wt), respectively. The Pacific oysters (C. gigas) ranged from 0.20-2.10 ppm cadmium. By comparison east coast oysters (C. virginica) ranged from 0.10-7.80 ppm and averaged 3.10 ppm. The Atlantic coast animals were collected from Maine through North Carolina, therefore, in addition to other

environmental factors the range of cadmium concentration in these shellfish may reflect the differences in cadmium concentrations along the coast. Even though these factors are all taken into account, it is not clear why the values I obtained for the freshwater mussel, M. margaritifera, (0. 19 ppm, wet wt) fall within the ranges for the marine bivalves cited. In addition, experimental concentration used for these mussels was about 10 times the reported value (0. 11 ppb) for cadmium in seawater (Bowen, 1966).

Chronic exposure to a metal may lead to higher tissue concentrations in an organism than an acute exposure (Krumholz, Goldberg and Boroughs, 1957). If the organism has a very long life span, toxic concentrations of many metals may be accumulated and eventually a state of equilibrium is reached in organisms chronically exposed. By contrast, in an acute exposure, such as a single feeding or injection, only a small amount of the substance is accumulated in the body. This lower accumulation may be due to the short time of exposure in relation to the rate of accumulation.

The following C_f (concentration factors) values for cadmium are taken from extremely long term environmental exposures. The scallop (Pecten novae-zelandiae), the oyster (Ostrea sinuata) and the mussel (Mytilus edulis aoteanus) were collected from the Tasman Bay, Australia, and analyzed by Brooks and Rumsby (1965). They reported C_f values of 2, 260, 000, 318, 000 and 100, 000 for the scallops, oysters

and mussels, respectively. Comparable values were obtained by Mullin and Riley (1956) for cadmium in Pecten maximus collected from the Irish Sea. Pringle et al. (1968) found a somewhat lower concentration factor (226,000) for cadmium in American oysters (C. virginica) and an even lower C_f (800) for the mussel (Mytilus edulis). They also obtained C_f values for cadmium in the quahaug (Mercenaria mercenaria) and the soft-shell clam (Mya arenaria) of 750 and 800, respectively. The difference in concentration factors among the shellfish reflect differences in species, environmental cadmium concentration and other environmental parameters. Schroeder and Balassa (1961) reported C_f values of 2000-10,000 for cadmium in marine shellfish.

The uptake experiment described here for the freshwater mussel was, in fact, a chronic exposure under constant conditions unlike the environmental conditions. Thus, the levels reached at near equilibrium may not correspond well with extremely long term exposures under natural conditions. The edible tissues of the freshwater mussel, M. margaritifera, showed a C_f of about 20. There was significant adsorption of cadmium to the shell which resulted in a whole-body C_f of 150.

Since the concentration factor is a measure of maximum accumulation of a metal it follows that the same environmental and biological factors which influence the rate of uptake may be used to

discuss the large differences in C_f values for cadmium among freshwater and marine bivalves. As mentioned earlier, Polikarpov (1966) and Patel and Ganguly (1969) have shown that concentration factors for animals taken from the natural environment are often 100-1000 times greater than laboratory chronic exposure data. In addition to differences in exposure time this phenomenon may be a result of laboratory experiments being unable to simulate true environmental conditions adequately. Thus, laboratory C_f values should be considered minimum values only.

Tissue Distribution

Brooks and Rumsby (1967) analyzed the tissues of the oyster, Ostrea sinuata. They found that the highest concentration of cadmium was in the visceral mass, followed by lower concentrations in the gills and the mantle and labial palps. Mullin and Riley (1956) found essentially the same distribution in the lamellibranch, Chlamys opercularis, except for the gills, which contained more than four times the cadmium concentration of the muscle. They also examined the tissue distribution of cadmium in scallops (P. maximus) freshly collected from the Irish Sea and found comparable values to those previously cited.

The distribution of cadmium in the soft tissues observed in my study of the mussel, M. margaritifera, at termination of chronic

exposure generally compares quite closely with that found by Brooks and Rumsby (1967) and by Mullin and Riley (1956). This agreement among the tissue distributions suggests that the specificity for these tissues is quite tolerant of differences in the chemical and physical form of the metal.

Neither Mullin and Riley (1956) nor Brooks and Rumsby (1967) found significant amounts of cadmium associated with the shells of the marine bivalves they tested. This study found more than 75% of the total whole-body cadmium content associated with the shells of the freshwater mussel. This could be due to chemical differences in the shell or possibly a function of the chemical and physical state of the available cadmium from the environment allowing high physical binding activity.

The possible ecological significance of this difference is speculative. However, if deposition of cadmium in inedible shells is a metabolic process of the freshwater mussel, then it would be an efficient means for the removal of cadmium from the freshwater food chain. If this high shell concentration is strictly a physical adsorption process, there would be very little ecological significance.

Retention Studies

The retention data presented in this thesis represent the only

retention data reported for marine and freshwater bivalves. A two-component retention curve was demonstrated for cadmium in the freshwater mussel, M. margaritifera. The shorter component in this excretion curve represents unassimilated metal and the proportions of assimilated radioactive cadmium which are turned over rapidly. The long term component is the most important quantitatively since the major residence time of assimilated cadmium is determined by the slow rate component. As reported previously the biological half-life, T_b, of the long term component was found to be 835 days. This implies an extremely long turnover time for the cadmium bound in the tissues. Thus, a very long time would be required for mussels to eliminate a majority of their initial body burden of cadmium even if the animals were transferred to water low in cadmium.

Toxicity

Many cadmium toxicity studies on aquatic organisms have been reported in the scientific literature, but they have primarily been limited to fish with only a few on marine bivalves. The units and terminology used in these studies needs clarification. In the United States, the term "median tolerance limit" (TLm) has been customarily used in fish toxicology studies (Sprague, 1969). The TLm estimates the median level of the metal that a group of animals can tolerate for any given period of time, i.e., the 96-hour TLm.

Another very useful measure of toxicity is the "median survival time" (MST). This is used to measure survival (mortality) times for a fixed concentration of the metal. A group of animals is exposed to a lethal level of a contaminant and the percentage surviving at different times is noted. The relationship between cumulative % survival and survival time will usually be a skewed sigmoidal curve. A linear relationship may be obtained by plotting the cumulative % survival (mortality) on a normal probability scale and the survival (exposure) time on a logarithmic scale. From this graph the length of time 50% of the animals were able to survive (the median survival time) is estimated.

Eisler, (1971) reported a 96-hour TLm of 2.2 ppm Cd^{+2} for the soft-shell clam (Mya arenaria) maintained at 20°C and 20‰ salinity. The marine mussel, Mytilus edulis, was shown by Eisler to be much more resistant, with a 96-hour TLm of 25.0 ppm Cd^{+2} and the same conditions of temperature and salinity. Because of a limitation of time and cadmium stock solution, I decided to measure the median survival time for two cadmium concentrations instead of the 96-hour mean tolerance limit. The median survival times of 88 and 61 hours were determined for cadmium concentrations of 2.0 and 4.0 ppm cadmium, respectively. A very close relationship may be seen in the 96-hour TLm of 2.2 ppm cadmium for the soft-shell clam and an 88-hour mean survival time for a cadmium concentration of 2.0 ppm for

the freshwater mussel. Considering the differences in species, salinity and temperature, an explanation for the similarity is not clear.

Pringle et al. (1968) reported cadmium to be extremely toxic to the east coast oyster (C. virginica) and somewhat less toxic to the hard-shell clam. Shuster and Pringle (1969) exposed east coast oysters to 0.2 ppm cadmium while being maintained at 20°C and 31‰ salinity. After 28 days of chronic exposure the slope of the uptake curve seemed to become constant and deaths started to occur. Tissue concentrations at this time were measured and found to be about 45 ppm cadmium (wet wt). After about 85 days of chronic exposure, tissue concentrations in the oysters were about 100 ppm cadmium and only 10% of the oysters had survived. From the data presented by Shuster and Pringle, a mediun survival time of about 1300 hours was determined.

These results suggest that if the cadmium concentration of the environment is low, a bivalve can accumulate more of the metal and live longer than a bivalve being exposed to a higher concentration of cadmium. This may indicate a physiological detoxification mechanism which binds excess cadmium and stores it in an inactive state until such time that the protective mechanism becomes saturated and toxicity then occurs.

Fish seem to be more resistant to cadmium toxicity than bivalves. Ball (1967) reported that all concentrations from 0.01-1.0 ppm

cadmium killed rainbow trout in about 144 hours. Cadmium was lethal to sticklebacks at 0.2 ppm and to goldfish (no times were reported) at a concentration of 0.017 ppm (Nilsson, 1970). Eisler (1971) found 96-hour TLm values of 21.0, 50.0 and 55.0 ppm cadmium for the striped killifish (Fundulus majalis), the sheepshead minnow (Cyprinodon variegatus) and the mummichog (Fundulus heteroclitus), respectively. The comparative toxicity of cadmium in soft and hard water was reported by Pickering and Henderson (1966). Flathead minnows (Pimephales promelas) showed a 96-hour TLm of about 0.84 ppm cadmium when maintained in soft water compared to about 73 ppm cadmium when maintained in hard water. The green sunfish (Lepomis cyanellus) showed similar results with a soft water 96-hour TLm of about 2.8 ppm cadmium and a hard water value of 66 ppm cadmium.

A possible influence on cadmium toxicity resulting from the presence of a metabolically similar element was studied by Hublou (1954). He found that a cadmium concentration of 0.03 ppm acted synergistically with 0.15 ppm zinc causing mortality of salmon fry (time not specified). Cadmium, in addition to its effects on zinc, interferes with the metabolism of iron, copper, manganese and calcium (Fox, 1971).

In summary, teleosts appear most resistant to cadmium toxicity, with 96-hour TLm values of between 21 and 55 ppm cadmium, and crustaceans seem most sensitive, with TLm values between 0.32-4.1

ppm cadmium. Molluscs are intermediate in sensitivity with corresponding 96-hour TLm values from 2.2-25.0 ppm cadmium (Eisler, 1971).

Mercury

It is not surprising that the scientific literature dealing with mercury metabolism in bivalves has originated almost entirely in Japan and the Scandinavian countries. Serious mercury contamination has occurred in both areas. General public awareness of mercury contamination in the United States and Canada is less than three years old. The bulk of research initiated during this period in North American has yet to be completed and fully reported.

As with cadmium, the results presented here on mercury metabolism in freshwater mussels appears to be the first reported for freshwater shellfish except for one retention study which is mentioned later. These data will be compared and contrasted with those from freshwater fish and marine fish and bivalves.

Uptake

In 1961 Uchida (as cited by Westöö, 1966) showed that the mercury compound in the shellfish that caused the Minamata disease was methylmercury. Matida and Kumada (1969) compared clams, oysters and mussels from a presumably uncontaminated area of Minamata Bay to oysters from a contaminated area of the bay. The clam, Mactra sulcateria, the oyster, C. gigas, and the mussel,

Mytilus edulis, contained 3.0, 3.3 and 0.6 ppm mercury, respectively. These values were compared to C. gigas collected from a contaminated station in Minamata Bay in 1961 contained 10.0 ppm mercury. Matida and Kumada reported methylmercuric chloride in one species of bivalve taken from Minamata Bay. They found the soft tissue of the mussel, Hormomua mutabilis (Gould), contained 48 ppm total mercury (dry wt) of which 16 ppm was methylmercury. Other workers found mercury levels in the mussel Hormomya mutabilis (Gould) also collected from Minamata Bay to be over 100 ppm (dry wt) until the end of 1959 (Irukayama et al., 1962). After waste disposal facilities were finished at the factory responsible for the mercury (January, 1960) these same workers found that the mercury content in these species decreased gradually. By April 1960 the value was about 30 ppm and it had dropped to 9 ppm by December 1961. Pringle and Shuster (1967) transformed the dry weight data of Irukayama et al. to a wet weight basis. These corrected values were over 20 ppm for the maximum observed mercury level in H. mutabilis and less than 2 ppm for December 1961. From the data presented by Irukayama et al. (1962), a biological half-life (T_b) of 135 days for the mercury excretion from these mussels while in the bay may be computed.

The mussel Mytilus edulis, the cockle Cardium edile, and the clam Macoma balthica, collected from the Wadden Sea, of northern

Holland, contained 0.13, 0.089 and 0.099 ppm mercury, respectively (Goeij and Houtman, 1971). These workers compared this area to an industrial, and presumably polluted, area along the Dutch Coast where mussels were found to contain 0.2-0.3 ppm mercury. Marine coastal ducks (Eiderducks) which feed on these bivalves contained from 1.0-3.2 ppm mercury in edible tissues with 14.1 ppm found in the liver and up to 2.3 ppm mercury in the brain. The form of the mercury was not mentioned.

The values I have observed for the freshwater mussel, M. margaritifera, agree closely with the data presented by Goeij and Houtman (1971) for the Wadden Sea. The tissue levels in these freshwater mussels were 0.024 ppm and 0.067 ppm for mercuric nitrate and methylmercuric chloride, respectively. These concentrations are, however, considerably lower than found in "control" areas of Minamata Bay.

Concentrations of 32 ppb mercury (as $HgCl_2$) affected the embryonic development of Mytilus at 13-17°C and Crassostrea at 27°C. However, levels of 10 ppb mercury did not affect these two organisms during embryonic development (Okubo and Okubo, 1962).

Using the short-necked clam (Venus japonica Deshayes), Irukayama et al. (1962) found that mercury compounds were highly toxic at seawater concentrations of 500 ppb mercury. Fujiki (1963) cultured the shellfish, V. japonica, in seawater containing

methylmercury among other mercury compounds. He was attempting to determine a route of the causative agent of Minamata Disease. From Fujiki's data, Lőfroth (1970) calculated that a methylmercury tissue concentration of 20-30 ppm on a wet weight basis was lethal for Venus japonica. These data compare closely with the tissue concentration of cadmium found by Shuster and Pringle (1969) to be toxic to the east coast oyster. The toxicity of mercury was not investigated in the freshwater mussel. However, after 57 days of chronic exposure to methylmercury the tissue concentration of mercury in the freshwater mussel was almost 300 times lower than the reported lethal concentration for V. japonica. Irukayama et al. (1962) concluded that "The content of mercury in the shellfish depends on the survival period of the shellfish, but not on the toxicity of mercury compounds." This would suggest an insufficient mechanism for the excretion of mercury compounds in shellfish. The shape of the uptake curve for methylmercury obtained for the freshwater mussel, M. margaritifera, tends to support this conclusion. It would appear that water concentrations of mercury in the part per billion range may be accumulated to the point of toxicity by Margaritifera margaritifera due to its long life span.

A moderate volume of data have been published on mercury levels in fish from natural environments, again mainly from Japan and Scandinavia. Westöö (1966) analyzed the mercury content in four species of freshwater fish from Sweden. He found that 75%, and

sometimes as much as 90%, of the total mercury content was in the form of methylmercury. Jernelöv and Lann (1971) studied food chain relationships to feeding behavior in fish. They reported that the percentage of mercury accumulated through the food chain was fairly constant (60%) for pike in the three bodies of water tested. However, for bottom feeding fish, direct uptake from the water is more important. In the same three bodies of water, less than 25% of the mercury accumulated in bottom feeders came through the food chain. The mercury concentration for fish of the same species and from the same fishing ground increases with the weight of the fish (Westöö, 1967). Bache, Gutenmann and Lisk (1971) reported that concentrations of both total mercury and methylmercury increased with the age of lake trout. The proportion of methylmercury to total mercury also increased with age. Conversely, Wobeser et al. (1970) found no association between mercury concentration and age in Saskatchewan River fish. This apparent discrepancy may be partially explained by biological factors such as differences in species, food habits, sex and many physical environmental factors.

Marine fish data have been collected from many locations of the world. Flounder and plaice from the Wadden Sea, contained 0.50 and 0.21 ppm mercury, respectively (Goeij and Houtman, 1971). Westöö (1966) reported that methylmercury had been found in marine fish, but only in small amounts. American plaice contained 0.07 ppm as

methylmercury, as did Greenland halibut (Zitko et al., 1971).

Of several mercury compounds studied under laboratory conditions, Hannerz (1968) found methylmercury was the most readily accumulated. The concentration factor, C_f, for pike muscle was about 2000 and that for pike kidneys was 9000. He postulated that the concentration of electrolytes in the water could exert an influence on the accumulation of mercury in fish. However, his experiments showed that this is probably not the true picture. Cod and pike accumulated both methylmercury and methoxyethyl mercury faster in saltwater than in brackish water. However, the relationship was not constant. The uptake rate in pike of methylmercury from brackish water was lower than that from freshwater. This variability and other fish data were the basis for Hannerz to conclude that "There is no general connection between the salinity and the accumulation of mercury in fish."

If we can assume this to be similar for shellfish, the order of importance of physical parameters on mercury uptake and accumulation could be a little more clearly listed.

Tissue Distribution

The mercury content was determined in the different tissues of the shellfish, Hormomya mutabilis (Gould), collected from Minamata Bay, Japan (Fujiki, 1963). The ganglion contained 181 ppm (dry wt)

followed by the gills (87 ppm), digestive gland (73 ppm), genital gland, mantle and ligament with about 63 ppm mercury each. This worker found the muscle contained about the lowest concentration (25 ppm), which is consistent with most shellfish findings.

Irukayama et al. (1962) found that the distribution of mercuric chloride in Venus japonica under laboratory conditions of exposure differed somewhat from that found in the mussels from Minamata Bay. This was especially the case in the relationship between the gills and ganglion. Gill concentrations were 30% higher than those in the ganglion for the laboratory experiment. By contrast, ganglion concentrations were 48% higher than those in the gills from Minamata Bay mussels. This difference might suggest a very slow turnover for the nervous system tissue. The animal from the natural environment having had a much longer exposure period would have had an opportunity to accumulate much larger concentrations in compartments with a slow turnover. However, the difference in species undoubtedly has some effect.

The freshwater mussel, M. margaritifera, showed the highest percentage of total body mercury in the visceral mass after chronic exposure to mercuric nitrate and methylmercuric chloride. The gills were next with approximately 23% and 40% of the amount in the visceral mass for the two mercury compounds, respectively. The adductor muscle had nearly the same amount of methylmercury as the gills.

The foot contained the lowest amount of both mercury compounds. The foot and the muscle generally contain the lowest concentration of mercury in bivalves.

A major portion of the scientific literature pertaining to the metabolism of mercury compounds by freshwater fish has come from Swedish investigators. Fish caught in the vicinity of two Swedish factories using inorganic mercury and phenylmercury showed a strong increase in methylmercury content, indicating that a synthesis of methylmercury had taken place (Norén and Westöö, 1967). In order to gather more data in this area, many laboratory studies were conducted, a few of which are reported below. Hannerz (1968) exposed pike and cod to methoxyethylmercuric hydroxide for about 90 days. At the end of chronic exposure, the kidneys and liver of the pike were nearly equal in mercury content and were the highest followed by the spleen, muscle, brain, bone and blood. Cod displayed a different pattern of distribution. The gills had concentrations of mercury 50-100 times those of other organs. Hannerz believed that the concentration of mercury in the blood was too low to be responsible for the high gill concentration, due to excretion alone. A more likely explanation for the high gill concentration of mercury would be a direct adsorption of methylmercury on the gill epithelium.

Hannerz (1968) exposed several species of fish to different mercury compounds while maintaining them in ponds. He concluded

that the mean concentration factors for methylmercury in all organs analyzed was 4.2 times higher than that for inorganic mercury and 1.7 times higher than that for methoxyethylmercury.

A comparison between fish and shellfish may be made here. Surface adsorption seems to play a dominant part in contaminated areas. Exposed tissue surfaces seem to accumulate large concentrations of mercury from the water. However, when a fish or bivalve has reached equilibrium or has been transported from a contaminated area, the digestive and excretory organs seem to display the highest concentration of mercury.

Retention Studies

A retention curve for a whole organism represents the mean of a number of individual compartments each with its own retention time. Miettinen et al. (1969) studied the retention of inorganic mercury (Hg^{+2}), phenylmercuric nitrate and methylmercuric nitrate when injected into the foot musculature of the freshwater mussel, Pseudanodonta complanata. Two-component curves were obtained in all experiments and in all cases, more than 90% of the injected activity was involved in the slower (long term) component. The short term component represents unassimilated mercury and that mercury which is turned over rapidly. The long term component is the more important ecologically since the major residence time of assimilated

mercury is determined by this set of compartments. Of the long term components found for P. complanata, inorganic mercury showed a T_b of 23 days, followed by a T_b of 43 days for phenylmercuric nitrate and a range of T_b's of 86-435 days for methylmercury. This range was the result of three separate experiments designed to observe the possible effect of animal age (size) on the retention of organic mercury. The youngest (smallest) animals gave a T_b of 86 days, for medium animals a T_b of 129 was found and for the oldest (largest) mussels a T_b of 435 days was reported.

The retention data for the freshwater mussel, M. margaritifera, I have obtained agree closely with those reported for the freshwater mussel, Pseudanodonta complanata, presented above. I observed a two-component retention curve for each mercury compound involving 91% and a T_b of 194 days and 93% and a T_b of 860 days in the long term component for mercuric nitrate and methylmercuric chloride, respectively.

Mercury retention data have been reported for only one species of marine bivalve. The Mediterranean marine mollusc, Tapes decussata, revealed a T_b of 174 days for methylmercuric chloride (Miettinen et al., 1969).

The most frequently used experimental fish in Sweden seems to be the pike, Esox lucius . Many workers have chosen this species for their investigations. Miettinen et al. (1969) found that 10-40% of

orally administered mercury was usually excreted within one or two days in the pike and the perch, Perca fluviatilis. The remainder followed an exponential function and showed T_b values of 100-600 days for methylmercuric nitrate and 60-150 days for phenylmercuric nitrate. When methylmercury was administered to fish the resulting retention curve always had two components. The long component for roach Leuciscus rutilus, flounder Pleuronectes flesus, the perch and the pike, displayed T_b values of 25, 430, 470 and 490 days, respectively. When phenylmercury was tested in the perch, flounder and pike the resulting T_b's were 157, 164 and 190 days, respectively. It is evident from Miettinen's data that the biological half-life for methylmercury in these four fish species is over twice as long as that for phenylmercury. This observation is supported by other fish data from Norén and Westöö (1967).

Hannerz (1968) found that the T_b for pike muscle, after chronic exposure to about 10 ppb phenylmercuric acetate, was about 65-70 days. He found the liver and spleen showed more rapid elimination rates with T_b values of only 35-40 days. Miettinen et al. (1969) found no significant difference in the biological half-life of pike in freshwater or brackish water. He concluded (supporting Hannerz's data) that "It is evident that when the mercury compounds are bound to the muscle protein, they have a much slower excretion rate than when bound to the digestive organs."

These fish data are remarkably consistent with those reported for the marine and freshwater bivalves. This is surprising to me because of the extreme difference in metabolic rates of the two phyla.

Zinc

Intensive research on zinc metabolism in freshwater vertebrates has been carried out for many years, but only in selected areas of the United States. The movement of zinc in the Columbia River and its estuary has been extensively studied for the past 27 years. The Clinch River and White Oak Lake located in eastern Tennessee have also been areas of concerted zinc research. More limited studies on the metabolism of zinc in freshwater bivalves have been undertaken.

Uptake

Many freshwater molluscs have been found to concentrate high levels of zinc and other heavy metals. Pauley and Nakatani (1968) found that the freshwater mussel, Anodonta californiensis, accumulated ^{65}Zn in proportion to the concentration of the isotope in the test water. Merlini, Girardi and Pozzi (1967) found that the freshwater bivalve, Unio mancus var. elongatulus, concentrated zinc and six other metals over the individual metal concentrations found in the water of Lago Maggiore located in northern Italy. Harvey (1969) found the freshwater clam, Lampsilis radiata (Gmel.), to be a very

efficient concentrator of zinc from the Savannah River. These animals reached equilibrium within 91 days of chronic exposure, revealing a C_f of 4080 for zinc.

The accumulation of zinc in the freshwater clam, Anodonta nuttalliana (Lea), was found by Harrison (1968) to be dependent upon the concentration of the stable element in the water, the temperature and the size of the animal. This concept, concerning the dependence of zinc uptake on the specific activity of the water, is very important. The uptake of radionuclides from water by an organism is usually faster than the rate of loss. The slower rate of loss is the result of ^{65}Zn constituting a smaller portion of the total zinc in the bivalve, compared with the larger portion of total zinc in the water represented by ^{65}Zn. For example, if the bivalve already contained stable zinc concentrations more than a thousand times greater than that of the water, then the ratio of ^{65}Zn to total zinc would be diluted upon entering the bivalve.

A significant amount of research has been conducted on zinc metabolism in marine bivalves. Commercially important species have received the most attention. Some of these bivalves include oysters (Crassostrea virginica and C. gigas), the razor clam (Siliqua patula), hard-shell clams (Mercenaria mercenaria), the bay scallop (Pecten irradians), and the mussel (Mytilus edulis). All these shellfish have been found to accumulate zinc many thousands of times above

the environmental seawater concentrations and, hence, serve as effective biological indicators of this metal in the marine environment (Chipman, Rice and Price, 1958; Watson, Davis and Hanson, 1961, 1963). In addition, Young and Folsom (1967) found the coastal mussel (Mytilus californianus) an efficient concentrator of silver as well as zinc.

The concentration of zinc in shellfish along the Atlantic coast varied over a range from about 10-40 ppm in the case of hard- and soft-shell clams to about 180-4100 ppm for the eastern oyster (Pringle et al., 1968). They also found concentrations of 90-350 ppm in the Pacific oysters, or only about 0.1 as high as related east coast species. The oysters with the highest zinc content were found in the upper Chesapeake Bay and contained 3200-4000 ppm zinc (McFarren, Campbell and Engle, 1961). These reported values of zinc concentration in oysters vary over almost two orders of magnitude. This could be a result of many environmental factors, most probably differences in species of shellfish (Wolfe, 1970b), variations in the zinc concentrations of the water (Pauley and Nakatani, 1968; Harrison, 1968), or seasonal variations in uptake (Galtsoff, 1964; Pringle et al., 1968). Compared to the zinc concentration found in hard- and soft-shell clams, the high levels in oysters suggest a possible species difference in the physiological role of zinc. This will be discussed in a later section.

McFarren, Campbell and Engle (1961) reported that mussels, clams and oysters differed considerably in their ability to concentrate and/or retain zinc. They found that oysters contained about 30-40 times more zinc than clams and about 90 times as much as mussels from the same or nearby areas. This may further support the hypothesis just mentioned, i.e., that these differences may be due to different metabolic requirements among shellfish.

A large number of concentration factors (C_f) values have been published for marine bivalves which are tabulated below:

Organism	C_f (total zinc)	Reference
Crassostrea virginica	250,000	Chipman, Rice and Price, 1958
" "	100,000-200,000	Wolfe, 1970b
" "	148,000	Pringle et al., 1968
Crassostrea gigas	14,600	Seymour, 1966
" "	5,600-12,000	Salo and Leet, 1967
Crassostrea angulata	250,000	Preston, 1966
Ostrea sinuata	110,300	Brooks and Rumsby, 1965
Ostrea edulis	250,000	Preston, 1966
Pecten novae-zelandiae	28,000	Brooks and Rumsby, 1965
Mytilus edulis aoteanus	9,100	" " "
Mercenaria mercenaria	2,100	Pringle et al., 1968
Mya arenaria	1,700	" " "
Mytilus edulis	2,200	" " "
surf clam	1,525	" " "

Concentration factors were determined for the freshwater mussel, M. margaritifera. The C_f for the edible tissues of these mussels were 16 and 26 for zinc concentrations of 624 and 64 ppb, respectively. A reduction in the C_f for an increase in the zinc

concentration of the water is evident. This trend has also been reported by Chipman, Rice and Price (1958) for the east coast oyster. The large differences in C_f values between this freshwater mussel and the marine bivalves tabulated above is probably due to the exposure time to the metal, differences in the zinc concentration of the water, species differences and the trend cited between laboratory experiments and natural environmental exposures previously discussed.

Marine molluscs are naturally rich in zinc and oysters contain greater amounts than other bivalves. As pointed out by Chipman, Rice and Price (1958), the amount of zinc concentrated in the soft tissues of oysters is directly related to the amount available in the water in which they live. This same concept has previously been mentioned pertaining to freshwater species. Even in a constantly changing system, where the ^{65}Zn concentration varied, Salo and Leet (1967) found that changes in oyster concentration rapidly followed zinc concentrations in discharge water from a boiling water nuclear reactor. This response increases the value of oysters as biological indicators of zinc pollution.

Tissue Distribution

Harrison (1968) investigated the tissue distribution of zinc in the freshwater clam, Anodonta nuttalliana (Lea), during chronic uptake. She reported that most tissues reached equilibrium after

about 100 days of chronic laboratory exposure. The mantle, gills and calcareous tissues, however, did not reach equilibrium by the termination of the experiment (147 days), when 80% of the total zinc was bound to the shell, mantle and gills. At the termination of chronic uptake, Pauley and Nakatani (1968) examined the tissue distribution of zinc in the freshwater mussel, Anodonta californiensis, and found that 75% of the ^{65}Zn was in the gills, mantle and palps. The adductor muscle and foot were low, containing only about 5% of the total activity. These same workers collected six mussels upstream from the Hanford reactor area, which showed a similar pattern of distribution.

McFarren, Campbell and Engle (1961) found that zinc was distributed fairly evenly throughout the tissues of the east coast oyster. They reported that the mantle and palps contained about 33% of the total zinc followed by the gills (23%), and the stomach and liver with about 21%. The adductor muscle was reported to contain about 5% of the total zinc. This distribution is consistent with that found in the same species of oyster, but in a different location by Wolfe (1970b). The soft tissue distribution of zinc that I found for M. margaritifera was similar to that found for both the freshwater and marine bivalves cited. The mantle and palps of the freshwater mussel contained about 44% of the total soft tissue zinc. The visceral mass contained 31%, the gills 12%, and the adductor muscle contained 3% of the total zinc

associated with the soft tissue. In addition, Pauley and Nakatani (1968) found about 60% of the total body zinc was associated with the shell of the freshwater mussel, Anodonta californiensis. This finding agrees well with mine which showed that 66% of the total zinc was bound to the shell of M. margaritifera. Gong et al. (1957) found much higher levels of zinc associated with the shell of the Japanese little-neck clam than with the soft tissue. However, many marine and freshwater bivalves have shown just the opposite trend. Wolfe (1970b) reported that the soft parts of east coast oysters contained an average of six times the shell concentration. However, he pointed out that, since the soft parts account for only 19.5% of the total live weight of the oyster, the shell actually contained nearly 45% of the total body zinc. Merlini, Girardi and Pozzi (1967) found that less than 1% of the total body zinc of the freshwater clam, Unio mancus, from Lago Maggiore was associated with the shell. This corraborated earlier work by Brooks and Rumsby (1965).

The temperatures of the respective collecting areas for the above studies were not included in the literature. Temperature may be the key to this discrepancy. Fitzgerald and Skauen (1961) reported that, at 5°C and low ^{65}Zn concentration, adsorption to the shell of the east coast oyster was major. However, at 18°C and low ^{65}Zn concentration, the metabolic activity of the oyster was rapid enough to cause maximum uptake of the metal before it was adsorbed onto the shell.

Harrison (1968) found that the ^{65}Zn activity on the unscrubbed shell of Anodonta nuttalliana (Lea) was always 50 to 100% higher than on the scrubbed shell. This suggests that a large portion of the zinc is loosely adsorbed to the shell. This same scrubbing technique was tried on the inside of the shells of M. margaritifera with similar results. The outside was covered with a plastic coating to reduce this phenomenon during uptake, as previously mentioned.

Retention Studies

Harvey (1969) found a two-component retention curve in the freshwater clam, Lampsilis radiata (Gmel.). The short term component, involving 40% of the initial activity, showed a T_b of 3.5 days. The long term component revealed a T_b of 40 days associated with 60% of the initial zinc body burden. Harrison (1968) reported a much longer component (650 days) for zinc retention in the freshwater clam, Anodonta nuttalliana(Lea). Seymour (1966) found a T_b of 255 days for zinc in the Pacific oyster, C. gigas. The east coast oyster, (C. virginica), showed a T_b of 150 days for zinc, and the T_b for zinc in the clam, Mercenaria mercenaria, was 51 days (Duke, 1963). A biological half-life of 76 days for zinc was determined for the coastal mussel, Mytilus californianus, by Young and Folsom (1967). These values also bracket the T_b of 103 days I found for the long term component of zinc retention in the freshwater mussel, M.

margaritifera. Thus, it appears that there are significant differences between the biological half-lives among freshwater and marine bivalves. Environmental factors influencing the metabolic rate of these organisms or species differences in the physiological requirements for zinc may be responsible for these differences.

Nuclear Reactors Monitored by Oysters

The oyster's ability to accumulate zinc combined with its commercial importance make it especially suitable as a biological indicator for ^{65}Zn in the environment. The level of ^{65}Zn in oysters is a major consideration in the assessment of potential radiation doses to resident human populations near nuclear reactors.

The maximum permissible release rates of ^{65}Zn-containing effluent from the fuel element cooling ponds of the Bradwell (Essex) nuclear power station in Britain are determined by the consumption of contaminated oyster flesh by the local population, i.e., oyster flesh is the critical material. Special layings of two commercial species of oysters (Crassostrea angulata and Ostrea edulis) have been installed in the Blackwater Estuary for use as biological monitors for the ^{65}Zn (Preston, 1966). Salo and Leet (1967) used the Pacific oyster to study the accumulation of ^{65}Zn into oyster tissues being maintained in the discharge canal of the Humboldt Bay boiling water reactor, Humboldt Bay, California.

The effective half-lives of elements in the environment for those radionuclides which are produced and released from nuclear power reactors are very important. Not only does the concentration of stable zinc in the water decrease by biological processes, but ^{65}Zn is also lost due to radioactive decay. The biological and radioactive decay constants are additive and together they yield a loss rate called the effective half-life (T_{eff}) which describes the actual rate at which the ^{65}Zn concentration would decrease in organisms growing in an uncontaminated natural environment. For example, Young and Folsom (1967) cite a T_b of 76 days for ^{65}Zn in the mussel, Mytilus californianus. If the radioactive half-life ($T_{1/2}$) of 245 days was taken into account, the T_{eff} is only 58 days.

Inhibition Studies

Despite the many extensive studies of zinc metabolism in marine and freshwater shellfish, very little is actually known about the physiological role of the metal in bivalves (Wolfe, 1970). As previously discussed, zinc plays an essential role in many enzymes, and it has been shown that large quantities of zinc are accumulated by shellfish, especially the oyster, C. virginica. This prompted Wolfe (1970) to analyze oyster tissue for zinc-containing enzymes. He found three: alkaline phosphatase, carbonic anhydrase and malic dehydrogenase. Recent computations by Pequegnat, Fowler and Small

(1969) showed that 2.7 ppm zinc probably represents a maximum enzymatic requirement for marine organisms. On this basis, shellfish, and oysters in particular, concentrate zinc several orders of magnitude over that required for essential metabolic functions.

This conclusion was supported by Wolfe (1970) who removed 96% of the protein-bound zinc from oyster homogenates without affecting the activity of alkaline phosphatase. However, removal of more than 96% resulted in a decrease in the activity of this enzyme. Wolfe concluded, "If alkaline phosphatase is considered representative of the metabolic functions of zinc in oysters, most of the zinc accumulated by oysters must be superfluous to the animal's requirements." Much of this excess zinc is found adsorbed to the exposed surfaces of the gills, mantle and labial palps of oysters, as evidenced by the usually higher zinc concentrations in these tissues than in internal tissues. This suggests that an adsorption-exchange mechanism accounts for much of the zinc accumulation in these organisms.

The functional value of this large excess of zinc in shellfish is not clear. One hypothesis, based on studies of terrestrial vertebrates, is that this excess may act to minimize uptake of other chemically similar, but toxic metals. Cadmium would be an example. The Cd^{+2} ion interacts physiologically with zinc, to which it is chemically similar. This is demonstrated by the fact that Zn^{+2}, administered simultaneously with Cd^{+2} to the rat, counteracts the toxic

effect of subcutaneously administered cadmium (Underwood, 1971). Also reported in this work is that cadmium uptake in rats was enhanced by small concentrations of zinc, but uptake was depressed at higher zinc concentrations.

Competition between the two metallic ions for the same binding sites on blood proteins might occur when a sufficient concentration of both ions was present to saturate the sites. Such competition could result in reduced absorption of zinc, the magnitude of the effect being dependent on the proportion of cadmium to zinc ions. This was demonstrated by Lease (1968) who reported that cadmium did not interfere with the uptake of 3 μg of zinc, when cadmium was administered simultaneously at about 5, 10 and 50 times that amount of zinc. This may lend support for Underwood's findings, that below a certain zinc concentration, cadmium shows no inhibitory effect. However, Lease also reported that cadmium did interfere with zinc metabolism when 18 or 27 μg of zinc were administered along with cadmium levels of about 5, 10 and 50 times those of zinc. Therefore, it appears that the level of zinc in an organism is a controlling factor on the inhibitory effects of cadmium. Also exerting a simultaneous but less dominant effect may be the ratio of zinc to cadmium ions.

Both of these points were demonstrated in the freshwater mussel, Margaritifera margaritifera. When 0.6 ppm cadmium was administered to test water which contained the mussels and different

concentrations of zinc ranging from 0.2-1.6 ppm no significant inhibition of zinc uptake occurred. However, when 2.0 ppm cadmium was administered to the same concentrations of zinc, a significant inhibition occurred in zinc uptake in the mussels for six of the seven zinc concentrations. The general trend showed that the greater inhibition occurred (82%) when the concentration of cadmium was 10 times higher than the zinc concentration, which was the largest difference studied. When the concentrations of zinc and cadmium were both 2.0 ppm, zinc uptake was inhibited about 22%.

This finding appears consistent with the hypothesis that the high levels of zinc found in shellfish may act as a protective mechanism successfully out-competing cadmium for enzyme binding sites until the cadmium concentration approaches and exceeds the zinc concentration. This study is the first reported demonstration of inhibition of zinc uptake by cadmium in any aquatic organism.

CONCLUSIONS

I have presented several possible routes for the entrance of heavy metals into the aquatic environment. Once these metals have been released, man is generally at the mercy of the aquatic organisms to transport, modify and biologically remove these pollutants from their environment.

To realistically evaluate the biological effects of heavy metals in streams, it is necessary to realize that natural concentrations of these metals occur widely in unpolluted streams. Likewise, all aquatic organisms possess some degree of tolerance to these metals. Different groups of animals vary in their resistance to metals when present in quantities above their minimum physiological demand. The freshwater molluscs, in general, are the least resistant to excesses of heavy metals in their environment (Wurtz, 1962). Therefore, molluscs would likely be the first animals eradicated when a stream became polluted with heavy metals. For this reason, these animals are of particular importance in field observations on streams where heavy metal pollution is suspected.

Molluscs in the natural environment appear to accumulate trace metals at different rates according to the environmental concentrations of the particular metal, the temperature, the duration of exposure and the species of shellfish, as well as the physiological activity of the

animal itself. The apparent selectivity of trace metals among various molluscan species appears to depend upon the availability of the metals in the environment, i. e., their chemical and physical properties, the number and kind of organic chelating agents available for attracting and binding metallic ions, their transport, and storage and the stability of the complex formed.

There are many individual physiological compartments within the animal each with its own turnover rate. The whole-body retention pattern follows a mean of these turnover rates. There appears to be a direct relationship between the uptake rate for a given metal and its retention for any molluscan species (Pringle et al., 1968).

When the environmental concentration of a particular toxic metal persists over a sufficient period of time, the animal may accumulate the metal to such an extent that the animal becomes physiologically nonfunctional and dies. The relative toxicity of a particular metal varies from species to species for any given concentration and identical conditions.

Because of the concentrating ability of shellfish, they should be closely monitored in areas of suspected heavy metal pollution. Not only could the bivalves themselves become intoxicated, but they could pass on the already concentrated metals in their tissues to consumers. Bivalves could be used to predict levels of heavy metals in the water and also in many species of fish which may have undetectable

concentrations of the metals. For example, as previously shown, shellfish generally possess C_f values of about 100,000 for the heavy metals studied in this thesis. If a cadmium concentration of 0.5 ppm was found in a typical bivalve, this could reflect a water concentration as low as 0.005 ppb cadmium, which is clearly undetectable by modern analytical techniques. Fish generally possess C_f values on the order of 1000. The same water concentration may provide a tissue concentration of 5 ppb cadmium which is on the borderline of statistically significant detection by modern methods. Therefore, the human intake from fish and bivalves could be monitored and the magnitude of the hazards determined.

It would be unreasonable to leave the problem of heavy metal contamination of marine and freshwater organisms without considering some of its far-reaching effects. The community intoxications reported earlier in this thesis should not be viewed as "someone else's problem" just because they were confined to relatively small geographical areas and the specific sources were identified and controlled. The important point is that the actual concentration of the metals in fish, shellfish and other food items responsible for these human deaths are only about 10 times higher than the concentration in similar food items over much of the northern hemisphere. This "safety factor" is much too small. However, the variety of foods

eaten, especially in the U.S., would tend to reduce drastically the intake of the heavy metals associated with fish and shellfish.

Human dependence upon fish and shellfish for food is increasing. Industrial uses and releases of heavy metals have increased drastically over the last several decades. Therefore, man may be headed in a collision course with heavily contaminated fish and shellfish as a significant source of his food. We have already observed the effect in Japan.

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APPENDIX

Appendix Table 1. Artificial stream water (Doudoroff, 1956).

NaCl	0.0058 g/l
$MgSO_4$	0.0185 g/l
K_2SO_4	0.0011 g/l
$CaHCO_3$	0.0175 g/l

Appendix Table 2. Uptake of $^{115m}CdCl_2$ (dpm/g) by the freshwater mussel during chronic exposure. Each value represents the mean of 15 animals ± one standard deviation.

Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)
1	18,625 ± 4,779	15	383,792 ± 67,994
2	38,632 8,326	19	487,386 93,862
3	74,182 15,699	23	569,175 107,444
4	98,092 17,723	27	617,292 116,194
6	162,250 34,241	31	657,698 131,285
8	198,872 38,137	36	714,658 137,395
10	256,014 47,336	41	763,380 143,533
12	319,002 57,710	45	770,736 146,195

Appendix Table 3. Uptake of $^{203}Hg(NO_3)_2$ (dpm/g) by the freshwater mussel during chronic exposure. Each value represents the mean of 11 animals ± one standard deviation.

Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)
2	3,073 ± 840	13	21,084 ± 6,856	27	49,429 ± 13,722
3	4,261 1,288	14	22,706 6,558	28	51,684 13,726
4	6,225 1,941	16	25,702 6,641	30	50,580 13,544
5	6,833 2,188	17	28,197 8,109	31	52,564 14,231
6	9,789 3,153	18	28,085 7,911	33	56,904 14,491
7	11,670 3,738	19	30,657 9,307	35	62,460 15,937
9	15,194 5,181	20	33,741 10,212	37	64,197 15,585
10	17,954 6,372	21	36,036 11,163	39	67,988 16,428
11	17,816 5,908	23	39,450 11,961		
12	19,960 6,758	25	46,523 12,959		

Appendix Table 4. Uptake of $CH_3{}^{203}HgCl$ (dpm/g) by the freshwater mussel during chronic exposure. Each value represents the mean of 11 animals ± one standard deviation.

Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)
1	9,047 ± 6,837	14	79,681 ± 29,908	31	145,376 ± 53,491
2	13,243 7,186	15	86,468 34,293	33	153,434 55,031
3	17,996 7,693	16	91,382 35,188	36	166,315 58,423
4	23,286 9,683	18	103,181 41,640	39	180,286 65,081
5	28,122 10,905	19	106,034 41,556	42	188,339 68,915
6	33,254 12,511	20	109,693 43,986	45	202,212 70,604
7	37,682 13,044	21	114,448 46,355	48	211,699 75,670
8	38,571 13,539	22	116,411 46,705	51	227,650 80,516
9	45,170 16,428	23	118,780 46,958	54	242,038 86,297
10	59,415 25,597	25	121,732 46,564	57	254,416 92,096
12	70,009 27,645	27	128,097 49,202		
13	74,376 28,566	29	134,497 50,387		

Appendix Table 5. Uptake of $^{65}ZnCl_2$ (dpm/g) by the freshwater mussel during chronic exposure to 64 ppb Zn^{+2} nominal. Each value represents the mean of 12 animals ± one standard deviation.

Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)	Time	Whole-body activity (dpm/g)
1	546 ± 55	19	7,545 ± 986	49	17,336 ± 2,322
2	1,127 85	23	9,172 1,229	53	17,897 2,393
3	1,824 140	30	11,972 1,972	58	18,220 2,371
5	2,651 269	34	12,751 1,681	63	18,286 2,792
7	3,595 369	38	14,395 1,948	68	19,719 2,788
11	4,891 530	42	15,134 2,031	73	18,891 2,481
15	6,422 818	46	16,363 2,345	78	20,458 2,896

Appendix Table 6. Uptake of $^{65}ZnCl_2$ (dpm/g) by the freshwater mussel during chronic exposure to 624 ppb Zn^{+2} nominal. Each value represents the mean of 15 animals ± one standard deviation.

Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)	Time (days)	Whole-body activity (dpm/g)
1	4,834 ± 1,292	19	53,414 ± 13,940	54	104,214 ± 25,448
2	9,825 2,389	23	61,180 15,076	58	107,270 25,505
3	13,792 3,161	27	66,091 15,562	62	110,255 25,796
5	21,551 5,494	31	74,515 18,704	66	116,651 29,306
7	27,330 7,128	35	76,870 18,659	73	127,291 30,753
9	33,760 8,651	39	84,278 19,584	80	128,322 31,580
12	38,821 9,854	43	86,350 20,120		
15	44,183 11,522	47	92,400 21,011		

Appendix Table 7. Retention of $^{115m}CdCl_2$ (% of original whole-body activity) by the freshwater mussel. Each value represents the mean of 14 animals ± one standard deviation.

Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity
0	100.0	22	86.48 ± 1.84	69	74.55 ± 3.55
1	96.82 ± 1.07	27	87.57 2.39	76	75.91 3.98
2	96.09 1.05	31	85.98 2.24	83	76.31 4.51
3	95.64 1.29	35	84.04 2.40	90	75.20 4.47
5	97.33 1.40	39	81.85 2.72	97	76.60 4.38
7	91.85 1.22	44	81.04 3.15	104	73.75 5.15
10	95.44 1.32	49	78.69 3.32	111	74.57 5.01
14	90.04 1.61	54	80.04 3.35	118	76.00 5.83
18	90.88 1.66	62	78.67 3.64	125	76.59 5.47

Appendix Table 8. Retention of $^{203}Hg(NO_3)_2$ (% of original whole-body activity) by the freshwater mussel. Each value represents the mean of eight animals ± one standard deviation.

Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity
0	100.0	52	77.65 ± 5.78	95	67.70 ± 6.13
7	90.43 ± 2.47	59	74.97 5.98	108	69.68 8.33
14	88.86 3.65	66	72.99 5.23	122	62.18 5.70
21	84.65 3.63	73	71.22 5.79	136	60.35 5.69
30	82.76 5.09	80	69.64 5.38	150	57.25 6.08
47	79.48 6.19	87	68.82 5.94		

Appendix Table 9. Retention of $CH_3{}^{203}HgCl$ (% of original whole-body activity) by the freshwater mussel. Each value represents the mean of 12 animals ± one standard deviation.

Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity
0	100.0	32	90.06 ± 3.33	107	87.37 ± 4.96
1	98.58 ± 1.18	39	90.14 3.20	112	86.54 5.26
2	97.99 1.36	50	91.84 3.43	119	85.87 5.41
3	97.02 1.87	57	91.19 3.51	126	85.32 5.64
4	97.03 1.57	62	88.74 4.47	133	84.55 5.96
6	95.40 2.05	67	88.71 3.75	140	87.60 6.41
8	93.16 2.39	72	87.91 3.91	147	82.62 6.69
11	96.08 1.97	76	86.36 4.34		
16	92.96 2.40	81	86.58 4.35		
21	91.42 2.81	85	85.35 4.67		
26	94.49 3.15	92	87.42 4.84		

Appendix Table 10. Retention of $^{65}ZnCl_2$ (% of original whole-body activity) by the freshwater mussel. Each value represents the mean of nine animals ± one standard deviation.

Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity	Time (days)	% of Original whole-body activity
0	100.0	13	83.22 ± 1.78	46	68.19 ± 2.56
1	97.71 ± 0.87	16	79.93 1.96	51	65.59 2.74
2	96.43 1.15	20	76.52 2.01	57	63.99 2.95
3	93.63 1.07	24	77.13 2.27	63	61.35 2.79
4	92.48 1.50	28	75.48 2.22	69	59.90 2.93
6	89.54 1.52	32	73.53 2.19	75	58.03 3.05
8	86.78 1.76	37	71.20 2.60	81	57.08 3.10
10	85.03 1.86	42	68.68 2.42		