NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

ACKNOWLEDGMENTS

I would like to acknowledge the help of the following people to whom I am indebted:

To my supervisor, Dr. Sami Qadri, who initiated this study and gave me much advice and assistance.

To Dr. A.S.W. de Freitas, for his encouragement and guidance throughout my research at the National Research Council of Canada.

In addition I would like to thank Dr. T. Moon for his helpful comments while serving on my advisory committee, Mrs. Eva Jaworsky for performing some of the mercury analyses, and Ms. Margaret Anne Gidney and Mr. Ken Trudel for technical assistance.

Finally I wish to thank the National Research Council of Canada for allowing me to utilize their facilities for part of the study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Uptake from both food and water of organic (methylmercuric chloride) and inorganic (mercuric chloride) mercury and its subsequent retention by the fresh water amphipod, Hyalella azteca (Saussure) were estimated by radiotracer techniques. To study uptake of mercury from food, the natural food items used were three species of algae, Scenedesmus quadricauda, Anabaena flos-aquae, and Navicula pelliculosa, and pulverized autumn maple leaves, as well as the artificial food, trout chow. The net assimilation of methylmercuric chloride ranged from 77 to 87% of the amount present in the ingested diet, compared to 3 to 17% for mercuric chloride. Clearance of methylmercuric chloride from the body tissues of Hyalella azteca was a first order process with a half life of 30 to 50 days, while that for mercuric chloride was 6 to 12 days. A positive relation was observed between dose rate and whole body retention. Clearance rate does not increase with temperature, over a range in temperature from 10 to 20°C.

An uptake rate of $\sim 0.4 \times 10^{-3}$ µg Hg per amphipod per hour was obtained at 20°C when the amphipods were exposed to water containing methylmercuric chloride at a concentration of 0.8×10^{-3} µg Hg/ml. A similar rate was observed for uptake of inorganic mercuric chloride. The uptake efficiency or bioavailability of mercury for transfer from water to the amphipod was independent of mercury concentration over the ranges 0.0008 to 0.014 µg Hg/ml for CH_3HgCl, and 0.0005 to 0.005 µg Hg/ml for $HgCl_2$. The uptake of mercury from the water

decreased by about 60% as temperature decreased from 20 to 10°C. Water quality affected direct uptake of methylmercuric chloride and mercuric chloride with uptake rates for both mercury compounds being lower in Ottawa River water than in dechlorinated tap water.

The experimental values for assimilation efficiency, uptake rate and elimination rate of both methylmercuric chloride and mercuric chloride, are used to explain levels of mercury found in amphipods in their natural environment.

RÉSUMÉ

L'absorption du mercure organique (chlorure de mercure méthylé) et inorganique (chlorure de mercure) contenu dans la nourriture et l'eau et sa rétention ultérieure par l'amphipode d'eau douce, *Hyalella azteca* (Saussure), ont été estimées par dépistage radioactif. Pour permettre l'étude de l'absorption du mercure contenu dans la nourriture, les aliments naturels utilisés furent trois espèces d'algues, *Scenedesmus quadricauda*, *Anabaena flos-aquae*, et *Navicula pelliculosa*, des feuilles d'érable, collectionnées en automne et pulvérisées, ainsi qu'un mélange de nourriture déséchée préparée spécialement pour les truites ("trout chow"). L'assimilation nette de chlorure de mercury méthylé a varié dans une proportion de 77 à 87% de la quantité présente dans la diète ingérée, et de 3 à 17% pour le chlorure de mercure. L'élimination du chlorure de mercure méthylé des tissus de *Hyalella azteca* a été un processus de premier ordre avec une demi-vie de 30 à 50 jours alors que celle du chlorure de mercure a été de 6 à 12 jours. Un rapport positif a été observé entre le taux de la dose et la rétention par le corps entier. Le taux d'élimination n'a pas augmenté avec la température entre 10° et 20°C.

Un taux d'absorption de - 0.4×10^{-3} µg Hg par amphipode par heure a été obtenu à 20°C, quand les amphipodes furent placés dans de l'eau contenant du chlorure de mercure méthylé à une concentration de 0.8×10^{-3} µg Hg/ml. Un taux inférieur de 50% a été observé pour

l'absorption du chlorure de mercure inorganique. L'efficacité de l'absorption ou la disponibilité biologique du mercure pour le transfert entre l'eau et l'amphipode était indépendante de la concentration du mercure variant de 0.0008 à 0.014 µg Hg/ml pour CH_3HgCl et 0.0005 à 0.005 µg Hg/ml pour $HgCl_2$. L'absorption du mercure de l'eau a diminué d'environ 60% lorsque la température passait de 20°C à 10°C. La qualité de l'eau a affecté l'absorption directe du chlorure de mercure méthylé et du chlorure de mercure. Les taux d'absorption pour les deux composés de mercure ont été inférieurs pour l'eau de la rivière des Outaouais, comparativement à celui de l'eau d'aqueduc déchlorinée.

Les valeurs expérimentales pour l'efficacité d'assimilation, les taux d'absorption et d'élimination du chlorure de mercure méthylé et du chlorure de mercure ont été utilisés pour expliquer les niveaux de mercure trouvés chez les amphipodes dans leur environnement naturel.

INTRODUCTION

The general tendency for lake and river sediments to act as a major sink for chemical pollutants is well documented. The processes of pollutant absorption/desorption indicate that most heavy metals, metaloids, and many organic zenobiotic compounds are tightly bound to sediments. The organic detrital material is usually associated with a disproportionately large fraction of the toxic compounds, particularly in the case of mercury pollutants. In this sense, sediments and the natural processes of floc formation and its aggregation and deposition act as an efficient decontamination mechanism for reducing the concentration of water born mercury and hence its availability for direct uptake from water by biota. Despite the fact that mercury is largely immobilized in the sediment and does not often exist in hazardous concentrations in solution, such 'locking up' of pollutants by sediment material may be less secure than the observed desorption rates indicate. Fish are able to obtain and to concentrate enough mercury to render them hazardous for food consumption in places where mercury has been released into the environment. The mercury found in fish is predominately methylmercury, whereas the mercury found in detritus is less than 1% methylmercury (Jernelov and Lann, 1971; Zitko et al., 1971). The contaminating methyl-mercury originates in the sediments by bacterial conversion of inorganic mercury to methylmercury. This methylmercury then finds its way into the water phase for uptake by aquatic

organisms, resulting in a food chain biomagnification process. Sediment bacteria and fungi as well as benthic invertebrate organisms derive a major part of their calories from sediment associated nutritive material. Benthic feeders have been shown to have a high mercury body burden, often higher than the sediment which they inhabit, (Hannerz, 1968; Jernelov and Lann, 1971; Bissonnette, 1977). Since such sediment-bound mercury could continue to be available for several years (Jernelov, 1969) it is very important to define the role of organisms dwelling in or upon the sediment, in mediating mercury flux.

The role of aquatic invertebrates in mercury ecodynamics is not at all well understood, particularly the benthic invertebrate community, which should be of paramount importance, since this community accounts for most of the animal biomass production in aquatic ecosystems. To help define the role of benthic invertebrates in mediating mercury flux, the following questions need to be investigated: in what form is mercury present in the biotic and abiotic detritus; how is mercury made available from the sediment for concentration by aquatic organisms; what forms of mercury do the organisms accumulate and subsequently concentrate; and at what rates do mercury uptake and depuration occur by the aquatic organisms.

Since mercury is a natural component element in aquatic environments, it can be assumed that its naturally synthesized derivative, methylmercury, has been present in

aquatic ecosystems for many millions of years, and it is rather surprising that its ubiquitous presence in aquatic biota was not established until the late 1960's (Westoo, 1967). Mercury is able to exist in the natural environment in contact with water in three oxidation states: as the metal itself, in the 1+ (mercurous) state, and in the 2+ (mercuric) state. The mixture of forms which will occur in a given assemblage, or predominate in solution, depends upon the redox potential and pH of the environment and upon the nature of the anions and other groups present with which mercury could form stable complexes. The solubility of metallic mercury in water had been determined as long ago as 1934 by Stock and is about 25 $\mu g\ l^{-1}$ at 25°C. In oxygenated water the solubility of mercury increases as $Hg(OH)_2$ forms in solution, and in chloride-rich acidic water it increases markedly as undissociated $HgCl_2$ forms. In most surface waters $Hg(OH)_2$ and $HgCl_2$ are the predominant species. At low redox potentials observed in reducing sediments, mercury is effectively immobilized by sulfide ion, due to its low solubility.

Mercury in the zero oxidation state in solution exhibits the property of being more soluble in hydrocarbons than in water (Reichardt and Bonhoeffer, 1930). Thus this form of mercury in solution is preferentially soluble in the lipid-rich cell membranes of living organisms. Hem (1970) has suggested that this facilitates removal of mercury from water by aquatic organisms and leads to more rapid accumulation

in their tissues. $HgCl_2$ is also soluble in organic solvents, although its solubility in aliphatic hydrocarbons is lower than that in water (Sidgewick, 1950).

When considering the chemistry of mercury, an important factor is its high affinity for sulfide sulfur. Many of the substances that constitute protoplasm contain sulfhydryl groups (e.g. proteins contain available free sulfhydryl groups through incorporation of the amino acid cysteine in their structures). The binding of mercury to sulfhydryl groups is probably basic to the toxicity of mercury compounds, since the result would be inhibition of enzyme action (Webb, 1966). Mercury also has a high affinity for other functional groups incorporated in proteins, such as the amino group ($-NH_2$). The large number of these groups increases the capacity of proteinaceous organic matter to bind mercury and may also increase the strength with which mercury is held.

A reaction of importance in the aquatic chemistry of mercury is that in which mercury becomes attached to organic groups through covalent bonds to carbon atoms. One example is methyl mercuric chloride ($CH_3Hg^+Cl^-$). This compound rapidly penetrates animal membranes due to its high lipid solubility. Methylmercury also binds tightly to tissues, particularly proteinaceous tissue (Brown and Kulkarni, 1967). Its bioaccumulation potential has led to levels in fish dangerous to man as well as to other piscivorous mammals and birds. Recent investigations have shown that

mono-methylmercury and di-methylmercury compounds can be synthesized from metallic, inorganic, and organo-mercurials, by sediment dwelling bacteria (Wood et al., 1968; Jensen and Jernelov, 1969; Jernelov, 1969; Fagerstrom and Jernelov, 1971; Landner, 1971; and Spangler et al., 1973). Methylmercury is therefore available for concentration by the biota regardless of the chemical form of mercury introduced to the aquatic system. Shin and Krenkel (1976) reported that microorganisms capable of methylating mercury do not seem to be rare in the natural environment because methylation is apparently a detoxification procedure. However, there are many species that are capable of degrading methylmercury. Such species may be very abundant, surpressing the accumulation of methylmercury in sediments (Spangler et al. 1973). High levels of methylmercury reported in fish have drawn attention to mercury biomagnification via the food chain: microbial flora - benthos - small benthophagous fish - predatory fish (Jarvenpaa et al., 1970). This food chain becomes contaminated by the methylmercury originating by bacterial conversion of inorganic mercury.

In general terms, benthic invertebrates can be expected to take up both inorganic and organic forms of mercury directly from the water and by ingestion of mercury contaminated detrital material and other food items. The availability of either form of mercury, from food and by direct uptake from the water, may be similar for different species, but the absolute rates of uptake should vary according to

physiological differences among species, and also on the type and amount of food eaten (de Freitas et al., 1977, Davies, 1978). One problem complicating mercury uptake, is the differences in membrane permeability that probably occurs among taxa. Luoma (1977), studying a polychaete worm and a shrimp, suggested that interspecies differences in bioconcentration and toxicity are strongly influenced by differences in physiological permeability. In field samples of various aquatic organisms, it is expected that the level of mercury in the organisms will vary from species to species, due to the differences in physiological structure and condition of the organisms. Thus for any given taxon the following need to be defined: the amount of mercury ingested as contaminated food, and its chemical form; the efficiency of assimilation of ingested mercury; the amount of mercury absorbed from the water; and the elimination rate for organic and inorganic mercury from tissues into the environment.

Uptake rate constants for inorganic and organic forms of mercury and whole body clearance rates have been reported for various species of fish (Jarvenpaa et al., 1970; Sharpe et al., 1977; de Freitas et al., 1977) and have been used to explain 1) the levels of mercury observed in wild populations of organisms (Fagerstrom and Asell, 1973; Norstrom et al., 1975) and 2) the relative magnitude of the food and water vector (de Freitas et al., 1974; Fagerstrom et al., 1974; Norstrom et al., 1976). For example, for fish, water concentrations below presently detectable levels of

0.01×10^{-3} µg Hg/ml. water, can account for methylmercury levels in fish tissue in excess of 0.5 ppm (de Freitas and Hart, 1977), a concentration that is often observed in nature (Lockhart et al. 1972). Benthic organisms also contain methylmercury, and the question arises then as to the similarity between invertebrates and fish in their ability to assimilate and eliminate mercury.

There are many factors that affect the availability of mercury for bioassimilation from water, clearance and food chain transfer. These control factors can be divided into two groups: biological factors intrinsic to the organism, and physical factors external to the organism (de Freitas, 1977). Mercury uptake from food can be affected by external factors such as mercury concentration in the food, chemical form of mercury in the food, food type, food availability and water temperature. Intrinsic factors would include food consumption, assimilation efficiency of the ingested mercury, metabolic rate and voidance time. External factors controlling mercury uptake from water would be the assimilation efficiency of mercury from water, pollutant concentration in the water, chemical form of mercury in the water, and water quality parameters such as pH, hardness, suspended or dissolved material, and temperature related factors which affect the organism's growth and metabolic rate.

One important taxonomic group of benthic invertebrates is the Class Crustacea. Corner and Sparrow (1957) and Hannerz (1968) have shown that mercury compounds do enter a

crustacean and that methylmercury enters faster than inorganic mercury. The cuticle (exoskeleton) of crustaceans has many properties in common with insects, being basically a chitin-protein structure, stiffened and hardened by deposition of calcium salts (Guarino et al. 1976). This cuticle is lipophilic and readily allows methylmercuric chloride to cross the biological membrane (Hughes, 1957). In addition, Corner and Sparrow (1957) showed methylmercuric chloride to be 4 to 15 times more toxic than mercuric chloride in crustacea, due at least in part to the higher assimilation efficiency of methylmercury compound relative to that of inorganic mercury.

This study examines the role of the benthic crustacean, Hyalella azteca (Order Amphipoda) in the mobilization and transport of mercury from sediments. Hyalella azteca is widely found in shallow, freshwater lakes and rivers throughout North and Central America (Pennak, 1953; Bousfield, 1973). It is most abundant in shallow-water sediment, living on aquatic vegetation or buried in organic debris to a depth of 2 cm. (Jackson, 1912; Bovee, 1949; Cooper, 1965; Hargrave, 1970). Laboratory experiments have shown that Hyalella is a discriminate particle feeder, digesting bacteria and algae from ingested sediment particles (Hargrave, 1970).

Hyalella azteca is 6 to 8 mm. in length with a dry weight of approximately 700 µg. The head bears two pair of antennae, a pair of mandibles, two pair of maxillae and one pair of maxillipeds. Epiphytic algae are scraped off aquatic plants and other surfaces, and transferred via mandibles and

maxillipeds to the mouth. The digestive system is composed of a foregut, midgut, and hindgut. The foregut consists of a cardiac or chewing stomach located in the anterior peraeon connected to the mouth by a short esophagus. From the anterior end of the midgut, directed posteriorly, are two elongate, tube shaped digestive caeca and a short anteriorly directed caecum. The hindgut is a short straight tube emptying via the anus (Bousfield 1973). Respiration takes place partly through the body surface but mainly through the thin walls of the coxal gills located on the inner bases of the walking limbs. Action of the pleopods maintains a steady circulation of oxygenated water over the gills.

The life cycle of Hyalella can be divided into an immature stage (consisting of 5 instars), a juvenile stage (including instars 6 and 7) and an adult stage (the eighth instar and older). Adult females are capable of producing broods of young (ranging from 1 to 50 amphipods at every moult following the onset of reproduction). Eggs are carried in a thoracic brood pouch. Newly hatched amphipods resemble the parents and do not undergo a larval metamorphosis. Duration of development and hatching of eggs varies, but largely depends on temperature.

This study attempts to quantify the assimilation efficiency of mercury (organic and inorganic) ingested as contaminated food, the rate of uptake of organic and inorganic mercury from the water and elimination rates for organic and inorganic mercury from the tissues of Hyalella into the

environment. The effect of some control factors such as temperature and water quality are also examined. With such parameters defined, attempts can be made to quantitatively define the bioaccumulation potential of both methyl and inorganic mercury by the amphipod in its natural environment.

The experimental protocols involve the use of ^{203}Hg labelled mercury compounds. Mercury-203 is a gamma emitting isotope and this allows easy measurements of the amount of mercury taken up by the amphipod from contaminated food and water and the amount retained in subsequent periods is estimated by doing whole body ^{203}Hg counts of live amphipods. The live amphipod can be quickly radioassayed and then replaced into the exposure or clearance situation for subsequent assay. The radioisotope also allows the use of low experimental levels of mercury, approaching those encountered in the environment.

MATERIALS AND METHODS

General Procedures

Maintenance of experimental animals

The amphipods, Hyalella azteca, were obtained from Carolina Biological Supplies[1]. They were maintained in a 10 gallon aquarium half-filled with dechlorinated tap water. This constantly aerated aquarium contained sediment (approximately 1 cm. deep) from the Ottawa River and the aquatic plants of the genera Myriophyllum, Elodea, and Lemna. Approximately half of the water in the aquarium was removed every 2 weeks and fresh dechlorinated tap water added. The system was maintained on a 14:10 hour light:dark cycle at a water temperature of 19±1°C. In all of the experiments the head length of the amphipods was measured to minimize differences in results due to body size, but amphipods were not separated by their stage of intermoult. Wildish and Zitko (1971) found that the stage of intermoult made no difference to uptake rate of PCB in amphipods.

Handling of mercury-203 labelled compounds

Mercury-203 labelled compounds were purchased from New England Nuclear[2] as aqueous solutions of methylmercuric chloride and mercuric chloride, with specific activities at time of purchase of 2.7 to 5.2 mC/mg and 4 mC/mg mercury respectively.

---

[1]Carolina Biological Supply Company, Burling, North Carolina

[2]575 Albany Street, Boston, Massachusetts, 02118, U.S.A.

Immediately following delivery, the mercury-203 labelled material was diluted with distilled water. The diluted N.E.N. stock was stored in 10 ml volumes, in 15 ml vacutainer tubes[1], for easy subsampling with disposable syringes when required for experimentation.

The initial concentration of radioactivity in the 10 ml volumes of diluted N.E.N. stock was usually 20 uC/ml. At this dilution of the original radioactivity, methylmercury does not undergo radiation induced chemical breakdown. The diluted N.E.N. stock of methylmercuric chloride can be used for up to six months after purchase without a significant increase in the level of mercury-203 labelled impurities (Sharpe et al., 1977).

Standardization of radioactivity measurements

The counting efficiency of the deep well scintillation counter[2] was determined by the use of a standard consisting of an accurately measured quantity of a mercury-203 labelled mercuric chloride solution in a sealed glass ampoule within a standard scintillation vial. This standard was used to determine the day to day variations in counting efficiency observed during the extended periods of measurements.

---

[1]Evacuated glass tubes, 127×16 mm., Becton-Dickinson, Division of Becton, Dickinson and Company, Rutherford, New Jersey.

[2]Ino-tech 5100 multichannel analyzer coupled to a 2 in. diameter NaI doughnut crystal detection unit. Counting efficiency for Hg-203 was approximately 40%.

Chemical fractionation of mercury

The purity of the diluted N.E.N. stock of methylmercuric chloride was tested from time to time using a modification of the Westoo procedure for rapid determination of methylmercury salts in fish tissue and other samples (Westoo 1967, 1968).

For a 10 ml sample of aqueous exposure medium, the following procedure was used for fractionation of organic (methyl) mercury and inorganic mercury. The 10 ml sample was placed in a 50 ml stoppered graduated cylinder. An aliquot (0.25 ml) of each of 1000 µg/ml solutions of methylmercuric chloride and mercuric chloride were added as carrier material. Sodium chloride (2.0 g), concentrated hydrochloric acid (3.5 ml), and benzene (20 ml) were then added and the mixture was stoppered and shaken for 30 sec. The mixture was then allowed to stand until the phases separated. The benzene layer was removed and placed in a 100 ml graduated cylinder. The benzene extraction was repeated two more times. Each time the benzene fraction was transferred to the 100 ml graduated cylinder. The final volumes of the aqueous phase (inorganic mercury) and benzene phase (organic mercury) were then recorded. Five ml of each phase were then placed in separate scintillation vials and the radioactivity levels in each were assayed using a deep well scintillation counter. The relative amounts of organic and inorganic mercury in the sample were then calculated.

A similar procedure was used for fractionating the organic and inorganic mercury in the amphipods or mercury-contaminated food used. The amphipod was first homogenized in a modified 15 ml glass centrifuge tube. The bottom of the tube had been ground and a glass rod ground to fit it. If the foods were being analyzed, approximately 10 mg wet weight of the food was added directly to the centrifuge tube, but the food was not ground or homogenized. To the centrifuge tube the following were added: 2 ml distilled water, 2 ml benzene, 0.25 ml each of methylmercuric chloride (1000 µg Hg/ml) and mercuric chloride (1000 µg Hg/ml), 0.7 ml concentrated HCl and 0.4 g NaCl. The contents were mixed on a vortex mixer for 30 sec, then centrifuged. The benzene layer was removed and placed in a scintillation vial and the benzene extraction repeated twice. The entire benzene fraction and aqueous fraction (each in separate scintillation vials) were then assayed for their radioactivity.

Mercury dosing procedures

Uptake from food

Two forms of mercury were used in the feeding experiments: the organic form, methylmercuric chloride, and the inorganic form, mercuric chloride. Foods contaminated with mercury were the green alga, Scenedesmus quadricauda (Indiana University Collection[1] #77), the bluegreen alga,

[1]Culture Collection of Algae, Dept. of Botany, Indiana University, Bloomington, Indiana. In the body of the thesis and in tables and figures, only the generic names for the algae and diatoms are used.

Anabaena flos-aquae, (I.U. #1444), the diatom, Navicula pelliculosa, (I.U. #668), air dried maple leaves and Purina trout chow[2]. Trout chow has been used in other experiments to feed Daphnia, mussels and minnows (Terhaar et al. 1977).

Prior to addition of the mercury-203 labelled solutions, the food was found to contain approximately the following levels of total mercury (on a dry weight basis): algae (consisting of a mixture of Scenedesmus, Anabaena and Navicula) 315 ppb; trout chow 49.5 ppb, and maple leaves 298 ppb. An adaptation of the Magos (1971) procedure for selective atomic absorption determination of mercury, as described by Norstrom and Peter (1972), was used for this analysis. These background levels are less than 5% of the mercury levels in the contaminated foods used in the feeding experiments (see Results).

To prepare the amphipods for a feeding experiment, twenty-four hours prior to an experiment, 15 to 30 amphipods were removed from their aquarium and placed in a 2 l. glass beaker containing 1800 ml dechlorinated tap water and the uncontaminated version of the diet to be used in the experiment. After this twenty-four hour acclimation period, the amphipods were placed in another 2 l. glass beaker containing 1800 ml dechlorinated tap water and were starved for 2 hours.

---

[2]Ralston Purina Co., General Offices, St. Louis, Mo. 63188. Analysis of Purina Trout Chow fish feed size #3, guaranteed by Purina: crude protein 40%, crude fat 8%, crude fiber 5%, ash 10%, added minerals 1%, carbohydrate (by difference) 30-35%.

Mercury contaminated food was prepared by adding approximately 35 mg (dry weight) of the food (live algae, finely ground trout chow (15 µm to 75 µm) or pulverized leaves) to a 20 ml glass test tube containing dechlorinated tap water and the mercury-203 labelled methylmercuric chloride or mercuric chloride. The tube was then shaken for 2 hours, centrifuged, the water layer decanted off, and fresh dechlorinated tap water added. The tube was then mixed for 30 sec on a vortex mixer and centrifuged again. Several similar washes were performed until stability of the mercury remaining on the food was established (see Results). The food and final rinse water were then filtered on Whatman #1 filter paper, the food scraped off and added in clumps to the beaker containing the amphipods. Samples of all foods used were examined microscopically for any abnormalities or rupturing that may have occurred during contamination or washing. An excess of food (approximately 30 times the amount required) was added. The concentration of mercury in the algae was estimated by radioactive assay of ^{203}Hg before addition of the contaminated food to the feeding beaker.

In order to correct for any uptake of mercury by the amphipods from the water, a 200 ml sample of water in the feeding exposure beaker was removed by pipet halfway through the exposure period and was centrifuged to remove any food particles. Three amphipods which had been acclimated with the amphipods used in the feeding experiment, were then added to it for the same length of time as exposure to the food.

Depending on the particular experiment, exposure to the food varied from 40 min. to 24 hours. At the end of the exposure period the amphipods were removed from the feeding beaker by a modified 5 ml pipet, which had the tip removed allowing an opening large enough through which an amphipod could easily pass. The amphipods were rinsed by transferral through three separate beakers containing 150 ml dechlorinated tap water (total time: 30 sec.) and then placed in separate beakers each containing another 150 ml dechlorinated tap water for a period of 6 1/2 min. At the end of this time the amphipods were transferred to individual glass counting vials along with 1 ml of water and were then assayed for their whole body content of Hg-203 in a deep well scintillation counter. For the study of subsequent whole body clearance of the mercury, the amphipods were then transferred to 2 l. beakers containing 1800 ml dechlorinated tap water, with 2 amphipods per beaker. Measurements of the Hg-203 in the amphipods were taken during clearance at post-exposure times of 2, 4, 8, 24 hours and then once daily for a maximum of 4 weeks. Water was changed in the clearance beakers each time the amphipods were assayed. Any moults found during clearance were also assayed for their Hg-203 content. During the clearance period, the amphipods were fed the same diet (but uncontaminated with Hg-203) as used in the experiment. Water temperature during all feeding experiments and subsequent clearance periods was maintained at 19±1°C except when the effect of temperature on clearance of methylmercuric chloride was being investigated.

Uptake from water

Dechlorinated tap water was used in all water uptake experiments, except for the study on the effect of water quality where unfiltered Ottawa River water was also used. Mercury contaminated water was prepared by adding a measured portion of the stock solution of mercury-203 labelled methylmercuric chloride (approximately 4 µg Hg/ml) or mercuric chloride (approximately 5 µg Hg/ml) to a glass beaker containing 1800 ml dechlorinated tap water. The water was stirred by a magnetic stirrer for 60 sec., and then left to stand for 2 hours before addition of the amphipods. All amphipods were removed from their culture tank just prior to their addition to the beaker containing contaminated water. Eight to ten amphipods were added to each exposure beaker and were exposed for 2 hours to the water, except when uptake over long periods of time was being studied. Throughout this exposure period the concentration of total mercury in the exposure water was monitored by measuring the amount of radioactivity in aliquots of the exposure water, using the Inotech 5100 multichannel analyzer system. To each of these aliquots (usually 10 ml), 0.25 ml each of methylmercuric chloride (1000 µg Hg/ml) and mercuric chloride (1000 µg Hg/ml) was added immediately after removal from the exposure beaker. The samples were counted, 1 ml of benzene was added, and the samples were then frozen and later fractionated to determine the chemical form of mercury present. At the end of the exposure period, the amphipods were removed from the

contaminated water by a modified 5 ml glass pipet, rinsed and assayed for their whole body content of mercury-203 as described in detail for the feeding experiments. Water temperature was maintained at 19±1°C during the uptake experiments and subsequent clearance periods except when studying the effect of temperature on uptake and clearance.

Calculation of mercury concentration per gram dry weight of food

A small sample of the contaminated food was assayed for its Hg-203 content immediately before each feeding experiment. It was then placed on a small, tared piece of aluminum foil, placed for 24 hours in a 40°C oven, then weighed on a Cahn Electrobalance Model G2.

Data processing

All of the individual whole body measurements of live organisms taken during the post-exposure clearance period of either water or food uptake were expressed as percent activity remaining. First order kinetics in the clearance of mercury from amphipod tissue was assumed, and the data for the second slow clearing part of the curve was treated as an exponential curve described by the general equation $y=ae^{-bt}$, where y is the body burden of ingested mercury, a is compartment size, t is the time, in days of clearance, and b is the fractional clearance rate per day. The biological half life, or the time required for half of the accumulated tissue mercury to be lost from the organism as a result of biological processes, was determined by $\frac{\ln 2}{b}$

with the units being days. The data points were fitted to a straight line by least squares linear regression (Cunningham and Tripp, 1975). For a specific example of these calculations, see Table 1 and Fig. 1. These numbers represent clearance of CH_3HgCl from one amphipod, following a 40 min. ingestion period of $CH_3{}^{203}HgCl$ contaminated Scenedesmus.

Calculations involving the second, slow clearing compartment start at 1.2 hours post-exposure. The correlation coefficient of the least squares line that best fits the data is given by the equation: $r = \frac{\Sigma XY - \Sigma X \Sigma Y/n}{\sqrt{[\Sigma X^2-(\Sigma X)^2/n][\Sigma Y^2-(\Sigma Y)^2/n]}}$

where X is the time, post-exposure, in hours, and Y is the ln corrected cpm. r for this specific example is 0.901. The slope, or fractional clearance rate, is calculated by:

$b = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\Sigma X^2-(\Sigma X)^2/n}$ and is 0.00105 h^{-1} or 0.0252 day^{-1}. $T_{\frac{1}{2}}$ calculated by $\frac{\ln 2}{b}$, is $\frac{0.693}{0.0252}$ = 27.5 days. The size of the slow clearing compartment is given by the y-intercept, and is defined by the equation $a = \frac{\Sigma Y - b\Sigma X}{n}$ and is 380.7 cpm. Assimilation efficiency of the ingested CH_3HgCl is calculated by: $\frac{a}{c} \times 100$ where a is the size of the slow clearing compartment (cpm), and c is the mercury body burden at the end of the exposure period. In this case, assimilation efficiency of CH_3HgCl is $\frac{380.7}{465.2} \times 100 = 81.8\%$.

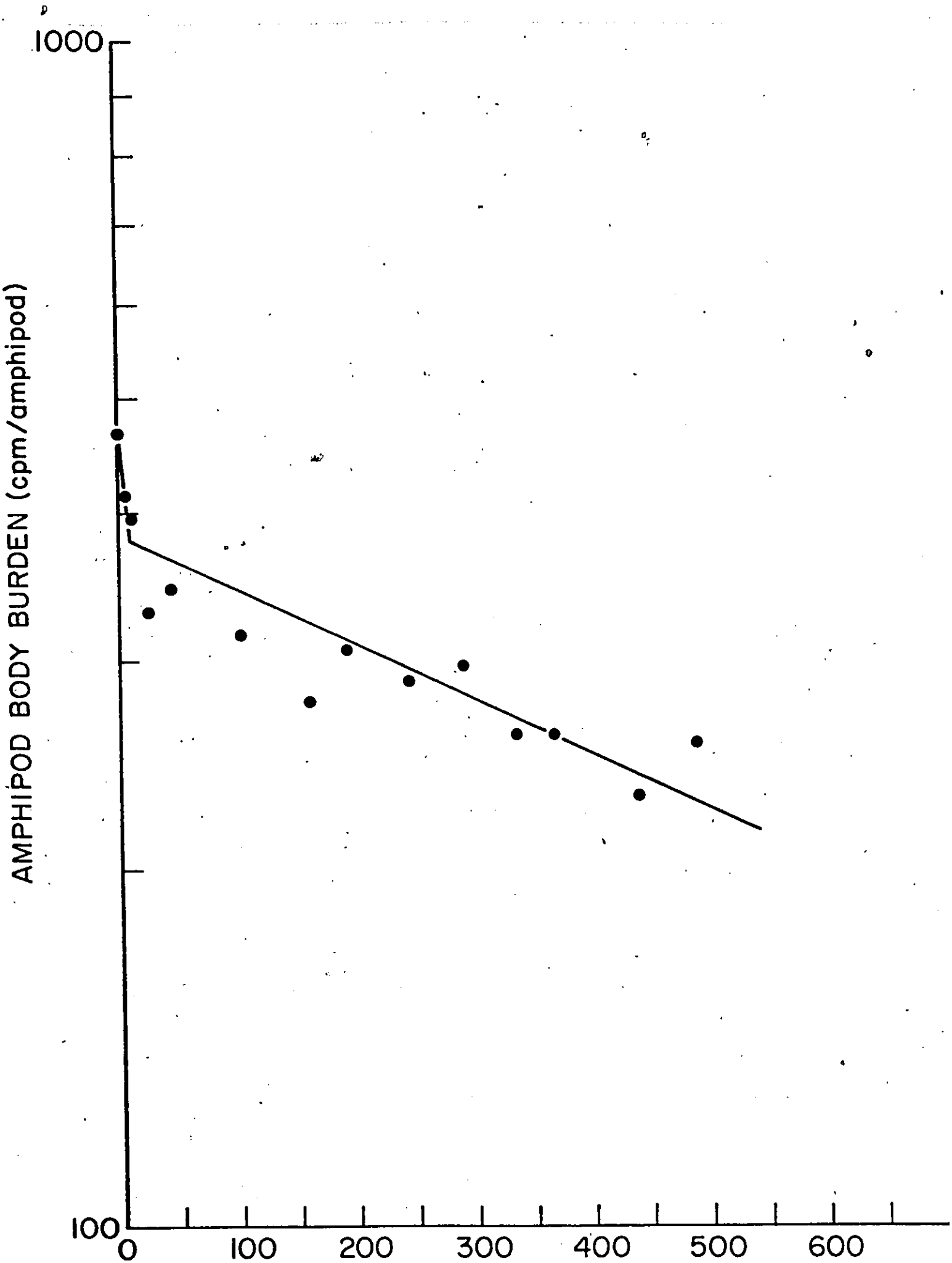
In studying uptake of CH_3HgCl and $HgCl_2$ from water by the amphipod, the term transfer coefficient is used. As

Table 1. An example to demonstrate the calculation of $T_{\frac{1}{2}}$ for CH_3HgCl following a 40 minute ingestion period of $CH_3{}^{203}HgCl$ - contaminated Scenedesmus; by Hyalella azteca.[1]

Time Post-exposure (hours)	Amphipod Body Burden of Hg-203 measured cpm	cpm corrected for radioactive decay	ln corrected cpm
0.0	465.2		6.14236
1.2	436.7		6.07925
2.3	412.2		6.02151
5.1	412.2		6.02151
9.6	395.4		5.9799
24.0	324.3	329.1	5.79636
45.6	335.0	345.0	5.84354
101.4	297.2	315.0	5.75257
148.1	253.2	276.8	5.6233
193.2	272.4	306.5	5.72522
244.3	249.0	288.6	5.66504
289.8	245.6	297.3	5.69474
336.8	211.4	259.7	5.55953
367.3	204.6	259.0	5.55683
439.8	174.6	231.0	5.44242
489.8	187.5	255.6	5.54361

[1]Water temperature during ingestion and clearance was 19±1°C. Specific activity of $CH_3{}^{203}HgCl$ was 2.0×10^5 cpm/µg Hg.

Fig. 1. An example illustrating clearance of CH_3HgCl from Hyalella azteca, following a 40 minute ingestion period of $CH_3{}^{203}HgCl$ contaminated Scenedesmus.


1000
100
AMPHIPOD BODY BURDEN (cpm/amphipod)
0
100
200
300
400
500
600

used in this text, transfer coefficient, (T_c), is defined as the amount of water (g) cleared of its mercury content by 1 g of amphipods in 1 hour. The following is an example of the calculation of the transfer coefficient for CH_3HgCl from the water to the amphipods at 19±1°C following a 2 hour exposure period (see Table 2).

$$T_c = \frac{1400 \text{ cpm/amphipod}}{13000 \text{ cpm/g water}} \times 200 \text{ amphipods/g} \div 2 \text{ hours}$$

$$= 10.8 \text{ h}^{-1}$$

All transfer coefficient values reported in the text (Tables 14, 15 and 16) were calculated on the basis of mercury body burden values obtained by extrapolating the clearance curve for mercury, measured during the post exposure period, back to zero time post exposure. This procedure for determining the mercury body burden results in the correct correspondence of uptake and clearance rate coefficients for use in determining the amount of mercury passing through the organism and the concentration of mercury in the body tissues of the organism.

Table 2. An example to demonstrate the calculation of transfer coefficient of CH_3HgCl, from the water to the amphipod.

Amphipod Number	Amphipod Body Burden[1] of CH_3HgCl at end of exposure period[2] (cpm)/Amphipod
1	1533
2	1500
3	1258
4	1727
5	1112
6	1848
7	1442
8	792
	$\tilde{x}$ ± S.E., 1400 ± 120

[1]Specific activity of CH_3HgCl was 2.74×10^6 cpm/µg Hg, and the level of radioactivity in exposure medium was 13000 cpm/ml dechlorinated tap water.

[2]Amphipods were exposed for a period of 2 h.

Specific Procedures

1. Determination of voidance time

The voidance time of Hyalella azteca has been estimated to be approximately 30 min. (Hargrave, 1970). To determine voidance time under the experimental conditions being used here, a feeding experiment using methylmercuric chloride contaminated trout chow was performed using 20, 40 and 60 min. exposure periods. The amount of contaminated food ingested by the amphipods, based on total body content of ^{203}Hg, was found to be linear between 20 and 40 min., but dropped off between 40 and 60 min., indicating that some of the mercury contaminated food was being egested during this period (see Results). In all of the following feeding experiments, exposure periods of 40 min. or less are used to estimate accurately assimilation efficiency of the mercury compounds.

2. The effect of food type on CH_3HgCl and $HgCl_2$ assimilation efficiency from the gastrointestinal tract of Hyalella azteca

Table 3 shows the exposure periods used, the number of amphipods per exposure situation, and the concentration of CH_3HgCl or $HgCl_2$ per µg dry weight of food, used in each feeding experiment.

3. The effect of temperature on clearance of CH_3HgCl following ingestion of $CH_3{}^{203}HgCl$ contaminated autumn maple leaves.

Thirty-five amphipods were removed from the culture aquarium and placed in three 2 l. glass beakers

Table 3. Mercury concentration in food types used to determine the effect of chemical form of mercury and food type on the assimilation efficiency of mercury from the gastrointestinal tract of Hyalella azteca.[1]

Chemical Form of Mercury	Experiment Number	Food Type	Mercury Concentration in Food (ng Hg/µg dry weight food)	Number of amphipods in each exposure period			
				20 min.	40 min.	4h	24h
CH_3HgCl [2]	1	Scenedesmus	0.34	10	10		
	2	Anabaena	0.34	12	10		
	3	Navicula	0.75	11	11		
	4	Trout chow	0.003		10	12	12
	5	Maple leaves	0.003		7	7	5
$HgCl_2$ [3]	6	Scenedesmus	0.09		15		
	7	Anabaena	0.27		15		
	8	Navicula	0.16		15		
	9	Trout chow	0.01		14		

[1] All experiments were performed at 19±1°C.

[2] Specific activity of $CH_3{}^{203}HgCl$ was approximately 2.0×10^5 cpm/µg Hg for experiments 1, 2 and 3. For experiments 4 and 5 the specific activity was 6×10^6 cpm/µg Hg.

[3] Specific activity of $^{203}HgCl_2$ was approximately 2.4×10^6 cpm/µg Hg.

containing 1800 ml dechlorinated tap water, as follows: beaker 1 and 3 - 10 amphipods each, beaker 2 - 15 amphipods. Beaker 1 was left at room temperature (19±1°C). Beakers 2 and 3 were placed in water baths and the water temperature in each was lowered 1°C per day until beaker 2 was at 15°C and beaker 3 at 10°C. The beakers were then held at these temperatures for 1 week before the feeding experiment was performed. During this acclimation period, pieces of Myriophyllum were placed in each beaker as well as pulverized autumn maple leaves. Water was changed once daily.

The feeding experiment was performed as described previously. As ingestion rate decreases with decreasing temperature, a 3 hour exposure period was used to allow the amphipods to accumulate enough Hg-203 to be assayed. Subsequent clearance was followed at the same water temperature as used in the exposure. A feeding experiment was also performed at 5°C, but the ingestion rate of the amphipods was too low to allow accumulation of measurable amounts of Hg-203.

4. Effect of temperature on uptake of CH_3HgCl from dechlorinated tap water.

Thirty amphipods were removed from the culture aquarium, placed in three 2 l. glass beakers (10 amphipods/ beaker), and acclimated to 5, 10 and 20°C as described in the previous section. To prepare the exposure media, a 1 l. beaker containing 500 ml dechlorinated tap water was placed in each water bath (5 and 10°C) and at room temperature (19±1°C). To each beaker 1 ml of diluted N.E.N. CH_3HgCl

stock (2.7×10^6 cpm/µg Hg) was added. The resulting concentration of mercury in each beaker was as follows: 5°C - 0.047 µg Hg/ml; 10°C - 0.045 µg Hg/ml; and 20°C - 0.053 µg Hg/ml. The amphipods were exposed for 2 hours.

5. Effect of water quality on direct uptake of CH_3HgCl and $HgCl_2$ by Hyalella azteca from water.

Dechlorinated tap water and unfiltered Ottawa River water were used in this experiment. Surface water from the Ottawa River was collected 24 hours prior to the experiment from the middle of the channel formed between Kettle Island and the Ontario shore. Two different concentrations of each mercury form were used to study uptake from each water type. All amphipods were exposed for 2 hours with 10 amphipods per exposure beaker. Water temperature was 19±1°C. Mercury clearance was followed for 24 hours post-exposure. This clearance was performed in the same type of water as used in the exposure, with the water being replaced at 2, 4 and 8 hours post-exposure.

6. Effect of different concentrations of CH_3HgCl and $HgCl_2$ on uptake from dechlorinated tap water by Hyalella azteca.

Three different concentrations of each mercury form were used (see Results). All amphipods were exposed for 2 hours with 10 amphipods per each CH_3HgCl concentration and 8 amphipods per each $HgCl_2$ concentration. Water temperature was maintained at 19±1°C. Subsequent clearance of mercury was followed for 24 hours.

RESULTS AND DISCUSSION

Uptake of mercury from food

When contaminating the various food types, stability of the $CH_3{}^{203}HgCl$ and $^{203}HgCl_2$ on each was determined. Each food type was rinsed until only 2% or less of the total cpm added, were being removed from the food. The number of rinses required to achieve this level are shown in Table 4. Samples of each algal type were examined microscopically after the final rinse. Algal cells were found to be intact, and clear supernatants were obtained in all of the final washes with each food type.

All whole body retention data from amphipods exposed to a single dose of ingested mercury, either organic or inorganic, demonstrated two compartment clearance. Two compartment clearance has also been shown for several species of fish by many other workers (Jarvenpaa et al. 1970; Giblin and Massaro, 1973; Weisbart, 1973; and Suzuki and Hatanaka, 1975), for the oyster (Cunningham and Tripp, 1973), and for the shrimp and polychaete worm (Luoma, 1977). Figure 2 represents a typical result from a single ingested dose (40 min. dosing period) of food (Scenedesmus) contaminated with either methylmercuric chloride or mercuric chloride. The initial post dose series of whole body measurements defining the first, fast clearing compartment coincides with the voidance time of the gastrointestinal tract and represents clearance of that portion of the ingested dose

Table 4. Stability of mercury on the different food types following exposure of the foods to CH_3HgCl and $HgCl_2$.

Chemical Form of Mercury	Food Type	Exposure Time of Food Types to Mercury (hours)	% of Total Counts Remaining on the food item					
			After Expos. Period[2]	After Rinse #1	After Rinse #2	After Rinse #3	Rinse #4	Rinse[1] #5
CH_3HgCl	Scenedesmus	1.5	90.4	88.8	88.0	87.4	86.9	86.3
		3.0	91.1	88.7	87.2	86.0	84.4	82.7
	Anabaena	1.5	89.4	87.0	85.7	85.1	84.2	83.3
		3.0	90.3	88.9	88.3	88.0	87.2	86.7
	Navicula	1.0	85.3	73.7	67.9	63.8	60.8	57.9
		2.0	73.8	69.1	65.0	62.1	59.2	55.3
	Trout chow	0.5	82.8	72.8	69.0	66.6	63.9	61.7
		1.0	87.4	80.5	77.2	75.1	73.3	71.7
	Maple leaves	2.0	94.1	92.9	92.3	91.9	91.5	91.2
$HgCl_2$	Scenedesmus	1.5	77.0	66.7	59.5	55.6	52.7	50.6
		3.0	72.9	69.8	63.2	59.0	53.7	50.5
	Anabaena	1.5	73.4	69.4	66.9	65.0	63.3	62.7
		3.0	83.6	79.9	79.0	77.7	77.1	76.7
	Navicula	1.5	54.3	43.3	37.5	35.0	31.6	29.7
		3.0	53.9	45.9	42.5	40.0	38.3	36.7
	Trout chow	1.0	88.5	83.2	78.2	74.4	70.1	68.3
		2.0	87.7	80.0	76.3	73.4	70.1	68.3
	Maple leaves	2.0	46.4	41.3	36.6	34.7	32.9	31.9

[1]After the final rinse, all food types were examined with a microscope. Cells were intact and clear supernatants were obtained after the final rinse.

[2]Values for the filtered food items before the food was rinsed with dechlorinated tap water.

of mercury not absorbed by the intestine. The second, slow clearing compartment represents the proportion of the ingested dose that remains associated with body tissues and thus its size relative to the size of the ingested dose is an effective measure of net assimilation efficiency. As stated earlier, this net assimilation efficiency can be calculated by extrapolating the y-intercept of the slow curve back to zero clearance time. In the case of methylmercuric chloride, the clearance curve (Fig. 2) demonstrates that about 25% of the ingested dose is rapidly lost and the remaining 75% of the ingested material becomes associated with body tissues and is lost at a very slow rate. Contrastingly, inorganic mercury is assimilated with only about 10% efficiency. These results seem to be independent of food type (Table 5). One exception to this is trout chow. A possible explanation for this low assimilation efficiency may be the high content of animal protein compared to the other natural plant foods used (see footnote p. 15). The mercury in the trout chow was fractionated prior to the feeding exposure and was found to be 97% organic mercury (Table 6). The natural levels of mercury occurring in the different food types were less than 2% of the levels used in the experiments (Table 7) and thus should not affect the results significantly.

Fractionation of the mercury in four amphipods from the trout chow feeding experiment gave only a 51.4% organic mercury content after 8 days in clearance (Table 8).

Fig. 2. The effect of chemical form of mercury on its assimilation efficiency from the gastrointestinal tract into body tissue of Hyalella azteca after ingestion of mercury contaminated Scenedesmus (40 min. exposure period).

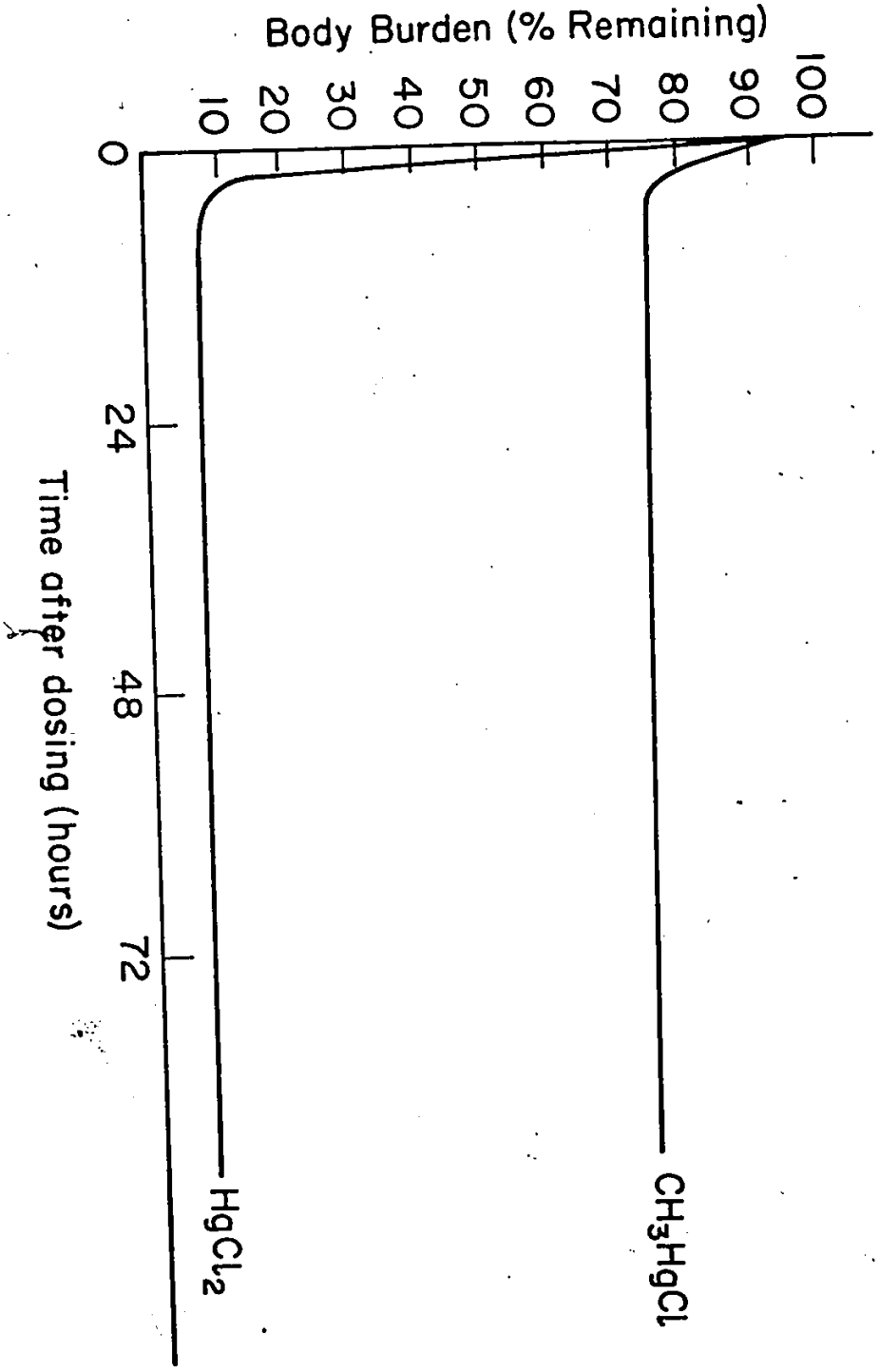

Body Burden (% Remaining)
0
10
20
30
40
50
60
70
80
90
100
Time after dosing (hours)
24
48
72
$HgCl_2$
CH_3HgCl

Table 5. Effect of food type on mercury assimilation from the gastrointestinal tract of *Hyalella azteca*

Food Type	Ingestion Period (min.)	Ingested Mercury[2] (ng Hg/amphipod)		Assimilation Efficiency[2] (%)	
		CH_3HgCl	$HgCl_2$	CH_3HgCl	$HgCl_2$
Scenedesmus	40	1.56±0.30(7)[1]	1.44±0.30(11)	77.2±2.4	5.0±0.9
Navicula	40	1.88±0.38(5)	1.44±0.30(9)	86.9±1.2	15.1±3.4
Anabaena	40	0.68±0.23(4)	6.88±2.15(5)	79.6±3.3	3.3±1.3
Trout chow	40	0.33±0.01(4)	0.10±0.01(10)	49.2±5.8	17.5±3.0
Autumn maple leaves	40	0.08±0.01(7)	--	82.7±6.2	--

[1]Values in parentheses refer to number of organisms

[2]Values are given as mean ± S.E.

Table 6. Chemical fractionation of the mercury from CH_3HgCl and $HgCl_2$ contaminated foods, used in the feeding experiments.

Chemical Form of Mercury added to Food	Food Type	Number of Food Samples Fractionated	% of Mercury in Benzene Layer (% organic)	% of Mercury in Water Layer (% inorganic)
CH_3HgCl	Scenedesmus	2	97.6±0.5[1] (97.1-98.0)[2]	
	Anabaena	2	98.1±0.05 (98.0-98.1)	
	Trout chow	2	97.0±0.1 (96.8-97.1)	
$HgCl_2$	Scenedesmus	3		99.5±0.1 (99.3-99.6)
	Trout Chow	2		99.3±0.0

[1]Values are presented as mean ± S.E.

[2]Values in parentheses represent the range of values obtained.

Table 7. Natural and experimental levels of mercury in the foods used in the feeding experiments.

Food Type	Natural Level of Mercury in the Foods (as total mercury) (µg Hg/g dry weight food)	Level of Mercury in the Foods in the Feeding Experiments (µg Hg/g dry weight food)		Natural Level as % of Experimental Level of Mercury	
		CH_3HgCl	$HgCl_2$	CH_3HgCl	$HgCl_2$
Algae[1]	0.315	340.0	160.0	0.1	0.1
Trout chow	0.050	3.0	12.9	1.7	0.4
Maple leaves	0.300	28.0	-	1.0	-

[1]The algal sample consisted of Scenedesmus, Anabaena and Navicula in approximately equal amounts.

These samples had been frozen for 2 months prior to their fractionation. Some demethylation may have occurred during the freezing period, however, fractionation of the mercury in the amphipods from other feeding experiments reported here showed that the chemical form of mercury in amphipod tissue remained unchanged during ingestion and subsequent tissue retention (Table 8). This agrees with several studies by Jernelov (1968, 1972), Pennacchioni et al. (1976) and Pentreath (1976). In contrast to this, Guarino et al. (1976) reported biotransformation rates $\leq$ 3% per day in lobsters and as much as 6% per day in rats.

Similar assimilation efficiencies for methylmercuric chloride and mercuric chloride have been shown using many other species of animals and foods (fish - Hannerz, 1968; Matida et al. 1971; de Freitas et al. 1974; de Freitas, 1976; aquatic invertebrates - Huckabee et al. 1975; Man - Miettinen et al. 1971). Thus there seems to be no major variation in the assimilation efficiency of either of these two mercury compounds due to food type or species of animal studied.

Table 9 shows the amount of mercury taken up directly from the water during ingestion of mercury contaminated diets. Since the mercury body burden resulting from the concurrent direct uptake of mercury from water was less than 3% of the total body burden the mercury body burdens resulting from the feeding experiments reported in this study were not corrected for uptake from the water.

Table 8. Fractionation[1] of the mercury taken up by the amphipods from ingesting CH_3HgCl and $HgCl_2$ contaminated foods.

Chemical Form of Mercury	Feeding Experiment	Number of Amphipods Tested	% of Total Mercury recovered Benzene Layer[2] (% organic)	% of Total Mercury recovered Water Layer[1] (% inorganic)
CH_3HgCl	Scenedesmus	3	84.4±1.2	
	Navicula	2	90.4±2.3	
	Trout Chow	4	51.4±5.6	
	Maple leaves	3	86.4±5.8	
$HgCl_2$	Maple leaves			
	40 min. exposure	2		76.1±3.3
	4 h. exposure	2		80.7±9.2
	24 h. exposure	3		87.8±7.6

[1]Fractionation was carried out on amphipods killed after a post ingestion period of days

[2]All values are presented as mean ± standard error.

Table 9. Direct uptake from water of CH_3HgCl and $HgCl_2$ during ingestion of mercury contaminated food by Hyalella azteca due to leaching of mercury from the food.

Mercury Form	Food Type	Total Uptake from Food and Water (40 min. exposure) (cpm/amphipod)	Direct Uptake from Water[1] (40 min. exposure) (cpm/amphipod)	Error due to Direct Uptake from Water (%)
CH_3HgCl[2]	Scenedesmus	310.0±60.2(7)[4]	3.0±2.0(3)	1.0
	Anabaena	131.3±45.0(9)	8.0±4.7(3)	6.1
	Navicula	334.7±70.4(11)	8.0±3.0(2)	2.4
$HgCl_2$[3]	Scenedesmus	3510.0±804.9(15)	29.0±2.7(4)	0.8
	Anabaena	14800.0±3954.3(7)	383.0±287.3(2)	2.6
	Navicula	3560.0±902.9(9)	96.0±10.5(2)	2.7

[1]Amphipods were exposed for 40 min. to filtered water samples removed from the beakers containing Hg-203 contaminated foods.

[2]Specific activity of $CH_3{}^{203}HgCl$ was 1.92×10^6 cpm/µg Hg.

[3]Specific activity of $^{203}HgCl_2$ was 2.7×10^6 cpm/µg Hg.

[4]All values represent the mean ± S.E.; values in parentheses refer to the number of organisms.

With continuous feeding organisms like Hyalella azteca, the time of exposure to the contaminated diet is important in determining net assimilation efficiency, since an ingestion period of shorter duration than the voidance time should be used. Table 10 shows the results of the experiment to estimate Hyalella azteca's gut clearance time (voidance time) using trout chow contaminated with $CH_3{}^{203}HgCl$. The amphipod's mercury body burden after a 40 min. ingestion period is approximately twice that achieved after a 20 min. ingestion period. However, there is a smaller increase in the amphipod's body burden of mercury from the 40 min. ingestion period to the 60 min. ingestion period, indicating that somewhere between 40 and 60 minutes of ingestion, the amphipod begins to egest a portion of the ingested mercury not absorbed by its gastrointestinal tract. This represents the fast clearing compartment. If we assume that the levelling off between 40 and 60 minutes of the mercury body burden does not represent a decrease in ingestion rate, as the amphipods had been starved for several hours before the experiment was performed then these results demonstrate that the voidance time is $\geq$ 40 min. Therefore to measure the entire size of the fast clearing compartment, and hence net assimilation efficiency, an ingestion period less than the voidance time should be used (i.e. 40 min.).

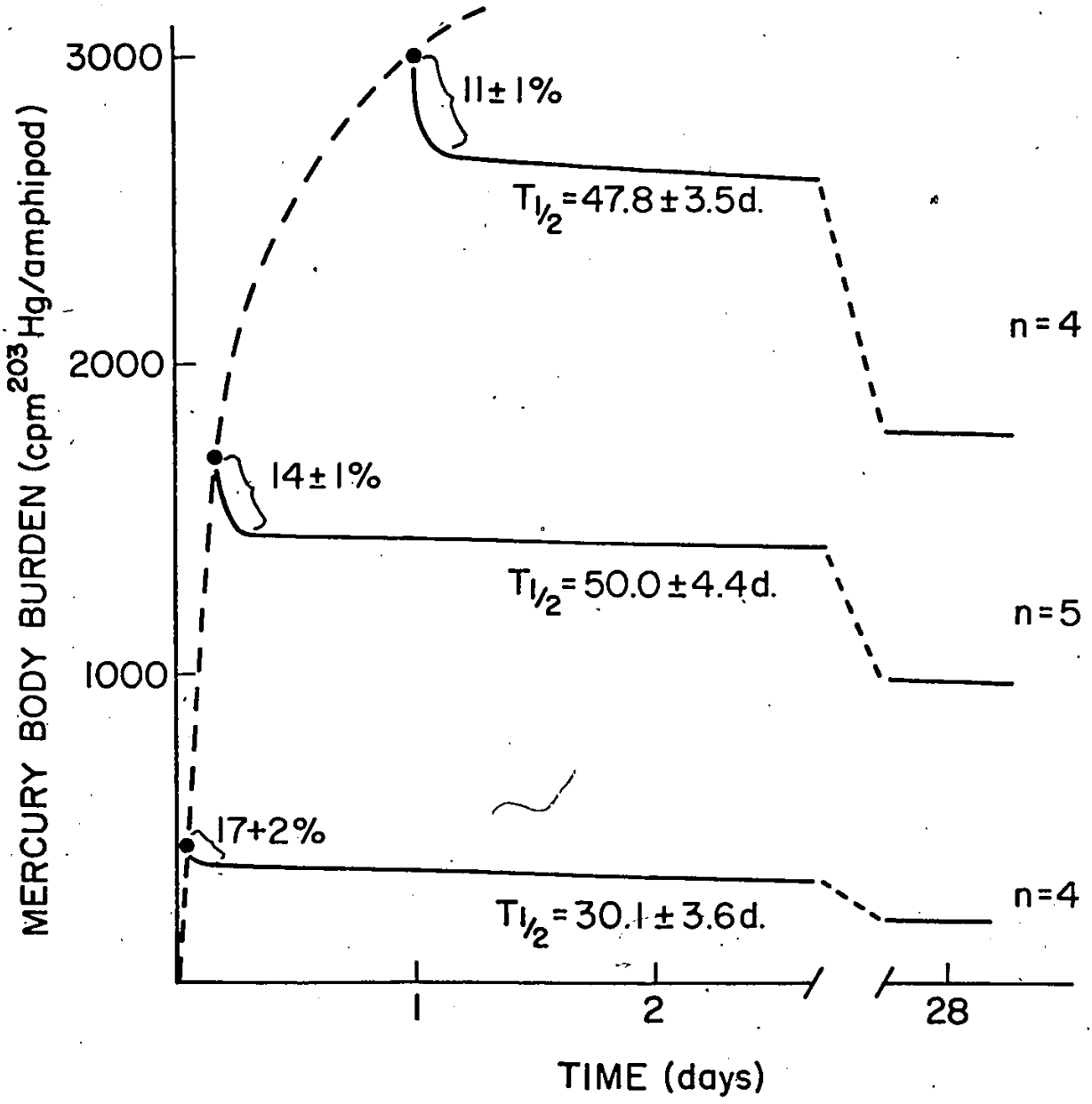
The results presented in Fig. 3 demonstrate that as exposure time increases, the measured size of the fast clearing compartment becomes less. After a 40 min. ingestion period,

Table 10. Estimation of gut clearance time in Hyalella azteca, using CH_3HgCl contaminated trout chow.

Exposure Time (minutes)	Amphipod Body Burden of Mercury at end of Exposure Period (cpm/amphipod)[1]	Number of Amphipods Used
20	699.3±108.6	4
40	1388.0±143.0	4
60	1481.7±.91.9	5

[1]Specific activity of $CH_3{}^{203}HgCl$ was 5×10^5 cpm/µg Hg.

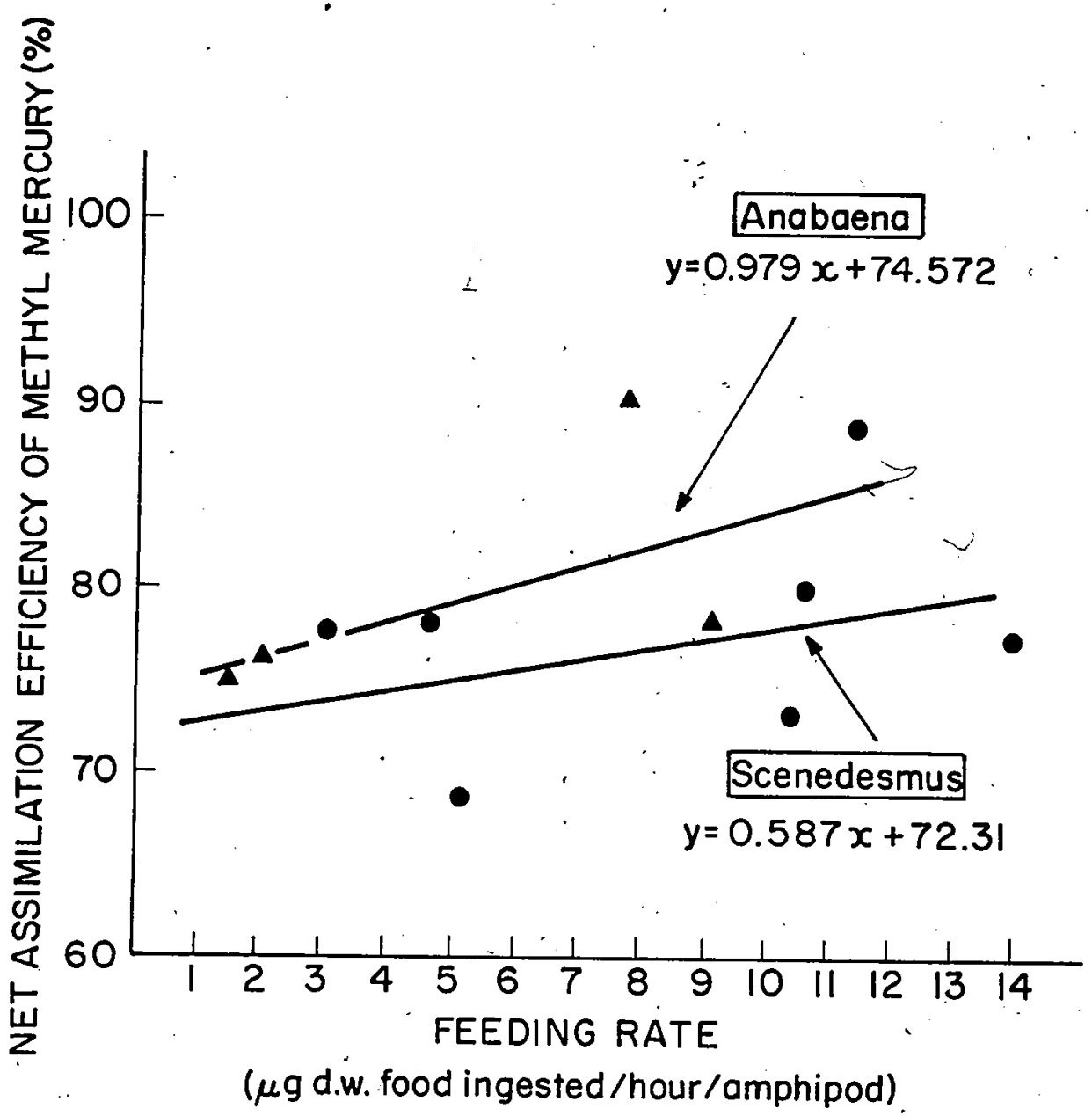
Fig. 3. Assimilation efficiency of CH_3HgCl from the gastrointestinal tract and whole body clearance of CH_3HgCl following ingestion of $CH_3{}^{203}HgCl$ contaminated autumn maple leaves. Specific activity of $CH_3{}^{203}HgCl$ was 5.9×10^6 cpm/µg Hg.


3000
2000
1000
MERCURY BODY BURDEN (cpm ^{203}Hg/amphipod)
11±1%
$T_{1/2}$=47.8±3.5d.
n=4
14±1%
$T_{1/2}$=50.0±4.4d.
n=5
17+2%
$T_{1/2}$=30.1±3.6d.
n=4
1
2
28
TIME (days)

the fast compartment represented 17% of the ingested methylmercuric chloride, whereas after 24 hours ingestion, it represented only 11%. This is as expected since as the body burden increases, with length of exposure time, the proportion of the body burden associated with the gut contents becomes less. On this basis, after about 4 to 5 days of continuous exposure to methylmercury, the size of the fast clearing compartment relative to the total body burden will be undetectable, being about 1 to 2% of the total body burden and hence lost in the scatter of the data.

Feeding rate may also affect assimilation efficiency of a compound as indicated by a model ecosystem study by Huckabee et al. (1975) and de Freitas et al. (1977). In Fig. 4 the effect of feeding rate on assimilation efficiency of methylmercuric chloride contaminated Scenedesmus and Anabaena is shown. With both foods there seems to be an increase of about 10% in assimilation efficiency with feeding rate ranging from 1 to approximately 14 µg food (dry weight) per hour per amphipod. These feeding rates represent only a moderate rate of feeding as Hyalella has shown a feeding rate in other feeding experiments (e.g. trout chow) of 60 µg food per hour. Hargrave (1971) found Hyalella to ingest 5 to 30 µg sediment per hour per amphipod, and 25 µg bacteria per hour per amphipod. Feeding rate as shown in Fig. 4 is based on the body burden of mercury in the amphipod and hence body burden could have been used as the x-axis instead of feeding rate. In the case of mercuric chloride contaminated foods, the assimilation

Fig. 4. Effect of feeding rate and type of food on assimilation efficiency of CH_3HgCl, from the gastrointestinal tract of Hyalella azteca.


NET ASSIMILATION EFFICIENCY OF METHYL MERCURY (%)
100
90
80
70
60
Anabaena
y=0.979 x +74.572
Scenedesmus
y= 0.587 x + 72.31
1 2 3 4 5 6 7 8 9 10 11 12 13 14
FEEDING RATE
(μg d.w. food ingested/hour/amphipod)

efficiencies were so low that even if they were affected by feeding rates, we were unable to detect an effect. Huckabee et al. (1975) studying fish also found assimilation efficiency of mercury to increase as ingestion rate increased. As ingestion rate increases, so does the residence time of the larger food mass in the gut, and the longer residence time may allow more complete digestion of mercury as well as ingested calories.

The effect of temperature on clearance of ingested methylmercuric chloride is presented in Table 11. Clearance rate decreases slightly (by about 25%) with increasing temperature over a range of 10 to 20°C. Other studies with many organisms including fish (Ruohtula and Miettenen, 1975) demonstrate that temperature is an important control factor in clearance of mercury compounds presumably resulting from the direct relationship between metabolic rate and temperature. However, no temperature effect was observed with methylmercury clearance from goldfish (Sharpe et al. 1977) on a maintenance diet, although rapidly growing trout are reported to clear mercury faster at higher temperatures than at lower temperatures (Ruohtula and Miettenen, 1975). Cunningham and Tripp (1975) also found that clearance rate of mercury increased as temperature increased with the oyster. The apparent reduction of clearance rate with temperature, shown by our results on amphipods may not be a temperature effect, but result from the much larger dose of ingested mercury acquired by the amphipods at 20°C (0.5 to 1.4 ng Hg/amphipod) compared to those at 10°C

Table 11. Effect of temperature on whole body clearance of methylmercuric chloride following ingestion by Hyalella azteca of $CH_3{}^{203}HgCl$ contaminated maple leaves.[2]

Water Temperature (°C)	Number of Amphipods	Amphipod Mercury Body Burden at end of Exposure (range in ng/amphipod)	Clearance $T_{\frac{1}{2}}$ (days)	Duration of Experiment (days)
10	4	0.031 - 0.242	70.3±17.0[1]	30
15	14	0.193 - 1.439	94.4±25.2	30
20	6	0.500 - 1.427	105.5±17.4	35

[1]Values are presented as mean ± S.E.

[2]Ingestion period was 3 hours.

(0.03 to 0.24 ng Hg/amphipod). This may suggest that the rate limiting step in clearance may be related to mercury tissue levels, particularly when high levels of mercury are acquired over a short period of time.

A comparison of the clearance times at a constant temperature for both methylmercuric chloride and mercuric chloride consumed with mercury contaminated foods of different types are shown in Table 12. The ingestion or feeding period used was considerably longer, 40 minutes, compared to 3 h in the previous experiment on the effect of temperature on clearance. The half-life of methylmercuric chloride ranged from 40 to 55 days for amphipods on algal diets, and 30 days for organisms feeding on maple leaves. Mercuric chloride was cleared much more rapidly with only 6 to 14 days. These results agree with other studies that have found that methylmercuric chloride is excreted more slowly than mercuric chloride (Berlin and Ulberg, 1963; Jarvenpaa et al. 1970; Miettinen et al. 1972; Smith et al. 1975). Clearance time of $HgCl_2$ for crabs has been found to be 25 days (Sloan et al. 1974) and for molluscs, 5 to 10 days (Unlu et al. 1972).

In most of these experiments there are large variations in food ingestion rate in animals of the same weight class that are comparably treated (as indicated by the large S.E.) This has also been found in studies of bivalves (Cunningham and Tripp, 1975) and fish (Fagerstrom et al. 1974; Huckabee et al. 1975). Such differences may result from the physiological state of the organism which may vary with sex or stage of intermoult, thus resulting in feeding rate variations.

Table 12. Influence of diet composition on the time required for half of the accumulated tissue mercury to be lost from Hyalella azteca after ingestion of CH_3HgCl and $HgCl_2$ contaminated foods.

Chemical Form of Mercury	Food Type	$T_{\frac{1}{2}}$ for Slow Compartment (days)	Amphipod Mercury Body Burden at End of Exposure[2] (ng Hg/amphipod)	Number of Amphipods	Duration of Experiment (days)
CH_3HgCl	Scenedesmus	40.8±3.7[1]	1.6	7	20
	Anabaena	55.6±9.0	0.7	4	17
	Nayicula	50.0±8.5	1.8	5	15
	Trout chow	49.5±10.1	0.3	7	8
	Maple leaves	30.1±3.6	0.1	4	7
$HgCl_2$	Scenedesmus	6.7±1.4	1.3	7	19
	Anabaena	14.4±3.8	6.7	5	34
	Navicula	6.1±1.5	1.5	12	34
	Trout chow	12.5±2.6	0.1	8	17

[1]Values are presented as mean ± S.E.

[2]Ingestion period of 40 minutes.

Mercury uptake from water

The maintenance of essentially steady state conditions with respect to both organic and inorganic mercury concentration in perfusate solution during an exposure period of 2 hours is shown by the data in Table 13. The observed reduction in the mercury concentration of only 2 to 3% after 24 hours in the case of methylmercuric chloride was in sharp contrast to the much greater concentration reduction of approximately 20% after 24 hours in the case of mercuric chloride. Such loss could have occurred due to adsorption on the walls of the glass beakers, mercury binding to ligands excreted by the amphipods, precipitation, and the reduction of ionic mercury to Hg° and its release to the atmosphere (Huckabee et al. 1975). The low concentration of mercury used in these experiments is likely an important factor also. Newton and Ellis (1974) found substantial amounts were lost at a concentration of 0.2 mg Hg/l and lower. Dokiya et al. (1974) showed similar results. The results in Table 13 also show the chemical stability of both mercury forms in the perfusate solution, as checked by the benzene fractionation procedure (see Methods). No significant methylation or demethylation of mercury was detected.

In studying uptake of mercury from water, the term transfer coefficient (T_c) was used by de Freitas and Hart (1975). As used here, this term is defined as the weight of water in grams completely cleared of its mercury content by 1 gram wet wt. of amphipods in 1 hour. In calculating these results, it is assumed that there are 200 amphipods/gram on

Table 13. Mercury concentration in perfusate water and its' chemical stability during exposure of Hyalella azteca.[1]

Chemical Form of Mercury	Duration of Experiment (hours)	Mercury Concentration in Water (µg Hg/ml)	Mercury Loss From Water During Uptake by Amphipods (%)	Mercury Composition % organic	Mercury Composition % inorganic
CH_3HgCl[2]	0	9.04×10^{-4}	0	78.2	
	8	8.96×10^{-4}	0.8 (5%)[4]	80.4	
	24	8.78×10^{-4}	2.9 (10%)	73.6	
	28	8.48×10^{-4}	6.2 (10%)	82.9	
$HgCl_2$[3]	0	6.09×10^{-3}	0		91.3
	4	5.94×10^{-3}	2.5 (10%)		89.8
	8	5.57×10^{-3}	8.5 (13%)		-
	24	4.86×10^{-3}	20.2 (40%)		87.8

[1] An average density of 5 amphipods/100 ml perfusate was used.

[2] Specific activity of $CH_3{}^{203}HgCl$ was 1.06×10^6 cpm/µg Hg.

[3] Specific activity of $^{203}HgCl_2$ was 1.66×10^6 cpm/µg Hg.

[4] Values in parentheses show mercury loss from water in absence of amphipods. These results were obtained with levels of CH_3HgCl being 4.2×10^{-3} µg Hg/ml and $HgCl_2$ being 2.3×10^{-3} µg Hg/ml, at 24°C in dechlorinated tap water (D.C. Mortimer, personal communication).

a wet weight basis. When transfer coefficient values are similar (i.e T_c values independent of concentrations) it can be assumed that uptake rate of mercury is directly dependent upon mercury concentration in the water and that uptake is a 1st order process. The results in Table 14, illustrate that the measured transfer coefficients for methylmercuric chloride were independent of concentration over a 20-fold range. The T_c values for methylmercuric concentrations of 0.8×10^{-3} µg Hg/ml, 6×10^{-3} µg Hg/ml and 1.4×10^{-3} µg Hg/ml were 13.3, 11.9 and 12.0 h^{-1}, respectively. Studies with fish of the same weight class also showed T_c to be independent of mercury concentration. For 2 gram fish (Notemigonus crysoleucus), T_c values are 3.8, 4.2 and 4.1 h^{-1}, at methylmercury concentrations of 0.01×10^{-3} µg Hg/ml, 0.1×10^{-3} µg Hg/ml, and 1×10^{-3} µg Hg/ml, respectively (de Freitas, 1977). For mercuric chloride, the transfer coefficients, over the range of 5×10^{-4} µg Hg/ml to 5×10^{-3} µg Hg/ml were similar (11.2 and 10.1), but when the concentration of mercuric chloride was as high as 5×10^{-2} µg Hg/ml the value of the transfer coefficient dropped to 5.9 h^{-1}. A mercury concentration of 1×10^{-4} to 1×10^{-3} µg Hg/ml is a realistic range for many contaminated waters, with a concentration of 5×10^{-3} µg Hg/ml being an extremely polluted situation (Gavis and Ferguson, 1972). Thus the highest concentration of $HgCl_2$ (5×10^{-2} µg Hg/ml) is unrealistic for the natural environment. Such a high concentration would very likely be lethal over a longer period of time than used in this experiment. After 2 hours of uptake

Table 14. Effect of different concentrations of CH_3HgCl and $HgCl_2$ on uptake[1] from dechlorinated water by Hyalella azteca.

Chemical Form of Mercury	Mercury Concentration in Exposure Medium (cpm/ml medium)	Mercury Concentration in Exposure Medium (µg Hg/ml medium)	Number of Amphipods	Uptake Rate (µg Hg/amp/h)	Transfer Coefficient[4] T_c (hour^{-1})
CH_3HgCl[2]	2170	0.0008	10	0.53×10^{-4}	13.3±1.9
	17317	0.0060	10	3.57×10^{-4}	11.9±1.3
	40462	0.0140	9	8.40×10^{-4}	12.0±1.1
$HgCl_2$[3]	748	0.0005	8	0.28×10^{-4}	11.2±2.0
	7267	0.0050	6	2.53×10^{-4}	10.1±1.3
	76760	0.0500	7	14.75×10^{-4}	5.9±0.8

[1]Amphipods were exposed for 2 hours at a water temperature of 19±1°C.

[2]Specific activity of $CH_3{}^{203}HgCl$ was 2.9×10^6 cpm/µg Hg.

[3]Specific activity of $^{203}HgCl_2$ was 1.5×10^6 cpm/µg Hg.

[4]Transfer coefficient values represent the weight of water in grams completely cleared of its mercury content by 1 gram wet wt. of amphipods in 1 hour. Each value represents the mean ± standard error.

of $HgCl_2$ the mean body burden of the amphipods was 1 µg Hg/g wet wt. at a concentration of 5×10^{-2} µg Hg/ml in the water. This concentration, although it did not result in death of any of the amphipods during the exposure period, may have affected the amphipods, thus resulting in a lower uptake rate by the amphipods. Many studies have resulted in 24 hour $HgCl_2$ LC50 for crustaceans of 4×10^{-3} µg Hg/ml for the grass shrimp (Ray and Tripp, 1976), 6×10^{-3} µg Hg/ml in the copepod (Barnes and Stanburg, 1948) and 4.5×10^{-3} µg Hg/ml in Artemia (Wisely and Blick, 1967).

In all of the uptake of mercury from water experiments, the amount of mercury taken up was too high to be a result of drinking mercury contaminated water. Lockwood and Andrews (1969) found the drinking rate of Gammarus duebeni to be less than 1 µl/hour. Hyalella azteca is a much smaller amphipod than Gammarus duebeni, but even if its drinking rate was as high as 1 µl/hour, at a methylmercury concentration of 8×10^{-4} µg Hg/ml, the resulting uptake rate would be 8×10^{-7} µg Hg/amphipod/h. The experimental uptake rate however was much higher, being 4×10^{-5} µg Hg/amphipod/hour (Table 14). Adsorption of mercury is very likely to occur over the amphipod's general integument, but uptake into tissues may occur predominately through the branchia, as was found with uptake of PCBs (Wildish and Zitko, 1971). Wildish and Zitko (1971) also found uptake to be unaffected by the amphipod's stage of intermoult. Although this was not investigated in the present study, moults occurring during clearance were

assayed for their ^{203}Hg content. No mercury was found in the moults.

The results of the effect of temperature on uptake of mercury from water are shown in Table 15. Pringle et al. (1968) have reported that temperature is closely related to uptake rate of a given metal. Our results support this observation. The transfer coefficient decreased from about .11 h^{-1} to 4 h^{-1} as temperature dropped from 20°C to 10°C, and uptake rate is thus likely to be directly related to the metabolism of the organism. At 5°C however, the transfer coefficient increases. Organ specific temperature dependence has been suggested by Vernberg and O'Hara (1972). They have shown that the gills of Uca pugilator concentrate inorganic mercury to a greater extent at a low temperature (5°C) but at higher temperatures, mercury is transferred from the gills to the hepatopancreas. The increase in mercury toxicity to the fiddler crabs at low temperatures may be due to the diminished ability to transport the unbound mercury compound from the gills. Smith et al. (1975) found that over a temperature range of 10°C to 20°C, temperature had no effect on uptake rate of CH_3HgCl or $HgCl_2$ by the clam, suggesting that uptake rate was not related to metabolic rate. However several studies with fish have shown that uptake rate increases with increasing temperature (Murphy and Murphy, 1971; MacLeod and Pessah, 1973; Reinert et al. 1974; and Ruohtula and Miettinen, 1975). The extent of an increase in uptake rate from water with increasing temperature depend on such factors

Table 15. Effect of temperature on uptake of CH_3HgCl from dechlorinated tap water by Hyalella azteca.[1]

CH_3HgCl[2] Concentration in Water (µg Hg/ml)	Water Temperature (°C)	Number of Amphipods	Uptake Rate (µg Hg/amp/h)	Transfer Coefficient[3] ($hour^{-1}$)
0.047	20	8	25.4×10^{-4}	10.8±1.0
0.045	10	9	7.80×10^{-4}	3.5±0.6
0.053	5	8	17.8×10^{-4}	6.7±0.9

[1]Amphipods were exposed for 2 hours.

[2]Specific activity of $CH_3{}^{203}HgCl$ was 2.74×10^6 cpm/µg Hg.

[3]Transfer coefficient values represent the weight of water, in grams, completely cleared of its' mercury content by 1 gram of amphipods in 1 hour. Each value represents the mean ± S.E.

as the effects of water temperature on the organism's metabolic rate and uptake efficiency of mercury or its bioavailability. Because of seasonal influences on water quality parameters and their effect on bioavailability (de Freitas 1977), it is difficult to accurately predict the effect of water temperature on the amount of mercury accumulated by aquatic organisms in natural systems.

Water quality has been found to influence accumulation of mercury in aquatic organisms. Johnels et al. (1967), studying pike, found higher accumulation rates in oligotrophic lakes than in more eutrophic ones with the same degree of mercury pollution. Similar results were obtained by Nuorteva and Hasanen (1971). Water quality was also found to affect mercury accumulation in areas upstream and downstream of Hamburg in different species of mussels (Karbe et al. 1975). de Freitas (1977), studying fish, found an enhancement of two to three fold in assimilation efficiency of methylmercury from Ottawa River water compared to tap or reconstituted water. Using filtered river water, the transfer coefficient from water to fish for methylmercury was increased by 10 to 40% compared to unfiltered water. In the present study, two sources of water were used; dechlorinated tap water, and unfiltered, surface Ottawa River water. For both methylmercuric chloride and mercuric chloride, the transfer coefficient for mercury from water to the amphipod, was lower in the unfiltered river water (Table 16). One possible explanation for this is that the mercury in unfiltered river water is not as available

Table 16. Effect of water quality on direct uptake of CH_3HgCl and $HgCl_2$ from water by Hyalella azteca.[1]

Water Type	Chemical Form of Mercury	Mercury Concentration in Exposure Medium (µg Hg/ml)	Number of Amphipods	Uptake Rate (µg Hg/amp/h)	Transfer Coefficient[4] T_c (h^{-1})
Dechlorinated Tap water	CH_3HgCl [2]	0.0003	8	3.26×10^{-5}	21.7±1.6
Ottawa River water		0.0005	8	1.7×10^{-5}	6.8±0.9
Dechlorinated Tap water	CH_3HgCl	0.0580	10	6.8×10^{-4}	2.4±0.5
Ottawa River water		0.0350	9	2.6×10^{-4}	1.5±0.3
Dechlorinated Tap water	$HgCl_2$ [3]	0.0060	8	3.33×10^{-4}	11.1±1.2
Ottawa River water		0.0070	10	1.37×10^{-4}	3.9±0.5
Dechlorinated Tap water	$HgCl_2$	0.3300	8	4.62×10^{-3}	2.8±0.1
Ottawa River water		0.3700	8	2.78×10^{-3}	1.5±0.4

[1] Amphipods were exposed for 2 hours at a water temperature of 19±1°C.

[2] Specific activity of $CH_3{}^{203}HgCl$ was 2.16×10^6 cpm/µg Hg.

[3] Specific activity of $^{203}HgCl_2$ was 1.8×10^6 cpm/µg Hg.

[4] Transfer coefficient values represent the weight of water in grams, completely cleared of its' mercury content by 1 gram of amphipods in 1 hour. Each value represents the mean ± S.E.

for uptake by the amphipods because some of the mercury may be bound to soluble thiol ligands (Huckabee et al. 1975). There may be also more particulate organic material in river water, which, acting as strong chelators, makes mercury unavailable for uptake by the amphipod. The results presented in Table 16 also demonstrate that very high methylmercury concentrations have the effect of reducing the efficiency of mercury uptake from water. This was shown earlier for $HgCl_2$, but not for CH_3HgCl over the concentration range studied (Table 14). Mercuric chloride and methylmercuric chloride concentrations in the range of (0.05-0.5 µg Hg/ml) are much higher than that encountered in the environment. Thus for levels of mercury encountered in natural systems, transfer coefficient values can be assumed to fall in the range of 25 h^{-1} to 7 h^{-1} for methylmercury chloride and about 50% lower for mercuric chloride. Transfer coefficient values probably fluctuate to an even greater extent in natural environments due to the combined effects of seasonal changes in water quality parameters and temperature related changes in metabolic rate.

Results on uptake from water and release of mercury by Hyalella azteca are in close agreement with corresponding values for Daphnia magna and various fish species, when differences in body weight are taken into account. For example, transfer coefficient values, using CH_3HgCl, for Daphnia magna (wet weight 0.1 mg) range from 100 to 300 h^{-1} (Huckabee et al. 1975; Trudel, 1979). Hyalella azteca (wet weight 5.0 mg) has

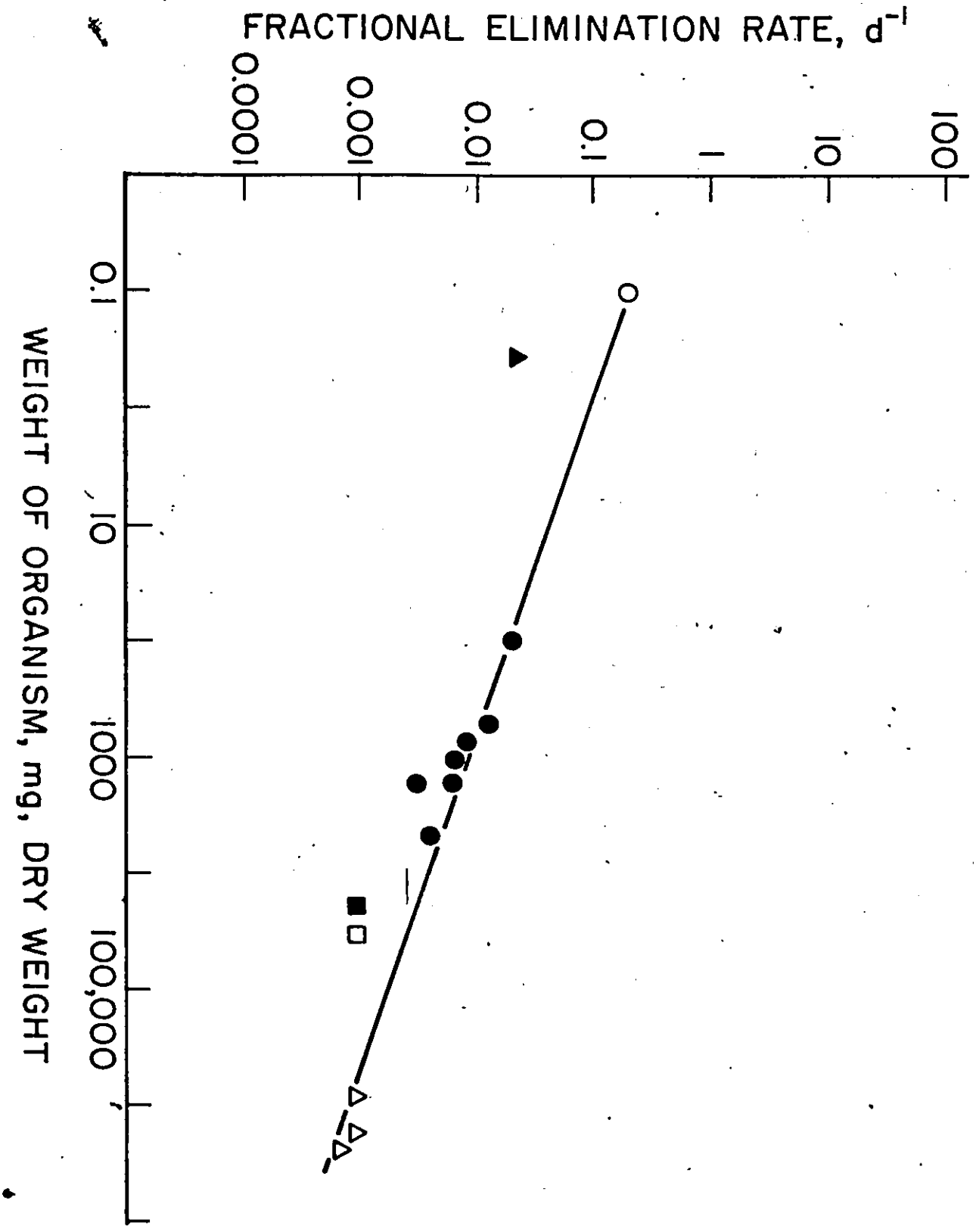
T_c values ranging from 8 to 25 h^{-1}. Studies on fish, Notemigonus crysoleucus, and Catosomius commensoni show T_c values of 4.4 h^{-1} for a 2 g fish, 3.0 h^{-1} for a 10 g fish and 1.7 h^{-1} for a 100 g fish (de Freitas, 1977).

It is useful to compare the efficiency of mercury uptake from water with that of O_2 uptake by the organism. Hyalella azteca requires approximately 1.2 mg O_2/g wet weight/hour, as reviewed by Schindler (1967). Assuming O_2 uptake from water is 100% efficient, at an oxygen concentration of 9 µg/ml water, Hyalella would be required to remove the O_2 from 133 ml water/g wet weight/hour (a T_c for O_2 of 133 h^{-1}). With a transfer coefficient for methylmercury of 20 h^{-1} (20 ml water/g wet weight/hour) uptake of mercury from water would be 15% (20/133) as efficient as oxygen uptake. This value agrees with other studies as reviewed by Norstrom et al. (1976) and more recently by de Freitas (1977) and Phillips and Buhler (1978) who found mercury uptake by fish to be 15 to 30% as efficient as oxygen uptake. Fractional clearance rates of CH_3HgCl plotted against whole body weights are given in Fig. 5, for Daphnia magna, Hyalella azteca and several fish species of various body weights, ranging from 1 g to 500 g. All species fit a common relationship described by the following equation originally developed by Sharpe et al. (1975) for mercury clearance from goldfish:

$$R_{pcl} = k_{cl} PW^{-0.58}$$

where R_{pcl} is the rate of clearance of mercury; k_{cl} is the

Fig. 5. Relation between elimination rate of methylmercury and body weight of aquatic organisms. Daphnia magna ○; Hyalella azteca ▲; Carassius auratus ●, ■, □; Esox lucius △.


FRACTIONAL ELIMINATION RATE, d⁻¹
1000.0
100.0
10.0
1.0
—
10
100
WEIGHT OF ORGANISM, mg, DRY WEIGHT
0.1
10
1000
100,000

clearance constant, for an organism weighing 1 g = 0.029 d^{-1}; P is the body burden of methylmercury; and W the weight of the organism (g). The value for Hyalella azteca falls below the regression line for fish and Daphnia magna but within the general scatter of the relationship. This result suggests that metabolic rate, although an important control factor in mercury clearance, operates in conjunction with other factors affecting clearance related functions.

Fractional clearance rates of CH_3HgCl and $HgCl_2$ after uptake from water and food, by Hyalella azteca are presented in Table 17. The clearance times established for $HgCl_2$, following uptake from water were longer than those established following uptake from food in agreement with other studies on fish reported by de Freitas et al. (1974). Elimination rates for CH_3HgCl are similar regardless of route of uptake, as was also found by Huckabee et al. (1975) and Trudel (1979) using Daphnia magna.

Applications to wild populations of Hyalella azteca

Mercury dynamics of an invertebrate in the broadest general sense is relatively simple to understand. Mercury is taken up by individuals from the diet or by direct absorption from the aqueous environment. Some of the mercury taken up by tissues is excreted and the remainder is retained in the tissues to be (1) returned to the sediment upon the death of the individual or (2) removed from the population by predators which assimilate a portion of the mercury content of their prey.

Table 17. Fractional clearance rates of CH_3HgCl and $HgCl_2$ following (1) ingestion of mercury-contaminated food and (2) uptake of mercury from contaminated water, by Hyalella azteca.[1]

Chemical Form of Mercury	Source of Mercury	Exposure Time	Number of Amphipods	Amphipod Body Burden of Mercury (ng Hg/org.)	Clearance Half Life[3] $t_{\frac{1}{2}}$ (days)
CH_3HgCl	Water[2]	2 hours	3	0.7	33.0±6.5
	Food -Scenedesmus	40 min.	7	1.6	40.8±3.7
	Anabaena	40 min.	4	0.7	53.3±9.0
	Navicula	40 min.	5	1.8	49.5±8.3
	Trout chow	40 min.	7	0.4	49.5±10.1
$HgCl_2$	Water[2]	40 min.	6	0.4	43.3±9.2
	Food -Scenedesmus	40 min.	7	1.3	6.7±1.4
	Anabaena	40 min.	5	1.6	14.4±3.8
	Navicula	40 min.	12	1.5	16.1±3.4
	Trout chow	40 min.	8	0.1	12.5±2.6

[1]Water temperature during all uptake and clearance experiments was 19±1°C.

[2]Dechlorinated tap water.

[3]Each value represents the mean ± S.E.

At the outset it is necessary to impose a simplified status on the complexity of real systems. A simplifying and probably valid generalization is that uptake from both food and water should be quantitatively related to metabolic rate, and should therefore fall within limits set by species-specific factors controlling metabolic rate and growth as modified by environmental factors such as water temperature and food availability. A knowledge of the concentration of mercury in water and its chemical speciation is therefore of central importance, but this information is seldom documented, although a massive literature exists on mercury levels in fish and other organisms. This lack of documentation of water concentration of methylmercury or the ability to predict it from sediment levels, imposes severe limitations on the quantitative application of uptake rate constants and other kinetic parameters to mercury bioaccumulation phenomena in the field. Our results quantitatively define the bioaccumulation potential of both methylmercury and inorganic mercury in terms of three parameters; (1) efficiency of uptake from food, (2) efficiency of uptake from water, and (3) elimination or fractional clearance from the whole body, but many gaps still exist in understanding the detail of the effects of many potentially important controlling factors such as seasonal variations in water quality and its effect on the bioavailability of mercury present in the water. It is important, nevertheless, to determine whether the experimental results obtained in this study can be used to predict mercury levels in wild populations of

Hyalella azteca. The following example is presented to illustrate the use of these laboratory results in predicting the probable magnitude of the food and water in the real world of Hyalella azteca.

The magnitude of the water vector and food vector can be calculated as follows: At a methylmercury concentration in water of 5 ng Hg/l (20% of total Hg conc. in water of 25 ng/l) and a T_c value of 20 h^{-1}, Hyalella azteca would accumulate 2.4 ng CH_3Hg/g/amphipod/day at 20°C by direct uptake from water. The concentration of total mercury in sediments from shallow areas of the Ottawa River in 1976 was 0.024 ppm g/wet weight of sediment of which 0.0024 ppm was probably methylmercury (Miller et al. 1977). At an ingestion rate of 0.003 g wet weight of sediment per day per amphipod, (50% Body wt/day) and an assimilation efficiency from the G.I. tract of ~0.8, uptake of methylmercury via the food vector should be ~1.0 ng CH_3Hg/g amphipod/day. Under these conditions, the food vector would account for considerably less than 50% of the total uptake of methylmercury ~3.4 ng Hg/g amphipod/day A continuous uptake of methylmercury at a constant rate of ~3.4 ng Hg/g amphipod/day (value for food and water uptake) will equal loss rate from the organism when its' mercury body burden is ~230 ng Hg/g amphipod; based on a fractional clearance rate of methylmercury from body tissues of 1.5% per day. This means that the concentration of methylmercury in Hyalella azteca should approach an equilibrium value of about 0.23 µg/g wet weight of tissue after about four months of exposure.

Table 18. Levels of total mercury in amphipods and other benthic invertebrates found in their natural environment.

Amphipod Total Mercury Body Burden (ppm dry weight)	Location of Amphipod Collection		Reference
2.16	Kettle Island Bay, Ottawa River	April, 1977	Present study
2.75		July, 1977	
0.29	Upstream, Upper Duck Island	June, 1976	Ottawa River Project, 1976 (Final Report)
2.92	Downstream, Upper Duck Island	July[3], 1976	
5.22	Lake Temiscaming, Quebec	May, 1976	
0.05 - 0.07[1]	above mill, Sweden		
1.90 - 17.0	below mill, Sweden		Johnels et al. (1967)
0.12 - 0.33[2]	Fraser River, B.C.		Parsons et al. (1973)

[1]This study does not specify the species of benthic invertebrates studied.

[2]These values refer to molluscs, only.

[3]Organic mercury accounts for ~77% of the total mercury.

Corresponding calculations for uptake of inorganic mercury results in water and food vectors of about 5.0 ng Hg and 1 ng Hg per g organism per day respectively. Inorganic mercury is cleared from body tissues at about 4% per day, a much faster rate than that for methylmercury, and the concentration of inorganic mercury on Hyalella azteca should approach an equilibrium value of about 150 ng Hg/g amphipod after about two months of exposure.

These calculations do not take into account growth during this period. Amphipod mercury body burdens would thus be somewhat lower, than calculated above, due to growth dilution. Table 18 shows actual field values for the mercury content in amphipods, and other benthic invertebrates, on a ppm dry weight basis and the above predictions agree quite well with these field samples. It should be noted that the values for methylmercury in water and sediments used in the above example are close to levels observed in the Ottawa River and many other areas of Canada, (Miller, 1977). It should also be stressed that this simple treatment of the uptake process can be used to distinguish between food and water uptake only when the concentration of mercury in the water or diet is known. The amphipod example used here clearly demonstrates that the water vector is probably of equal or greater magnitude than the food vector, particularly in areas with low background type contamination levels. One can speculate that the relative magnitude of the water vector will decrease during periods of decreasing mercury pollution

and conversely, in areas of increasing pollution, the relative magnitude of the water vector will increase. If the water vector does in fact account for 50% or more of the mercury taken up by Hyalella azteca, then the hypothesis that biological magnification in aquatic environments is controlled by mass transfer of trace substances through the food web may have only limited application to the problem of high mercury levels in aquatic organisms. However, some degree of biomagnification in the benthic food web is virtually assured in the transfer of methylmercury from prey to predator solely on the basis of its high assimilation efficiency of 80%, even in the case of a rapidly growing predator in which 10-20% of ingested calories are deposited as new tissue. In this context, it is obvious that a food conversion efficiency for growth of 10%, for example, precludes the food chain biomagnification of any compound including inorganic mercury whose assimilation efficiency is $\leq$10%, regardless of how slowly it is eliminated from body tissues.

From this treatment of mercury bioaccumulation it should be possible to make some abstractions to the mercury problem in particular if not to pollutants in general. The important abstraction that emerges is that bioaccumulation of trace substances can be usefully considered a "growth driven" process in which both uptake and clearance is defined by bioenergetic related parameters. This abstraction has been successfully applied to mathematical models for mercury

and PCB's in yellow perch (Norstrom et al. 1976). In spite of the level of sophistication built into this model, it is relatively simplistic in an ecological context, hence meaningful specific conclusions that are not in error because they exclude important relations are very difficult to make.

GENERAL CONCLUSIONS

The results on mercury dynamics obtained in this study quantitatively define the bioaccumulation potential of methylmercury and divalent inorganic mercury in terms of three parameters: 1) efficiency of uptake from food; 2) efficiency of uptake from water; and 3) elimination or fractional clearance from the whole body. Many gaps exist in understanding the detail, especially as applied to real ecosystems, particularly the effect of seasonal variation in water quality factors on the bioavailability of methylmercury present in the water. Our results on mercury dynamics in the amphipod Hyalella azteca are presented in the following summary:

Item 1

Assimilation from water favours the preferential bioaccumulation of methylmercury compared to divalent mercuric ion. The efficiency of removal of methylmercury from water is equivalent to approximately 15-20% of the efficiency of oxygen uptake from water. Inorganic mercury is taken up from the water by Hyalella azteca at a slower rate (2-3 times more slowly) than methylmercury, depending on water composition.

Item 2

Assimilation efficiency from food also strongly favours the preferential bioaccumulation of methylmercury compared to inorganic mercuric ion. Assimilation from the gastrointestinal tract ranged from 60% to 80% of the amount

of methylmercury ingested. The variation in assimilation efficiency was not associated with type of food including various species of algae and leaf material from terrestial plants. Unlike organic mercury, most of the absorbed inorganic mercury (5-15% of ingested amount) is cleared at a much faster rate than methylmercury.

Item 3

Data on elimination rate of methylmercury (whole body clearance) is in general agreement with the relationship originally developed for mercury clearance from fish (Sharpe et al. 1977). This relationship is defined by the equation $R_{pcl} = K_{cl} P W^{\zeta}$, when P is the body content of mercury, K_{cl} is the clearance coefficient and the clearance rate, R_{pcl}, is related to the body weight W raised to a negative power exponent ζ. It may be reasonable to conclude that the value for ζ of about -0.6 is applicable to invertebrates as well as fish, since it may be related to the well established relationship between body size and metabolic rate. However, although the value for the clearance coefficient, K_{cl}, of about 0.03 d^{-1} for various species of fish appears to be also applicable to Hyalella azteca it may not apply to other invertebrate taxa and its' value can certainly be expected to vary widely with differences in chemical structure of pollutant.

Item 4-

Conversion of ingested inorganic mercury to organic mercury and its subsequent absorption from the gastrointestinal tract into body tissues was not observed, nor was there any

evidence of an appreciable "in vivo" conversion of inorganic mercuric ion, present in tissues, to organic forms of mercury. However, slow "in vivo" conversion of methylmercury to inorganic mercury may be an important factor in elimination of methyl-mercury from the whole body.

Item 5

Values for uptake rates from food and water estimated on the basis of mercury concentrations in river water and in the natural diet of Hyalella azteca in the Ottawa river, coupled to assimilation efficiency factors, demonstrate that the water vector may be as important or even more important than the food vector in accounting for the mercury body burden in amphipods. Under these circumstances biomagnification along a food chain could be an over-simplification, since tissue levels of methylmercury may depend as much on the longevity of the organism and its growth rate, as on its position in the food web. The magnitude of each vector therefore, will be markedly time dependent in a seasonal environment. However, on an annual or "lifetime" basis, the water vector is probably as large or larger than the food vector especially at the lower trophic levels of the food web.

REFERENCES

Barnes, H. and F. Stanbury. 1948. The toxic action of copper and mercury salts both separately and when mixed on the harpactiad copepod, Nitocra spinipes (Boeck). J. Exp. Biol. 25: 270-275.

Berlin, M. and W. Ullberg. 1963. Accumulation and retention of mercury in the mouse. 1. An autoradiographic study after a single intravenous injection of mercuric chloride. 11. An autoradiographic comparison of phenylmercuric acetate with inorganic mercury. 111. An autoradiographic comparison of methylmercuric dicyandiamid with inorganic mercury. Arch. Environmental Health 6: 589-616.

Bissonnette, P. 1977. Extent of mercury and lead uptake from lake sediments by chironomids. In Biological Implications of Metals in the Environment. ERDA Symposium Series 42 pp. 609-622.

Bousfield, E.L. 1973. Shallow-water Gammaridean Amphipoda of New England, Cornell University Press, Ithaca, N.Y.

Bovee, E.C. 1949. Studied on the thermal death of Hyalella azteca. Biol. Bull. (Woods Hole) 96: 123-128.

Brown, J.R. and M.V. Kulkarni. 1967. A review of the toxicity and metabolism of mercury and its compounds. Med. Serv. J. Can. 23(5): 786-808.

Cooper, W.E. 1965. Dynamics and production of a natural population of a freshwater amphipod, Hyalella azteca. Ecological Monographs 35: 377-394.

Corner, E.D.S. and B.W. Sparrow. 1957. The modes of action of toxic agents. 11. Factors influencing the toxicities of mercury compounds to certain crustacea. J. Mar. Biol. Ass. U.K. 36: 459-472.

Cunningham, P.A. and M.R. Tripp. 1973. Accumulation and depuration of mercury in the American oyster, Crassostrea virginica. Marine Biol. 20: 14-19.

_____. 1975. Factors affecting the accumulation and removal of mercury from tissues of the American oyster Crassostrea virginica. Mar. Biol. (Berl) 31(4): 311-320.

Davies, A.J. 1978. Pollution studies with marine plankton Part II. Heavy metals. Adv. Mar. Biol., Vol. 15, pp. 381-508.

de Freitas, A.S.W. 1976. Mercury uptake and retention by fish. In Distribution and Transport of Pollutants in Flowing Water Ecosystems. Ottawa River Project Report No. 3, Div. Biol. Sciences, National Research Council of Canada, Ottawa.

de Freitas, A.S.W. 1977a. Mercury uptake and retention by fish. In Distribution and Transport of Pollutants in Flowing Water Ecosystems. Final Report. Ch. 30, 61 p. National Research Council of Canada, 1977. Library of Congress Classification QH 541-5, F7,089.

de Freitas, A.S.W., S.U. Qadri and B.E. Case. 1974. Origins and fate of mercury compounds in fish. In Proceedings of the International Conference on Transport of Persistent Chemicals in Aquatic Ecosystems. Ottawa, Canada May 1-3, 1974. p. 3: 31-36.

de Freitas, A.S.W. and J.S. Hart. 1975. Effect of body weight on uptake of methylmercury in fish. Water Quality Parameters ASTM STP 573. p. 356-363.

de Freitas, A.S.W., M.A.J. Gidney, A.E. McKinnon and R.J. Norstrom. 1977b. Factors affecting whole-body retention of methylmercury in fish. In Biological Implications of Metals in the Environmental. Proceedings of the 15th Annual Hanford Life Sciences Symposium, Richland, Washington, Sept. 29-Oct. 1, 1975. p. 441-451.

Dokiya, S., S. Yamazaki, and K. Fuwa. 1974. Loss of trace elements from natural water during storage. Environ. Lett. 7: 67-81.

Fagerstrom, T. and A. Jernelov. 1971. Formation of methylmercury from pure mercuric sulfide in aerobic organic sediment. Water Res. 5: 121-122.

Faterstrom, T. and B. Asell. 1973. Methylmercury accumulation in an aquatic food chain. A model and some implications for research planning. Ambio 2: 164-171.

Fagerstrom, T., B. Asell and A. Jernelov. 1974. Model for accumulation of methylmercury in northern pike Esox lucius. Oikos 25(1): 14-20.

Gavis, J. and J.F. Ferguson. 1972. The cycling of mercury through the environment. Water Res. 6: 989-1008.

Giblin, F.J. and E.J. Massaro. 1973. Pharmacodynamics of methylmercury in the rainbow trout (Salmo gairdneri): Tissue uptake, distribution, and excretion. Toxicol. Appl. Pharmacol. 24(18): 81-91.

Guarino, A.M., J.B. Anerson, J.B. Pritchard and D.P. Rall. 1976. Tissue distribution of (^{14}C) methylmercury in the lobster, Homarus americanus. J. Toxicol. Environ. Health 2(1): 13-24.

Hannerz, L. 1968. Experimental investigations on the accumulation of mercury in water organisms. Sweden Inst. Fr. Water Res. Drottningholm 48: 120-176.

Hargrave, B.T. 1970a. The utilization of benthic microflora by Hyalella azteca (Amphipoda). J. Anim. Ecol. 39: 427-437.

Hargrave, B.T. 1970b. Distribution, growth and seasonal abundance of Hyalella azteca in relation to sediment microflora. J. Fish. Res. Bd. Can. 27: 685-699.

Hem, J.D. 1970. Chemical behaviour of mercury in aqueous media. p. 19-24. In Mercury in the Environment. U.S. Geol. Surv. Prof. Pap. 713.

Huckabee, J.W., R.A. Goldstein, S.A. Janzen and S.E. Woock. 1975. Methylmercury in a freshwater food chain. In International Conference on Heavy Metals in the Environment. Toronto, Ontario, Canada. Oct. 27-31, 1975. p. 199-216.

Hughes, W.L. 1957. A physiochemical rationale for the biological activity of mercury and its' compounds. Ann. N.Y. Acad. Sci. 65: 454-460.

Jackson, H.H.T. 1912. A contribution to the natural history of Hyalella knickerbockeri Bate. Bull. Wis. Natur. Hist. Soc. 10: 49-60.

Jarvenpaa, T., A.M. Tillander, and J.K. Miettinen. 1970. Methylmercury half-time of elimination in flounder, pike and eel. Suom. Kemistil B43: 439-442.

Jensen, S. and A. Jernelov. 1969. Biological methylation of mercury in aquatic organisms. Nature; London 220: 753-754.

Jernelov, A. 1968. Laboratorieforsok rorande kvicksilvrets omvandling mellan olika forekomstformer. Vatten 4: 360-362.

_____. 1969. Conversion of mercury compounds. In M.W. Miller and G.G. Berg (eds.) Chemical Fallout. C.C. Thomas, Publisher, Springfield, Ill., p. 68-74.

Jernelov, A. 1972. Mercury in food chains. In R. Hartung and B.D. Dinman (ed.). Environmental Mercury Contamination. Ann Arbor Science Publishers Inc., Ann Arbor, Mich. p. 174-177.

_____ and H. Lann. 1971. Mercury accumulation in food chains. Oikos 22: 403-406.

Johnels, A.G., T. Westermark, W. Berg, P. Persson and B. Sjostrand. 1967. Pike and some other aquatic organisms in Sweden as indicators of mercury contamination of the environment. Oikos 18: 323-333.

Karbe, L.N. Antonacopoulos, and C. Schnier. 1975. The influence of water quality on accumulation of heavy metals in aquatic organisms. Verh. Internat. Verein. Limnol. 19: 2094-2101.

Landner, L. 1971. Biochemical model for the biological methylation of mercury suggested from methylation studies in vivo with Neurospora crassa. Nature 230: 542-453.

Lockhart, W.L., J.F. Uthe, A.R. Kenny, and P.M. Mehrle. 1972. Methylmercury in northern pike (Esox lucius): distribution, elimination and some biochemical characteristics of contaminated fish. J. Fish. Res. Board Can. 29: 1519-1523.

Lockwood, A.P.M. and W.R.H. Andrews. 1969. Active transport and sodium fluxes at moult in the amphipod, Gammarus duebeni. J. Exp. Biol. 51: 591-605.

Luoma, S.N. 1977. Physiological characteristics of mercury uptake by two estuarine species. Mar. Biol. (Berl). 41(3): 269-274.

MacLeod, J.C. and E. Pessah. 1973. Temperature effects on mercury accumulation, toxicity and metabolic rate in rainbow trout (Salmo gairdneri). J. Fish Res. Board Can. 30: 485-492.

Magos, L. 1971. Section atomic-absorption determination of inorganic mercury and methylmercury in undigested biological samples. Analyst 96: 847-853.

Matida, Y., H. Kumada, S. Kimura; Y. Saiga, T. Nose, M. Yokote and H. Kawatsu. 1971. Toxicity of mercury compounds to aquatic organisms and accumulation of the compounds by the organisms. Bull. Freshwater Fish. Res. Lab Tokyo 21: 197-225.

Miettinen, J.K., T. Rahola, T. Hattula, K. Rissanen, and M. Tillander. 1971. Elimination of ^{203}Hg-methylmercury in man. Ann. Clin. Res. 3: 116-122.

Miettinen, J.F., M. Heyraud and S. Keckes. 1972. Mercury as hydrospheric pollutant. 11. Biological half-time of methylmercury in four Mediterranean species: a fish, a crab, and two molluscs. In M. Ruivo (ed.). Marine Pollution and Sea Life. Fishing News (Books), London p. 295.

Miller, D.R., Akagi, H. and Kudo, A. 1977. Generation of methylmercury in river sediments. In Ottawa River Project. Final Report, N.R.C.C., Ottawa, pp. 191-19-24.

Murphy, P.G. and J.V. Murphy. 1971. Correlation between respiration and direct uptake of DDT in the mosquito fish Gambusia affinis. Bull. Environ. Contam. Toxicol. 6: 581-588.

Newton, D.W. and R. Ellis, Jr. 1974. Loss of mercury (11) from solution. J. Environ. Qual. 3: 20-23.

Norstrom, R.J. and J.D. Peter. 1973. Chemical analysis of field samples. In J.S. Hart (ed.). Distribution and Transport of Persistent Chemicals in Flowing Water Ecosystems. Interim Report No. 1. Ottawa River Project. Div. Biol. Sci., National Research Council of Canada, Ottawa, p. 1-38.

Norstrom, R.J., A.E. McKinnon, A.S.W. de Freitas, and D.R. Miller. 1975. Pathway definition of pesticide and mercury uptake by fish. Environmental Quality and Safety 4: 811-816.

Norstrom, R.J., A.E. McKinnon and A.S.W. de Freitas. 1976. A bioenergetics based model for pollutant accumulation by fish. Simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (Perca flavescens). J. Fish. Res. Board Can. 33: 248-267.

Nuorteva, P. and E. Hasanen. 1971. Observations on the mercury content of Myxocephalus quadricornis (L.) (Teleostei, Cottidae) in Finland. Ann. Fennici. 8: 331-335.

Pennacchioni, A., R. Marchetti and G.F. Gaggino. 1976. Inability of fish to methylate mercuric chloride in vivo. J. Environ. Qual. 5(4): 451-454.

Pentreath, R.J. 1976. The accumulation of mercury from food by the plaice, Pleuronectes platessa L. J. Exp. Mar. Biol. Ecol. 25(1): 51-56.

Phillips, G.R. and D.R. Buhler. 1978. The relative contributions of methylmercury from food or water to rainbow trout (Salmo gairdneri) in a controlled laboratory environment. Trans. Am. Fish. Soc. 107(6): 853-861.

Pringle, B., D. Hissong, E. Katz and S. Mulawka. 1968. Trace metal accumulation by estuarine mollusks. J. Sanit. Engng. Div. Am. Soc. Civ. Engrs. 94(5970): 455-475.

Ray, G.L. and M.R. Tripp. 1976. The uptake of mercury from water by the grass shrimp, Palaemonetes vulgaris (Say). J. Environ. Qual. 5(2): 193-196.

Reichardt, H. and Bonhoeffer. 1930. Absorptionspectrem von geldstem Quecksilber. Z. Electroch. 36: 753.

Reinert, R.E., L.J. Stone and W.A. Willford. 1974. Effect of temperature on accumulation of methylmercuric chloride and p,p.'DDT by rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 31(10): 1649-1652.

Ruohtula, M. and J.K. Miettinen. 1975. Retention and excretion of ^{203}Hg-labelled methylmercury in rainbow trout. Oikos 26(3): 385-390.

Sharpe, M.A., A.E. McKinnon, and A.S.W. de Freitas. 1975. The effect of body weight on clearance of methylmercury in the goldfish, Carassius auratus. Abstracts of Int. Conf. on Heavy Metals in the Environment. Oct. 27-31, 1975, Toronto, Ontario. p. C26-C28.

Sharpe, M.A., A.S.W. de Freitas and A.E. McKinnon. 1977. The effect of body size on methylmercury clearance by goldfish. Env. Biol. Fish. 2(2): 177-183.

Shin, E.B. and P.A. Krenkel. 1976. Mercury uptake by fish and biomethylation mechanisms. J. Water Pollut. Control Fed. 48(3 Part 1) 473-501.

Sidgewick, N.V. 1950. The Chemical Elements and their Compounds. Vol. 1. Oxford Univ. Press, London. p. 301, 324.

Sloan, J.P., J.A.J. Thompson, and P.A. Larkin. 1974. The biological half-life of inorganic mercury in the Dugeness crab (Cancer magister). J. Fish. Res. Board Can. 31(10): 1571-1576.

Smith, A.L., R.H. Green and A. Lutz. 1975. Uptake of mercury by freshwater clams (family Unionidae). J. Fish. Res. Board Can. 32: 1297-1303.

Spangler, W.J., J.L. Spigareli, J.M. Rose, R.S. Flippin and H.H. Miller. 1973. Degradation of methylmercury by bacteria isolated from environmental samples. Appl. Microbiol. 25: 488-493.

Stock, A. 1934. Uber Verdampfung, Loslichkeit und Oxidation des metallischen Quecksilbers. Z. Anorg. Chem. 217: 241.

Suzuki, T. and M. Hatanaka. 1975. Experimental investigation on the biological concentration of mercury-11. On the origin of mercury found in the body of young yellowtail. Bull. Jap. Soc. Scient. Fish 41: 225-231.

Terhaar, C.J., W.S. Ewell, S.P. Dziuba, W.W. White and P.J. Murphy. 1977. A laboratory model for evaluating the behaviour of heavy metals in an aquatic environment. Water Res. 11(1): 101-110.

Trudel, B.K. 1979. Biokinetics of mercury in Daphnia magna. Ph.D. Thesis, University of Ottawa (in preparation).

Unlu, M.Y., M. Heyraud and S. Keckes. 1972. Mercury as hydro spheric pollutant. 1. Accumulation and excretion of $^{203}HgCl_2$ in Tapes decussatus L. In M. Ruivo (ed.). Marine Pollution and Sea Life. Fishing News (Books), London. p. 292.

Vernberg, W.B. and J. O'Hara. 1972. Temperature-salinity stress and mercury uptake in the fiddler crab, Uca pugilator. J. Fish. Res. Board Can. 29: 1491-1494.

Webb, J.L. 1966. Enzyme and metabolic inhibitors. Vol. 11. Malonate, analogs, dehydroacetate, sulfhydryl reagents. O-iodobenzoate, mercurials. Academic Press, Inc. New York, N.Y. p. 635-651, 729-963.

Weisbart, M. 1973. The distribution and tissue retention of mercury-203 in the goldfish (Carassius auratus). Can. J. Zool. 51: 143-150.

Westoo, G. 1967. Determination of methylmercury compounds in foodstuffs 11. Determination of methylmercury in fish, egg, meat and liver. Acta Chem. Scand. 21: 1790-1800.

Westoo, G. 1968. Determination of methylmercury salts in various kinds of biological material. Acta Chem. Scand. 22: 2277-2280.

Wildish, D.J. and V. Zitko. 1971. Uptake of PCB by Gammarus oceanicus. Mar. Biol. 9: 213-218.

Wisely, B. and R. Blick. 1967. Mortality of marine invertebrate larvae in mercury, copper and zinc solutions. Aust. J. Mar. Freshwater Res. 18: 63-72.

Wood, J.M., F.S. Kennedy and C.G. Rosen. 1968. Synthesis of methylmercury compounds by extracts of a methanogenic bacterium. Nature 220: 173-174.

Zitko, V., B.J. Finlayson, D:J. Wildish, J.M. Anderson and A.C. Kohler. 1971. Methylmercury in freshwater and marine fishes in New Brunswick, in the Bay of Fundy, and on the Nova Scotia Banks. J. Fish. Res. Board Can. 28: 1285-1291.