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ABSTRACT

Uptake of methylmercury from food and water and its retention by Daphnia magna Straus were determined using radioactive tracer methods. Accumulation of methylmercury via the water vector could be described mathematically as a two-compartment system, with no apparent exchange between compartments. Kinetic parameter values for these two compartments differed dramatically, with the slower-clearing compartment reaching 90% equilibrium after about 14 days of exposure compared to only eight hours for the faster-clearing compartment. Adult D. magna took up methylmercury from water at a rate of 2000 grams of water cleared of mercury per gram of organism per hour. Most of this methylmercury was taken up into the rapidly-equilibrating compartment with only about 25% reaching the slowly-equilibrating compartment. The uptake rate of inorganic mercury from water was similar to that of methylmercury. Accumulation of inorganic mercury was a multicompartmental process with faster fractional clearance rates than for methylmercury.

Uptake rate of methylmercury accumulated from water increased proportionately with the .63 power of body weight, which is similar to the value that would be expected if uptake rate had increased in proportion to metabolic rate. The effect of temperature on methylmercury uptake supported the direct

relationship between metabolic rate and uptake rate of methylmercury from water. The fractional clearance rate of methylmercury accumulated via the water vector was independent of both body size and temperature.

Assimilation efficiency from food strongly favoured the preferential uptake of methylmercury over inorganic mercury. Assimilation ranged from 60 - 90% for methylmercury and 5 - 20% for inorganic mercury. Assimilation efficiency of methylmercury was independent of body size but was inversely related to food ingestion rate. Methylmercury assimilation efficiency was temperature-dependent with an optimum value at 20°C. Inorganic mercury assimilated from the diet was lost much more quickly (biological half-time = 12 hours) than methylmercury (biological half-time = 5 days). The fractional clearance rate of methylmercury assimilated from food was independent of body size and temperature.

A model was developed based on pollutant bioaccumulation kinetics coupled to Daphnia energetics. This model was used to estimate the concentration of methylmercury in an individual Daphnia exposed continuously from birth to constant conditions of methylmercury contaminated food and water. A possibly important simulation result was that very high concentration factors for methylmercury between water and Daphnia tissue (C.F. $\approx$ 300,000) were achieved by age five days, with only a 30% further increase in tissue concentration by the time the individual was a senior adult at age 40 days.

RESUME

L'absorption du mercure méthylé à partir de la nourriture et de l'eau et sa rétention par Daphnia magna Straus one été estimées par dépistage radioactif. L'accumulation du mercure méthylé par le vecteur d'eau peut être décrite mathématiquement par un système à deux compartiments, avec aucun échange apparent entre les deux compartiments. Les valeurs des paramètres cinétiques pour ces deux compartiments varièrent de façon dramatique, un compartiment atteignant 90% de son équilibre après environ 14 jours d'exposition comparativement à huit heures pour le compartiment à élimination rapide. Les D. magna adultes absorbèrent le mercure méthylé à partir de l'eau à un taux de 2000 grammes d'eau par gramme d'organisme par heure. La majeure partie de ce mercure méthylé fut absorbé par le compartiment qui s'équilibre rapidement, environ 25% seulement atteignant le compartiment qui s'équilibre lentement. Le taux d'absorption de mercure inorganique à partir de l'eau fut semblable à celui du mercure méthylé. L'accumulation du mercure inorganique fut un processus multicompartimental avec des taux d'élimination fractionnelle plus grands que pour le mercure méthylé.

Le taux d'absorption du mercure méthylé accumulé à partir de l'eau augmente d'une façon proportionelle avec le poids du corps porté à la puissance 0.63. Cette valeur est similaire à

la valeur attendue si le taux d'absorption augmentait d'une façon proportionelle au taux métabolique. L'effet de la température sur l'absorption du mercure méthylé confirme la relation entre le taux métabolique et le taux d'absorption du mercure méthylé de l'eau. Le taux d'élimination fractionnelle accumulé par le vecteur d'eau fut indépendant de la grosseur du corps et de la température.

L'efficacité d'assimilation du mercure méthylé à partir de la nourriture est beaucoup plus grande que celle du mercure inorganique. L'assimilation s'échelonne de 60 - 90% pour le mercure méthylé et de 5 - 20% pour le mercure inorganique. L'efficacité d'assimilation du mercure méthylé fut indépendante de la grosseur du corps mais inversement proportionelle au taux d'ingestion de nourriture. L'efficacité d'assimilation de mercure méthylé fut dépendante de la température atteignant une valeur optimale à 20°C. Le mercure inorganique assimilé à partir de la diète fut perdu beaucoup plus rapidement (demie-vie biologique = 12 heures) que le mercure méthylé (demie-vie biologique = 5 jours). Le taux d'élimination fractionnelle du mercure méthylé assimilé à partir de la nourriture fut indépendant de la grosseur du corps et de la température.

La cinétique de la bioaccumulation d'un polluant en rapport avec l'énergétique de Daphnia fut utilisée pour développer un modèle. Ce modèle fut utilisé pour estimer la concentration de

mercure méthylé dans un individu Daphnia soumis d'une façon continue dès la maissance à des conditions constantes de contamination de la nourriture et de l'eau par du mercure méthylé. Un résultat de cette simulation qui peut être d'importance fut que des facteurs de concentrations très élevées de mercure méthylé entre l'eau et les tissus de Daphnia (C.F. 300,000) furent atteints à l'âge de cinq jours avec seulement une augmentation de 30% dans la concentration des tissus lorsque l'individu adulte atteint l'âge de 40 jours.

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INTRODUCTION

Development of modern technology has been accompanied by a dramatic increase in production and consumption of chemicals. In many cases benefits of using a chemical have been associated with unanticipated damage to human health and environmental quality. Chemicals such as DDT, PCB's and mercury are a few examples that demonstrate the necessity for exercising close control over use of all chemicals. The degree of restriction to be imposed on the use of chemicals is usually determined by a tradeoff between economic and social costs and hazard to human health and environmental quality. Decisions in this area have traditionally favoured actions that have minimized economic costs at the expense of environmental quality. This is due, in part, to a general lack of confidence in the reliability of biological impact predictions. Hence, the need to improve methods of evaluating environmental hazard is critical and this problem represents a difficult challenge to biologists.

Assessment of chemical hazard requires the ability to predict in quantitative terms: 1) the concentrations of pollutant to which the organism is exposed; 2) the dose of pollutant experienced by a target organism under natural conditions; 3) the effects of that dose on the well-being of the organism; and 4) the role of the organism in maintaining ecosystem normalcy. Information on chemical effects in controlled dose/response systems can be

obtained from fairly standard laboratory protocols in toxicology. Extrapolation of this type of information to natural environments, however, will usually not result in valid conclusions for obvious reasons. A particularly important and yet relatively unresolved problem involves predicting exposure levels and resulting dose rates to organisms in aquatic environments. Methods involving a biomathematical approach are currently being developed, but a lack of suitable data on movement and bioaccumulation of any specific problem pollutant usually limits the useful application of the biomathematical approach.

This thesis takes a biomathematical approach to the problem of assessing the exposure/dose relationship in a fresh water ecosystem. It concentrates on questions of dose definition, i.e., how much and what type of information is needed to establish quantitative estimates of pollutant dose to filter feeding animals exposed to the pollutant in the water and food of their natural environment? This was done by focusing on various aspects of the accumulation of methylmercuric chloride and mercuric chloride, using Daphnia magna Straus as a model component of an aquatic ecosystem.

The following few brief statements will serve to introduce the subject of mercury accumulation in aquatic organisms, but no formal literature review will be made here since the

extensive literature on mercury has been thoroughly reviewed in at least two recent publications (National Research Council of Canada, 1979; Sherbin, 1979). Mercury occurs naturally in aquatic systems and must always have been present in these systems although at trace levels. Mercury can exist in three oxidation states: the metal; monovalent mercurous ion; and the divalent mercuric ion (Jonasson and Boyle, 1971). It can be assumed that methylmercury has also been present in aquatic systems for nearly as long as life forms have existed, since methylmercury is biosynthesized by aquatic bacteria (Jensen and Jernelov, 1969). The chemical stability of methylmercury and its greater lipophilicity compared to inorganic forms of mercury result in a higher bioaccumulation potential for methylmercury than inorganic mercury. This accounts, at least in part, for the greater toxicity of methylmercury than inorganic mercury. In the late 1960's mercury was found to be widely distributed in aquatic environments and it was found to occur in fish in high enough concentrations to pose a hazard to human health (Westoo, 1966). Since that time the vast majority of the research effort on mercury bioaccumulation has focused on the problem in fish. This work is reviewed by several authors (Armstrong, 1979; DeFreitas, 1979; Fagerstrom et al, 1974; Norstrom et al, 1976). Several attempts have been made to unify this body of information by the formulation of bioaccumulation models (Fagerstrom et al, 1974; Norstrom et al, 1976). Norstrom et al (1976) proposed a simple but general approach to modelling the pollutant bioaccumulation process in fish by assuming that the

uptake of all compounds via food and water vectors can be related to metabolic rate which can in turn be related to easily-measured parameters such as temperature, body size and growth rate. In the Norstrom model pollutant clearance was also related to body size but not to metabolic rate. The validity of the model and of the parameter values used in testing it appear to hold well for chemicals such as methylmercury and PCB's in fish. To date, however, it has not been possible to test the applicability of the model to other aquatic biota due to a lack of data on the pollutant dynamics in other taxa, particularly small metazoans.

The limited information regarding natural exposure conditions, accumulation kinetics and ecotoxicity of mercury and Daphnia can be summarized as follows. Concentrations of total mercury in Canadian fresh water lie in the range of .02 to .7 ug Hg l^{-1} (Sherbin, 1979). The few published estimates of methylmercury levels in fresh water, ranging from 0 - 4 x 10^{-3} ug Hg l^{-1}, are questionable since these values lie at or below the limits of detection of available analytical methods. The total mercury content of some fresh water plankton lies in the range of .01 to .1 ug Hg g^{-1}, d.w. There is a growing body of information suggesting that organic mercury makes up 10% or more of the mercury body burden. Huckabee et al (1975) suggested a single compartment first order model to describe methylmercury accumulation from water by Daphnia pulex with values for mercury uptake and loss rate constants of 300 - 400 ml of water cleared of mercury per gram d.w. of Daphnia per hour and .007 - .01 hr^{-1}, respectively. Little detailed work

has been done on mercury uptake via the food vector. Toxicity of mercury compounds to Daphnia has often been studied, but some recent work by Biesinger et al (1980) is of particular interest. Biesinger determined that a detectable increase in mortality rates in D. magna was caused by chronic exposure to ambient concentrations of methylmercury and inorganic mercury of .2 and 2 ug Hg l^{-1}, respectively, while detectable impairment of reproduction potential was observed at concentrations of .04 and .7 ug Hg l^{-1}, respectively. Daphnia magna populations appear to tolerate concentrations of methylmercury and inorganic mercury in their tissues as high as 75 and 23 ug Hg g^{-1}, d.w., respectively, without detectable increases in mortality rates, but tissue levels of only .9 and 15 ug Hg g^{-1}, d.w., respectively, cause reductions in reproduction rates.

As stated above, the aim of the present work was to study the accumulation of methylmercury and inorganic mercury by Daphnia magna. To this end the following studies were undertaken:

(a) a descriptive study of the accumulation of mercury by D. magna during brief exposures to mercury via food and water vectors;

(b) a study of the effects of body size and temperature on accumulation by D. magna of methylmercury via food and water vectors.

In order to relate this information to the problem of assessing pollutant dynamics under natural conditions, a bioaccumulation model was then formulated to calculate the concentration of mercury in the tissues of Daphnia magna at any time during its life history.

MATERIALS AND METHODS

Culturing of Daphnia magna.

Daphnia magna were cultured from a stock obtained from the Department of Zoology at the University of Toronto.

Daphnia magna were maintained in continuous culture in three litre volumes of dechlorinated Ottawa City tap water[1] with a specific conductivity of 95 - 125 $umho^{-1}$ cm^{-2}, pH 7.4 - 7.6 at room temperature of 20 ± 1°C at a light intensity of 40 - 60 ft-cdl with a 12L/12D photoperiod maintained automatically. Three-quarters of the water in each culture was replaced every second day. Daphnia were fed the Daphnia food stock described below at a rate of one ml of food stock per three litres of culture every second day.

Preparation of Daphnia Food Stock.

Daphnia food stock was prepared by soaking 10 g dry Purina Trout Chow and one g dry Fleischmann's yeast in 250 ml of a 4% solution of dextrose for one hour and then mixing vigourously in a Waring blender (model no. F.C.1.15) for five minutes. The resulting suspension was made up to 300 ml with water. Daphnia

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[1] Unless otherwise specified, all water used in these studies was dechlorinated Ottawa City tap water.

were fed at a rate of one ml of food stock per three litres of culture every second day or whenever the water in the cultures was replaced. The food stock was refrigerated and fresh stock was prepared every second week.

Preparation of Stock Yeast Suspension and Uncontaminated Feeding Suspension.

The stock yeast suspension used in the preparation of the uncontaminated feeding suspensions and the ^{203}Hg-contaminated feeding suspensions was itself prepared by adding one gram of Fleischmann's dry yeast (Standard Brands Canada Ltd.) to 100 ml of 4% glucose solution in tap water and incubating for four hours at 40°C. The resulting suspension was filtered through 25u pore size Nitex screening and then centrifuged gently for five minutes and the residue resuspended in 100 ml of 4% glucose solution at 40°C. The concentration of cells was then determined by optical density, using a Coleman spectrophotometer model 295 at 650 nm.

The uncontaminated feeding suspensions were prepared by adding enough of the stock yeast suspension to 3500 ml of water to yield a suspension of the required cell density.

Radioactive Labelled Mercury Compounds.

^{203}Hg-labelled compounds were purchased from New England Nuclear[1] (NEN) as aqueous solutions of methylmercuric

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[1]New England Nuclear; 575 Albany Street; Boston, Mass.; 02118

chloride and mercuric chloride with specific activities of 1 - 2 mCi/mg Hg and 5 - 7 mCi/mg Hg, respectively. Immediately following delivery, ^{203}Hg-labelled material was diluted with Ottawa City tap water. The diluted NEN stock was stored as 10 ml volumes in 20 ml vaccutainer tubes for easy subsampling when required.

The initial concentration of radioactivity in the diluted stock solution was usually 10 - 20 uCi/ml or less. At this dilution radiation-induced chemical breakdown of the CH_3HgCl is insignificant. The diluted NEN stock of methylmercuric chloride can be used for up to six months after purchase without a significant increase in the level of ^{203}Hg-labelled impurities (Sharpe et al, 1977).

Preparation of Mercury-contaminated Water.

The ^{203}Hg-contaminated water used in experiments was prepared by adding $^{203}HgCl_2$ or $CH_3{}^{203}HgCl$ stock solution to water that had been filtered (under vacuum through Whatman GF/C filters) to remove all particles of ingestible size. The solution was then stirred vigorously and was allowed to stand for at least one hour, thus ensuring that the concentration and bioavailability of Hg in the exposure medium remained as constant as possible during the period of exposure. The concentration of mercury in the exposure medium was measured at the beginning and end of each exposure

period by determining the radioactivity in aliquots of exposure medium. The volume of exposure medium used in each experiment was always adjusted according to the number of animals being tested, so that removal of mercury from the medium by D. magna would result a decline of 10% or less in the mercury concentration of the medium.

Preparation of ^{203}Hg-contaminated Food.

The ^{203}Hg-contaminated food was prepared by adding .5 to 2.0 ml of stock isotope solution of either ^{203}Hg-labelled CH_3HgCl_2 or $HgCl_2$ to 2.0 - 5.0 ml of stock yeast suspension containing 50 - 90 x 10^6 cells ml^{-1}. This mixture was incubated for 14 hours at 40°C. The mixture was then diluted to 20 ml with water at 20°C, centrifuged gently for five minutes, and the supernatant containing the unabsorbed ^{203}Hg was discarded. The ^{203}Hg-laden cells were then washed to remove loosely bound ^{203}Hg by suspending them in 20 ml of water, centrifuging, and decanting the supernatant. The cells were washed six times, the last wash removing only about 2% of the remaining ^{203}Hg content of the yeast. After the final washing, the cells were resuspended in 10 ml of water, the cell density determined, and the suspension diluted to the density required in the experiment. The suspension was allowed to stand for one hour prior to use to allow any remaining loosely bound mercury to partition between cells and the aqueous phase.

Measurement of Radioactivity.

The radioactivity in Daphnia, yeast, and water were measured using a Bicron deep-well NaI crystal detector and Inotech Multichannel Analyser. The approximate counting efficiency of this system was determined by measuring radioactivity of samples from newly arrived shipments of ^{203}Hg-labelled $HgCl_2$ or CH_3HgCl, the radioactivity of which was known from the supplier. Counting efficiency of the system was approximately 30 per cent. Constancy of counting efficiency over extended periods was verified from daily measurements of radioactivity of a standard consisting of ^{203}Hg-labelled CH_3HgCl adsorbed onto activated charcoal, sealed within a vial placed within a standard scintillation vial.

Radioactivity content of Daphnia was generally measured using groups of 5 to 10 animals in two ml of water in a scintillation vial. Counting times for Daphnia varied slightly according to the radioactivity content of the animals but never exceeded five minutes. Radioactivity of yeast suspensions and water were determined using 2 ml samples of either material in scintillation vials. Counting times for these samples were varied so that a minimum of 1000 counts were accumulated per sample. The error in counting due to natural uncertainty under these conditions is approximately 4% (International Atomic Energy Agency, 1966).

A Nuclear Chicago automated deep-well gamma counter was

used to measure the radioactivity of samples from the benzene-water extraction procedure. Approximate counting efficiency and day-to-day variation in counting efficiency were determined as with the Bicron/Inotech system. The counting efficiency of the Nuclear Chicago counter was about 39%.

Chemical Fractionation of Mercury.

Purity of diluted NEN stock of methylmercuric chloride was tested from time to time using a modification of the Westöö procedure for determination of methylmercury salts in fish tissue and other samples (Westoo, 1966).

For a 100 ml sample of aqueous exposure medium, the following procedure was used for separation of methylmercury and inorganic mercury. The 100 ml sample was placed in a 250 ml stoppered graduated cylinder. An aliquot (7.5 ml) of each of 1000 ug/ml solutions of methylmercuric chloride and mercuric chloride were added as carrier material. Sodium chloride (19 g), concentrated hydrochloric acid (30 ml), and benzene (60 ml) were then added and the mixture was stoppered and shaken for 60 seconds. The mixture was allowed to stand until the phases separated. The benzene layer was removed and placed in a 100 ml graduated cylinder. Benzene extraction was repeated two more times. The final volumes of the aqueous phase (inorganic mercury) and benzene phase (organic mercury) were then recorded. 10 ml of each phase

were then placed in separate vials and the radioactivity concentration in each was determined using a Nuclear Chicago deep-gamma counter. The relative amounts of organic and inorganic mercury in the sample were then calculated.

A similar procedure was used for separating organic mercury from inorganic mercury in Daphnia. Two Daphnia containing mercury-203 were placed in a modified 15 ml glass centrifuge tube. The bottom of this tapered tube had been ground and a glass rod had been ground to fit it. The sample in the tube was made up to 3 ml with tap water and 0.5 ml of each of methylmercuric chloride (1000 ug Hg/ml) and mercuric chloride (1000 ug Hg/ml). Daphnia were then crushed thoroughly with the glass rod. The rod was rinsed with 1.0 ml of tap water and was removed. The following were then added to the tube: 2 ml concentrated HCl, 1 g NaCl, and 4 ml benzene. The contents were then mixed vigourously in a vortex mixer for one minute and were then allowed to stand for five minutes. The benzene layer was removed and stored. The benzene extraction was repeated two more times. All of each of the benzene fraction and the aqueous fraction were then radioassayed for mercury-203.

Determination of Daphnia Body Size.

Unless otherwise stated, animals used in these studies

were young adult females ranging in body size from 2.5 to 3.0 mm in total length. Body length, the distance from the top of the helmet to the base of the shell spine, was measured to the nearest .07 mm using a dissecting microscope with an ocular micrometer. Values for body weight of Daphnia used in these studies were estimated using the body length-dry weight relationship determined for Daphnia magna by Schindler (1968).

Data Handling.

Ingestion rate, assimilation efficiency of mercury and retention of ingested mercury were estimated from the data using the methods outlined by Bourne (1959) for the study of ^{14}C-dynamics in Daphnia magna. Bourne assumed that the time course of changes in radioactivity content of Daphnia would be as illustrated in Fig. 1A. A suspension feeder such as Daphnia magna exposed to a suspension of food containing radioactive-labelled mercury would feed at a constant rate, and the whole body radioactivity content would increase at a constant rate, as illustrated by the straight line AB in Fig. 1A, as long as the duration of exposure was shorter than the minimum time required for a food particle to pass completely through the alimentary canal and be egested. When Daphnia are transferred to a suspension containing non-radioactive food, they should continue to feed at the same rate as they did when they were in the suspension of radioactive food and the

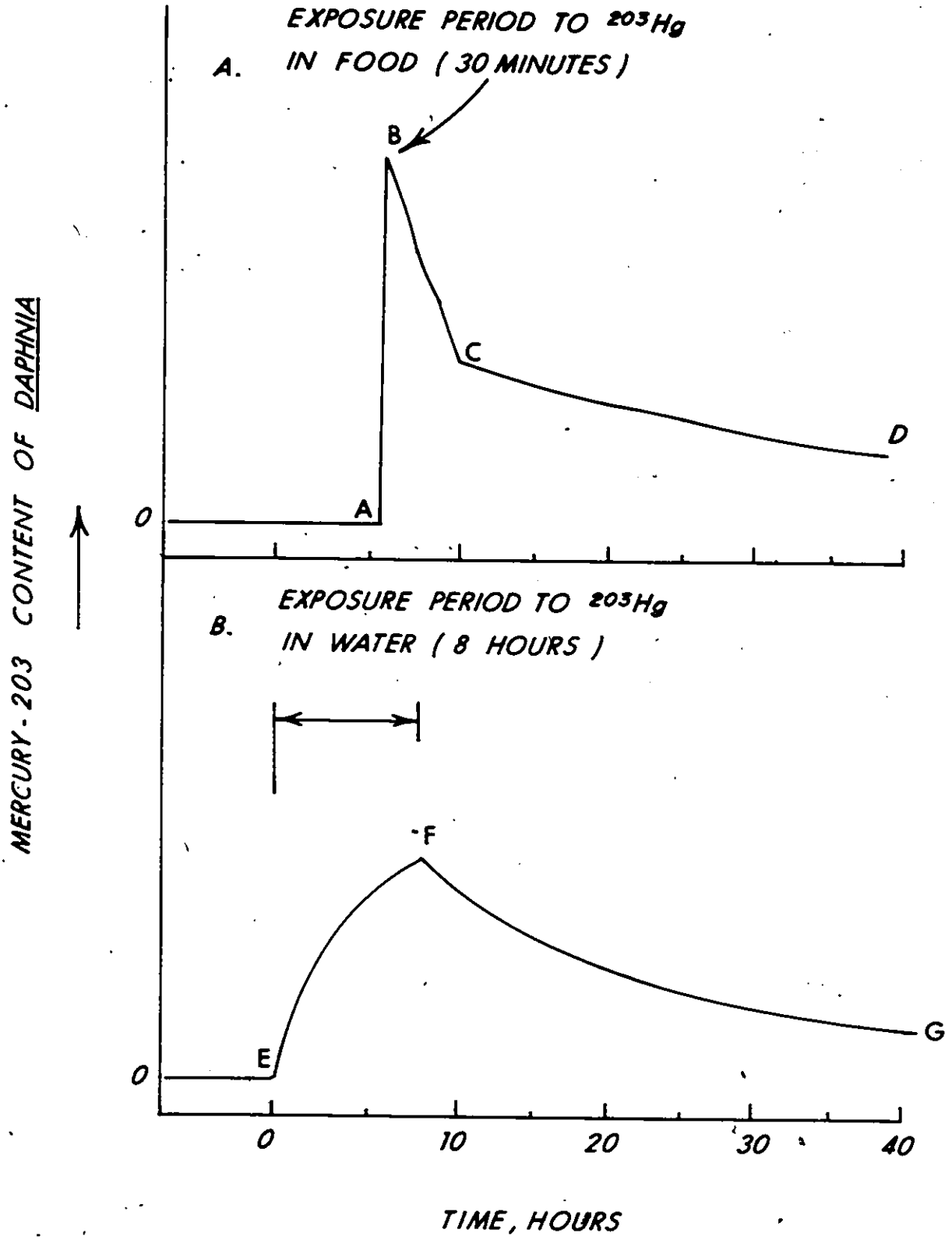
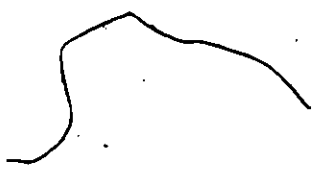

EXPOSURE PERIOD TO 203Hg
A. IN FOOD (30 MINUTES)
B
C
D
A
0
B. EXPOSURE PERIOD TO 203Hg
IN WATER (8 HOURS)
F
E
G
0
0
10
20
30
40
MERCURY-203 CONTENT OF DAPHNIA
TIME, HOURS

FIG 1

Fig. 1. Time course of changes in the radioactivity content of Daphnia during and after exposure to ^{203}Hg-labelled methylmercury in food (A) and water (B).



ingested non-radioactive food should replace the radioactive food in the gut. The radioactivity in Daphnia should drop rapidly, as illustrated by the line BC in Fig. 1A, because of the egestion of feces containing unassimilated radioactivity until all the unassimilated radioactivity has been voided from the gut. The radioactivity will not decline immediately to zero, however, since during the rapid loss of radioactive tracer from the gut a portion of the radioactive-labelled material is retained by Daphnia and this radioactivity is lost at a slower rate (CD, Fig. 1A). This radioactivity represents mercury that has been assimilated from the food into the tissues of Daphnia and the slope of the decrease of this curve will yield a measure of the rate of clearance of methylmercury from the tissues.

The anticipated time course of changes in mercury-203 radioactivity in Daphnia during an experiment on the uptake of mercury from water are illustrated in Fig. 1B. Daphnia exposed to radioactive-labelled mercury in water would absorb radioactivity at a constant rate. The increase in the radioactivity content of Daphnia would not be linear (line EF, Fig. 1B), however, because some of the mercury that is taken up is lost immediately. Since the rate of loss of radioactivity is proportional to the radioactivity content of Daphnia, the rate of mercury loss will increase as radioactivity content of Daphnia increases. If exposure to radioactive material continues for long enough, the

rate of loss of radioactivity will become equal to the rate of uptake and the radioactivity content of the organism will increase no further. Some compounds require very long exposures to reach this steady-state condition and it is not practical to continue exposure until steady state is reached. When Daphnia are transferred to a non-radioactive medium, the uptake of radioactivity ceases but the loss of radioactivity continues at the same fractional rate as during exposure. The radioactivity content of Daphnia may decline to zero in a smooth curvilinear fashion, a constant proportion of the body burden being lost per unit time. The slope of the decrease of this curve will yield a measure of the rate of clearance of methylmercury from the tissues.

A single compartment model is seldom sufficient to describe the kinetics of accumulation from water. Generally models of two or more compartments are required. For the results of studies of the uptake of methylmercury and inorganic mercury from water, the minimum number of compartments, compartment size, and fractional clearance rates were determined by means of compartmental analysis. Single and multiple exponential curves were fitted to the clearance data of experiments involving methylmercury and inorganic mercury in which the duration of exposure was varied from 2 to 12 hours. A curve described by a single exponential term was fitted first and then the number of exponential terms was increased until a further increase in the number of exponentials

did not result in a significant reduction in the sum of squares of residuals as determined by an F-test. Curve fitting was performed using the method of Marquardt (1963).

In most experiments mercury loss from Daphnia involved only two compartments. Clearance data for each replicate group was inspected to ensure that mercury loss after 24 hours postexposure involved only a single compartment and the best-fitting single exponential curve was then determined for the data beyond 24 hours postexposure by the method of least-squares. The equation

$$\ln Bt = \ln Bo - k.t$$

was used, where Bt is the mercury content of the slow-clearing compartment (ug Hg Daphnia) at time t (hours), Bo is the mercury content of the slow-clearing compartment at one minute postexposure, and k is the fractional clearance rate ($hours^{-1}$).

To determine the fractional clearance rate for the fast-clearing compartment in water vector studies the mercury content of the fast compartment at t hours postexposure, At, was estimated by subtracting the estimated mercury content of the slow compartment, Bt, from the observed mercury body burden of Daphnia, Yt, as

$$At = Yt - Bt$$

where

$$Bt = Boe^{-k.t}$$

The method of least-squares was then used to determine the slope of the mercury clearance curve for the fast compartment.

Procedure for Studying Accumulation of Mercury from Water.

The rate of mercury uptake from water by Daphnia was determined by exposing groups of adult female Daphnia ranging in size from 2.5 to 3.0 mm total length[1] to known concentrations of ^{203}Hg-labelled CH_3HgCl or $HgCl_2$ for eight hours at 20 ± 1°C. At the end of exposure the animals were washed for one minute by transferring them through a series of five x 200 ml volumes of water using a pipette. The amount of mercury accumulated by Daphnia during the exposure period was determined by radioassaying the animals for ^{203}Hg. All exposures were under static conditions.

After the ^{203}Hg body burden at one minute postexposure had been determined, animals were placed immediately into 3500 ml clean water containing food. Density of animals ranged from 5 - 20 animals per 3500 ml, depending on the size of the animals.[2] Mercury retention was determined from a series of measurements of the radioactivity content of the Daphnia taken over the next

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[1] Distance from the top of the helmet to the base of the shell spine.

[2] The density of animals was adjusted in order to minimize the possibility that the Daphnia would reabsorb significant levels of excreted ^{203}Hg. The densities used were such that the group of Daphnia would clear no more than 2% of the 3500 ml volume in 24 hours.

5 - 14 days. Measurements were generally made at hourly intervals for the first eight hours postexposure and at 24 hour intervals for the remainder of the study. Measurements were generally made on groups of animals. The medium was replaced at eight hour intervals during the first 24 hours and at 24 hour intervals for the remainder of the study.

Effect of Duration of Exposure on Mercury Uptake and Retention.

Five replicate groups of 20 - 24 adult female Daphnia of 2.4 - 2.7 mm body length were exposed to methylmercury in separate 1500 ml volumes of exposure medium containing approximately 2.3×10^{-3} ug Hg ml^{-1} as ^{203}Hg-labelled CH_3HgCl. From each group a sample of 5 - 8 animals was removed after 2, 4, 8, and 12 hours of exposure. Net uptake of mercury was determined for each group of Daphnia by radioassay for ^{203}Hg at one minute postexposure. Following the determination of net uptake of mercury, retention of accumulated ^{203}Hg was determined for each group of Daphnia from a series of radioassay made over the next six days.

Four replicate groups of 24 - 28 adult female Daphnia of 2.5 - 3.0 mm body length were exposed to inorganic mercury in separate 1500 ml volumes of exposure medium containing approximately 2.3×10^{-3} ug Hg ml^{-1} as ^{203}Hg-labelled $HgCl_2$. From each

group a sample of 6 or 7 animals was removed after 2, 4, 8, and 12 hours of exposure. Net uptake of mercury was determined for each sample by radioassay of Daphnia for ^{203}Hg at one minute postexposure.

Following the determination of net uptake of mercury, each sample of Daphnia was transferred to a separate 3500 ml volume of clean water. Retention of accumulated ^{203}Hg was determined for each sample from a series of radioassays made over the next 14 days.

Effect of Mercury Concentration in the Exposure Medium on Mercury Uptake and Retention.

Groups of 10 or 20 adult females ranging in body length from 2.5 to 3.0 mm were exposed to methylmercury in the water for eight hours in 2500 ml volumes of exposure medium containing methylmercury at five concentrations ranging from $.01 \times 10^{-3}$ to 5×10^{-3} ug Hg ml^{-1} as described above. At the end of exposure all groups, with the exception of the one exposed to the lowest Hg concentration, were split into two or four replicate subgroups of five animals per subgroup for measurement of net uptake of mercury at one minute postexposure. The group of 20 animals exposed to the lowest concentration of mercury, $.01 \times 10^{-3}$ ug Hg ml^{-1}, was measured as a group due to the extremely small quantity of Hg

which they had accumulated. Mercury retention was determined in the usual way for each subgroup that had been exposed.

Loss of Methylmercury on Moulting.

Twenty animals ranging in size from 2.0 to 3.3 mm body length were exposed for two hours to ^{203}Hg-labelled methylmercury in solution at a concentration of $.5 \times 10^{-3}$ ug Hg ml^{-1}. They were then transferred to clean water for 24 hours to allow complete loss of the fast-clearing compartment. Each animal was then transferred to a 20 ml glass vial containing 5 ml of water. Holes had been drilled in the walls of the vials to allow water to flow through the vial at a rate of 1 ml per minute. Daphnia were maintained in this system for four days without food. Mercury body burdens were determined daily for each individual. Shed exoskeletons and neonates were radioassayed separately for ^{203}Hg.

Effect of Body Size on Uptake and Retention of Methylmercury.

Three groups of 30 - 40 Daphnia per group composed of Daphnia of different sizes (1.0 - 1.3 mm, 1.9 - 2.3 mm, and 3.3 - 3.5 mm body length) were exposed for eight hours to methylmercury in 1500 ml volumes of exposure medium containing ^{203}Hg-labelled methylmercury at a concentration of approximately 0.8×10^{-3} ug Hg ml^{-1}. At the end of exposure groups were split into five replicate subgroups of 5 - 10 animals and net uptake of

mercury was determined by radioassay of animals at one minute postexposure. Following this determination mercury retention was determined for each group from a series of measurements of ^{203}Hg body burdens made over the next six days.

Effect of Temperature on Uptake from Water and Retention of Methylmercury.

In this study Daphnia magna cultures were maintained at temperatures of 5.5 ± 1, 10.5 ± 1, 15 ± 1, and 20 ± 1°C for four weeks prior to the experiment. This was sufficient time for completion of at least one generation at 10, 15, and 20°C. Only animals which had completed one generation at these temperatures were used. At 5°C reproduction and maturation proceeded so slowly that it was necessary to use animals that had been produced at 10°C but acclimated for four weeks at 5°C.

For this study 15, 30, 15, and 40 animals ranging in size from 3.1 to 3.3 mm body length were selected from cultures at 5.5, 10.5, 15, and 20°C, respectively. They were measured and placed in feeding suspension for 16 hours. One hour prior to the start of the experiment they were placed in food-free water at their acclimation temperatures. A single nine-litre batch of exposure medium was prepared containing ^{203}Hg-labelled methylmercury at a concentration of 2×10^{-3} ug Hg ml^{-1}. This was split into four volumes and placed in water baths at 5, 10, and 15°C,

respectively, for one hour to reach the necessary temperature.

At all temperatures Daphnia were exposed to methylmercury for eight hours. Net ^{203}Hg uptake was determined by radioassaying Daphnia for ^{203}Hg body burden at one minute postexposure in the usual way. Retention of this accumulated mercury was determined in the usual way over the next 15 days.

Procedures for Determining Ingestion Rate, Assimilation Efficiency and Clearance Rate of Mercury.

To determine the efficiency with which Daphnia assimilate ingested mercury, Daphnia were given a dose of mercury in the form of ^{203}Hg-contaminated food by having them feed on a suspension of ^{203}Hg-contaminated yeast. Daphnia were then transferred to a suspension of uncontaminated yeast cells where the unassimilated ^{203}Hg was voided from the gut. The amount of Hg assimilated by Daphnia was determined from the body burden of Daphnia following the loss of the unassimilated ^{203}Hg from the gut. Daphnia were maintained in the uncontaminated medium for an additional five days and the fractional clearance rate of the assimilated mercury was determined from the measurements of the radioactivity retained by Daphnia during this time. A more detailed description follows.

Daphnia, ranging in size from 2.5 to 3.0 mm body length, were selected from cultures and were held for 14 hours in a non-radioactive yeast suspension of .1 x 10^6 cells ml^{-1} to

precondition them to feeding on yeast cells at that concentration. Daphnia were given a dose of ^{203}Hg-labelled methylmercury or inorganic mercury by feeding them for 30 minutes in 70 ml volumes of ^{203}Hg-contaminated food (see page 9) at a density of $.1 \times 10^6$ cells ml^{-1}. Animals were generally fed in groups of 5 - 10 Daphnia per 70 ml volume of suspension in ground glass stoppered glass tubes. These tubes were inverted at 5 minute intervals during feeding to keep the cells in suspension. The mercury content of the suspension was determined by radioassaying 5 ml aliquots of exposure medium taken prior to exposure. As stated previously, the concentration of cells in the medium was estimated on the basis of the dilution of a stock suspension of known cell density.

At the end of the exposure period Daphnia were removed from the exposure medium using a wide-mouthed pipette and transferred through 5 x 200 ml volumes of uncontaminated feeding suspension in one minute. Daphnia were then radioassayed as a group before being transferred to a 250 ml volume of uncontaminated feeding suspension containing $.1 \times 10^6$ cells ml^{-1} where they were kept for eight hours. The amount of ^{203}Hg assimilated was determined from a series of measurements of the ^{203}Hg retained by Daphnia after 1, 2, 3, 4, 6, and 8 hours. The yeast suspension was replaced after 1, 2, 4, 6, and 8 hours.

Daphnia were then transferred to 3500 ml volumes of

water containing Daphnia feeding suspension. Clearance of assimilated mercury was determined from serial measurements of the ^{203}Hg body burden of Daphnia taken at 24 hour intervals for the next five days. This medium was replaced at 24 hour intervals.

Effect of Ingestion Rate on Assimilation and Clearance of Ingested Methylmercury.

Four replicate groups of five adult females, 2.5 - 3.0 mm in body length, were exposed for 30 minutes to radioactive yeast suspensions of different cell densities. Cell densities used were $.01 \times 10^6$, 0.1×10^6, and 0.5×10^6 cells per ml^{-1} as determined by dilution of a stock suspension of radioactive cells of known cell density. Yeast cells of each concentration contained the same concentration of ^{203}Hg-labelled methylmercury, about 1.1 mg Hg g^{-1} dry weight of food. At the end of the exposure period mercury content of Daphnia was determined by radioassaying each group of animals for ^{203}Hg. Groups were then transferred immediately to 250 ml volumes of uncontaminated yeast suspensions containing cell densities similar to those of the radioactive suspensions. The mercury content of Daphnia was measured at 1, 2, 4, 6, 8, and 24 hours postexposure and at 24 hour intervals for the next four days. The yeast suspension was replaced at two hour intervals for the first eight hours postexposure.

Effect of Body Size on Assimilation and Clearance of Ingested Methylmercury.

Three replicate groups of Daphnia selected from size ranges 1 - 1.3 mm, 1.7 - 2.3 mm, and 3.0 - 3.7 mm body length were exposed for 25 minutes to radioactive yeast suspensions of 0.1×10^6 cells per ml containing 1.4 mg Hg g^{-1}. At the end of the exposure period the mercury content of Daphnia was determined by radioassaying each group of animals for ^{203}Hg. Groups were then transferred immediately to 250 ml volumes of uncontaminated yeast suspensions of cell densities of 0.1×10^6 cells per ml. Mercury content of each group of Daphnia was determined at approximately 1, 2, 4, 6, and 8 hours postexposure and at 24 hour intervals for the next five days. Growth of animals from the different body size groups was determined by measuring 10 animals from each size range at 48 and 120 hours postexposure.

Effect of Temperature on Assimilation and Clearance of Methylmercury.

Two groups of six adult females of 2.5 - 3.0 mm body length were exposed for 30 minutes to a suspension of contaminated yeast of 0.1×10^{-6} cells per ml containing 1.7 mg Hg g^{-1} at temperatures of 10, 20, and 28°C. The mercury content of Daphnia at the end of the exposure period was determined by radioassaying each group of Daphnia for ^{203}Hg. Daphnia were then transferred

immediately to suspensions of uncontaminated yeast at their respective acclimation temperatures. Mercury content of Daphnia was determined by radioassay at 1, 2, 4, 6, and 8 hours postexposure and at 24 hour intervals for the next five days.

Assimilation Efficiency and Clearance of Ingested Inorganic Mercury.

Five groups of five adult females, 2.3 - 2.7 mm in body length, were exposed for 30 minutes to yeast suspensions of 0.1×10^6 cells per ml containing 1.6 mg Hg g^{-1} dry weight of yeast. Mercury content of Daphnia was then determined by radioassaying Daphnia in groups for ^{203}Hg. Daphnia were then transferred as groups to 250 ml volumes of yeast suspension containing 0.1×10^6 cells per ml for eight hours before being returned to 3500 ml volumes of water containing Daphnia food. Mercury content of Daphnia was determined at 1, 2, 4, 6, 8, 24, and 48 hours postexposure.

RESULTS

Uptake from Water and Clearance of Methylmercury.

Uptake of methylmercury from water by Daphnia was determined by exposing Daphnia to ^{203}Hg-labelled methylmercury in water at a concentration of $.44 \times 10^{-3}$ ug Hg ml^{-1} for up to 13 hours, removing groups of Daphnia after 2, 4, 8, and 13 hours of exposure to determine the amount of mercury that animals had accumulated. After body burdens of mercury had been determined, Daphnia were transferred to uncontaminated water, where clearance of methylmercury from Daphnia was determined from a series of measurements of mercury retained by Daphnia over the next five days.

Results in Fig. 2 show that mercury content of Daphnia increased steadily over 13 hours of exposure to ^{203}Hg-labelled methylmercury in water. The net uptake rate clearly declined by 50% over the thirteen-hour exposure period, from $.16 \times 10^{-3}$ ug Hg Daphnia^{-1} hr^{-1} over the first two-hour period to $.07 \times 10^{-3}$ ug Hg Daphnia^{-1} hr^{-1} over the interval from the eighth to the thirteenth hour of exposure. This decline in accumulation rate could not have been caused by a reduced concentration of ^{203}Hg in the exposure medium, since results in Table 1 show that the ^{203}Hg content of the exposure medium declined only 2% over the thirteen-hour exposure period.

Fig. 2. Uptake of methylmercury from water by Daphnia magna during 13 hours of exposure to ^{203}Hg-labelled methylmercury at a concentration of .44 x 10^{-3} ug Hg ml^{-1} at 20°C. Solid circles are the body burdens of methylmercury; triangles are the mean rates of Hg uptake between sampling times. Open circles are the mercury content of the exposure medium. Each point represents the mean ± S.E. of four groups.

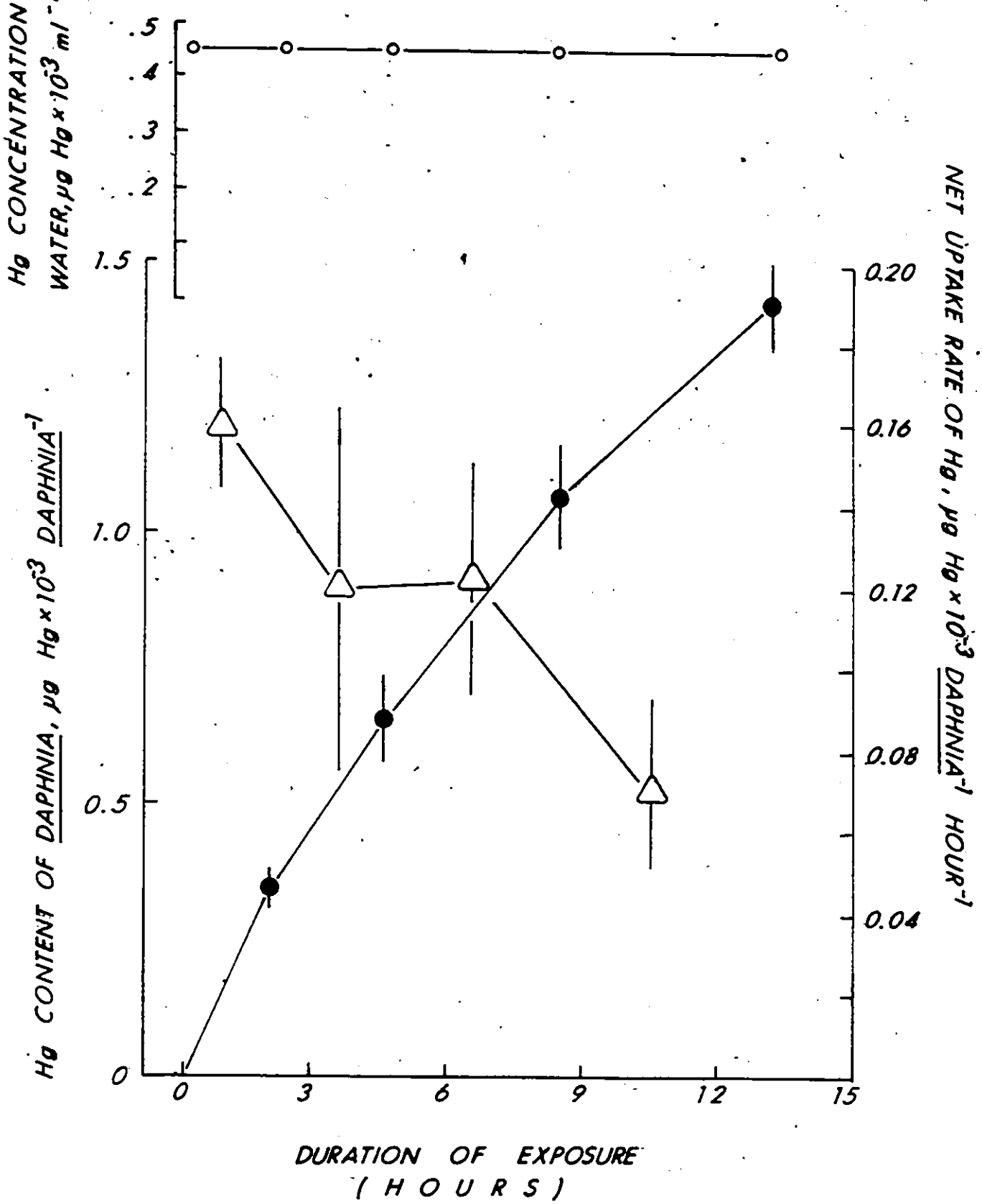

Hg CONCENTRATION WATER, μg Hg × 10⁻³ ml⁻¹
.5
.4
.3
.2
1.5
1.0
0.5
0
Hg CONTENT OF DAPHNIA, μg Hg × 10⁻³ DAPHNIA⁻¹
0.20
0.16
0.12
0.08
0.04
NET UPTAKE RATE OF Hg, μg Hg × 10⁻³ DAPHNIA⁻¹ HOUR⁻¹
0
3
6
9
12
15
DURATION OF EXPOSURE
(HOURS)

FIG. 2.

Table 1. The uptake of methyl mercury from water by Daphnia magna during periods of exposure varying in length from 2 to 13 hours at 20°C.

Duration of exposure, hours	Numbers[a] of Daphnia	Mean Hg concentration[b] in exposure medium, ug Hg ml^{-1}	Hg content[c] of Daphnia at one minute postexposure, ug Hg·Daphnia^{-1}	Transfer coefficient,[d] hr^{-1}
2.2	4 x 5	$.44 \times 10^{-3}$ (-2)	$.34 \pm .05 \times 10^{-3}$	3800±500
4.7	4 x 5	$.44 \times 10^{-3}$ (-2)	$.64 \pm .08 \times 10^{-3}$	3400±400
8.2	4 x 5	$.43 \times 10^{-3}$ (-2)	$1.04 \pm .08 \times 10^{-3}$	3300±300
12.9	4 x 5	$.43 \times 10^{-3}$ (-2)	$1.39 \pm .07 \times 10^{-3}$	2800±100

[a] Numbers of groups x numbers of animals per group.

[b] Hg concentrations based on measured radioactivity concentrations using a specific activity of 12.8×10^4 cpm ug Hg^{-1} based upon suppliers statement of specific activity at time of delivery, which was corrected for radioactive decay and the efficiency of the detector. (Values in parentheses refer to per cent changes in Hg concentration in exposure medium during exposure.)

[c] Hg content based on measured radioactivity content. Values represent mean ± S.E. for groups.

[d] The estimate of body weight used in these calculations, $.09 \times 10^{-3}$ g Daphnia^{-1}, was made from the mean body length using the length-dry weight relationship determined by Schindler (1968).

A decline in the net uptake rate of this kind might be caused in a number of ways. In a multi-compartmental accumulation system one or more compartments might be approaching equilibrium and might therefore accumulate mercury very slowly, while other compartments, which are far from equilibrium, continue to accumulate mercury at near maximum rates. Daphnia might be bioaccumulating a radiochemical contaminant present in the isotope stock solution which approaches an equilibrium either much more quickly or more slowly than methylmercury. Even though the concentration of methylmercury (measured as ^{203}Hg) remained constant throughout the exposure period, transformation of methylmercury to inorganic mercury or binding of mercury by excretory products of Daphnia may have resulted in a reduction in bioavailability of mercury to Daphnia. The possibility that one or more of these processes might be influencing the observed accumulation of ^{203}Hg was examined experimentally.

To determine whether any of the methylmercury in the exposure medium was transformed to inorganic mercury during the exposure period, samples of exposure medium were extracted with benzene, as described in the Materials and Methods Section. The benzene-water partitioning characteristics of ^{203}Hg in exposure medium, taken before and after an eight-hour exposure period, were compared to those of water samples spiked with $CH_3{}^{203}HgCl$. These results showed that over the eight-hour exposure period the

change in the ratio of benzene extractible ^{203}Hg to total ^{203}Hg in the exposure medium was negligibly small. This suggests that little, if any, demethylation of methylmercury took place and therefore could not account for the observed reduction in mercury uptake rate from water.

To determine how much, if any, of the ^{203}Hg accumulated in the tissues of Daphnia was converted from methylmercury to inorganic mercury, samples of eight-hour-exposed Daphnia were taken for tissue extraction at one minute postexposure and after 46 hours of clearance. The mercury in Daphnia was extracted using the extraction procedure described in the Materials and Methods Section.

The results in Table 2 show the fractionation of ^{203}Hg from water and Daphnia. Recoveries of mercury of about 89 - 100% were obtained, and mercury extractions from Daphnia were as complete as those from water. The ratios of benzene-extractible mercury to total mercury in water and in tissues of Daphnia at one minute and 46 hours postexposure were all similar.

If Daphnia had preferentially taken up inorganic mercury to methylmercury that was present in trace amounts in the exposure medium, the ratio of benzene-extractible mercury to total mercury would have been lower for Daphnia than for the exposure medium. This was clearly not the case. If any of the accumulated methylmercury had been converted by Daphnia to inorganic mercury and

Table 2. Benzene extraction of mercury from Daphnia and exposure medium.

Sample	Number of Replicates	Hg^a content of sample	Per cent of Hg recovered	Ratio of Hg^a in benzene fraction to total Hg recovered
Exposure medium prior to exposure of Daphnia	6	12×10^{-3} ug Hg ml^{-1}	93±2	.94±.01
Daphnia exposed for eight hours, one minute clearance	4	10×10^{-3} ug Hg Daphnia^{-1}	100±1	.96±.01
Daphnia exposed for eight hours, 46 hours clearance	4	4×10^{-3} ug Hg Daphnia^{-1}	89±7	.97±.07

[a] Amounts of mercury were calculated from measured radioactivity of samples using a value of 13×10^4 cpm ug Hg^{-1} as the specific activity.

retained, the ratio of benzene-extractible mercury to total mercury would have declined. This also was not the case, indicating that either no demethylation took place or that, if it took place, inorganic mercury was cleared quickly from Daphnia tissues.

To test for possible changes in the bioavailability of methylmercury during the exposure period, Daphnia were exposed to ^{203}Hg-labelled methylmercury in water for up to 12 hours, with samples of Daphnia being removed after 2, 4, 8, and 12 hours of exposure. A second group of Daphnia were exposed for two hours to a 500 ml sample of the exposure medium removed after the first group of Daphnia had been exposed to it for 10 hours. If the bioavailability of methylmercury had been reduced during the previous ten-hour exposure period, Daphnia would take up less methylmercury from the aged medium than from the fresh medium.

The results in Table 3 show that Daphnia exposed continuously for 12 hours to methylmercury in water exhibited the same decline in mercury uptake during exposure as did the animals in the earlier experiment. However, uncontaminated Daphnia took up mercury just as quickly from the aged (ten-hour-old) medium as from the fresh medium. This experiment demonstrated that the bioavailability of methylmercury did not change during the twelve-hour exposure period.

In addition this experiment demonstrated a very important

Table 3. The effect of the presence of Daphnia in exposure medium on the bioavailability of methyl mercury for uptake from water.

Condition	Number[a] of Daphnia	Duration of exposure, hours	Mean Hg concentration[b] in exposure medium, ug Hg ml^{-1}	Hg content[b] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Net uptake rate over the interval between samples,[c] ug Hg Daphnia^{-1} hr^{-1}
Routine exposure procedure	3 x 5	2	$.57 \times 10^{-3}$ (-3)	$.26 \pm .01 \times 10^{-3}$	$.13 \pm .01 \times 10^{-3}$
Routine exposure procedure	3 x 5	4	$.57 \times 10^{-3}$ (-3)	$.45 \pm .06 \times 10^{-3}$	$.10 \pm .04 \times 10^{-3}$
Routine exposure procedure	3 x 5	8	$.57 \times 10^{-3}$ (-3)	$.77 \pm .08 \times 10^{-3}$	$.08 \pm .01 \times 10^{-3}$
Routine exposure procedure	3 x 5	12	$.57 \times 10^{-3}$ (-3)	$.99 \pm .08 \times 10^{-3}$	$.05 \pm .01 \times 10^{-3}$
Exposure to used medium[d]	3 x 5	2	$.56 \times 10^{-3}$ (-1)	$.28 \pm .03 \times 10^{-3}$	$.14 \pm .02 \times 10^{-3}$

[a] Number of groups x number of animals per group.

[b] Mercury content was calculated from the measured radioactivity of samples using a specific activity of 12.7×10^{4} cpm ug Hg^{-1}.

[c] The net uptake rate over the interval between samples was calculated using the formula Uptake Rate = $\frac{Y(t) - Y(t-1)}{\text{length of interval, hr}}$ where Y (t) and Y (t-1) were the mercury content of Daphnia at the end and the beginning of the interval respectively.

[d] Five Daphnia were exposed to a 500 ml aliquot of exposure medium to which other Daphnia had already been exposed for 10 hours.

physiological property of Daphnia magna which is critical if Daphnia are to be tested in non-feeding situations. The animals that were held in uncontaminated media for 10 hours prior to exposure to used or aged medium had been without food for 10 hours but showed the same rate of mercury uptake as a freshly fed animal. Hence, in experiments as long as 12 hours food deprivation had no effect on mercury accumulation.

When the Daphnia that had been exposed to ^{203}Hg-labelled methylmercury in water for 2, 4, 8, and 13 hours were placed in uncontaminated water, the mercury loss was always clearly in two phases (Fig. 3). Mercury loss was initially rapid but after about 12 hours the rate of mercury loss declined markedly. This indicated that some of the mercury that had been taken up was only loosely associated with the organism and was quickly lost, while the remaining mercury was much more securely bound.

Assuming that first order kinetics obtain in the clearance of methylmercury, the whole body measurements of ^{203}Hg were analysed as outlined in the Materials and Methods Section in terms of the general exponential equation

$$Y(t) = Y(o)e^{-kt}$$

where Y(t) is the amount of mercury in Daphnia at any time, t is the clearance time in hours, k is the fractional clearance rate per hour, and Y(o) represents the mercury content of Daphnia when t is zero. It was found that the best-fitting curves were defined by

Fig. 3. Whole body clearance of methylmercury by Daphnia magna after uptake from water during exposures of varying duration to ^{203}Hg-labelled methylmercury in water at 20°C.

● Duration of exposure was 2.2 hours

$$Y_{(t)} = 28e^{-.42t} + 15e^{-.006t}$$

○ Duration of exposure was 4.7 hours

$$Y_{(t)} = 48e^{-.36t} + 33e^{-.006t}$$

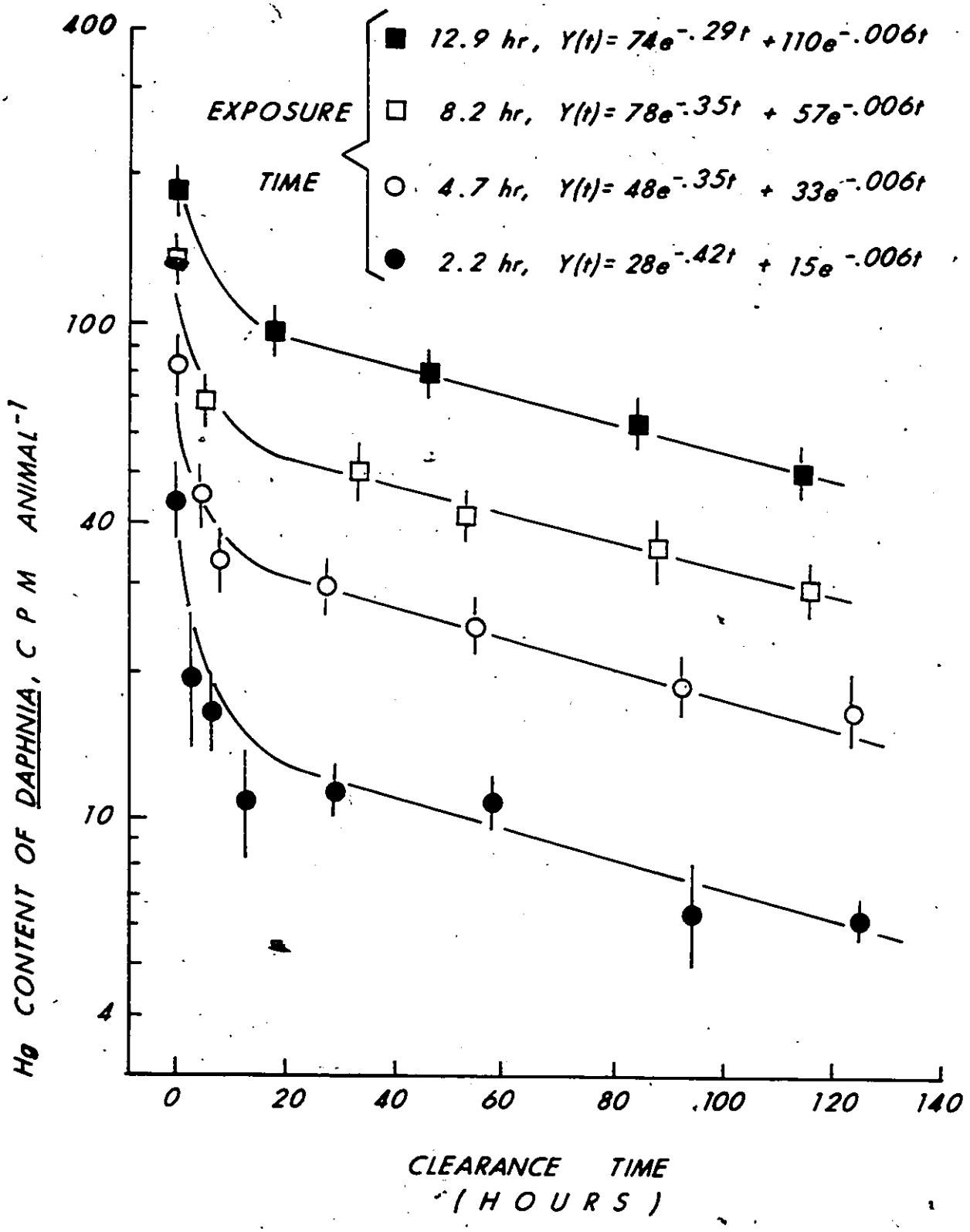
□ Duration of exposure was 8.2 hours

$$Y_{(t)} = 78e^{-.35t} + 57e^{-.006t}$$

■ Duration of exposure was 12.9 hours

$$Y_{(t)} = 74e^{-.29t} + 110e^{-.006t}$$

The curves represent the "best fitting" exponential curves defined by the equations where $Y_{(t)}$ is the body burden (cpm per Daphnia) retained by Daphnia and t is the clearance time in hours. Each point is the mean $\pm$ S.E. of four groups.


EXPOSURE TIME
12.9 hr, Y(t)= 74e^-.29t + 110e^-.006t
8.2 hr, Y(t)= 78e^-.35t + 57e^-.006t
4.7 hr, Y(t)= 48e^-.35t + 33e^-.006t
2.2 hr, Y(t)= 28e^-.42t + 15e^-.006t
400
100
40
10
4
0
20
40
60
80
100
120
140
Hg CONTENT OF DAPHNIA, C P M ANIMAL^-1
CLEARANCE TIME (HOURS)

F16 3

the sum of two exponentials. Curves were fitted separately to the data for each group of Daphnia at each exposure time and the regression parameters for groups with the same exposure duration were averaged. The curves defined by these average parameters are given with the data in Fig. 3.

The results in Table 4 show that as the duration of exposure increased to eight hours the mercury content of the fast-clearing compartment increased steadily to a value of $.61 \times 10^{-3}$ ug Hg Daphnia^{-1}, but no further increase in the content of the fast compartment was observed as the duration of exposure was increased to 13 hours. This suggests that after eight hours of exposure equilibrium had been reached between mercury uptake and loss from the fast-clearing compartment. This is consistent with the observed fractional clearance rates of the fast-clearing compartment, since a physiological compartment with a biological half-life of 2 - 4 hours would be expected to reach 95% of its equilibrium body burden within 7 - 9 hours of exposure if the rate of Hg uptake was constant.

The results in Table 4 show that the mercury content of the slow-clearing phase increased linearly over the first 13 hours of exposure at a rate of $.06 \times 10^{-3}$ ug Hg Daphnia^{-1} hr^{-1}. The mercury uptake rate into the slow compartment was therefore constant over the 13 hours of exposure. There was no apparent decline in the net uptake rate as there was in the fast compartment, because the fractional clearance rate of the slow compartment

Table 4. The effect of varying the duration of the dosing period on compartmental kinetics parameter values for methyl mercury accumulation from water. Animals of 2.4 - 2.7 mm body length were exposed to a concentration[a] of .44 x 10^{-3} ug Hg ml^{-1} as methyl mercury for periods of 2, 4, 8, and 12 hours at 20°C.

Duration of exposure, hours	Hg content[a] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Fast-clearing compartment[b]			Slow-clearing compartment[b]		
		Compartment size,[c] ug Hg Daphnia^{-1}	Hg uptake, transfer coefficient,[d] hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}	Compartment size,[c] ug Hg Daphnia^{-1}	Hg uptake, transfer coefficient,[d] hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}
2.2	.34±.05 x 10^{-3}	.22±.03 x 10^{-3} (.10±.01 x 10^{-3})	2500±300	.42±.09	.12±.02 x 10^{-3} (.055±.015 x 10^{-3})	1300±200	.007±.00[illegible]
4.7	.64±.08 x 10^{-3}	.30±.10 x 10^{-3} (.08±.02 x 10^{-3})	2000±200	.36±.05	.26±.05 x 10^{-3} (.055±.017 x 10^{-3})	1400±300	.006±.001
8.2	1.04±.08 x 10^{-3}	.61±.07 x 10^{-3} (.08±.01 x 10^{-3})	1900±200	.35±.09	.45±.03 x 10^{-3} (.056±.005 x 10^{-3})	1400±100	.006±.001
12.9	1.39±.07 x 10^{-3}	.58±.10 x 10^{-3} (.05±.01 x 10^{-3})	1200±200	.29±.14	.80±.05 x 10^{-3} (.062±.001 x 10^{-3})	1600±100	.006±.001

[a]Amounts of mercury were calculated from measured radioactivity content of samples using a value of 12.8 x 10^{4} cpm ug Hg^{-1} as the specific activity.

[b]The "best-fitting" exponential curves were determined for clearance data of each group. Values presented are means ± S.E. of groups having the same dose duration.

[c]Values in parentheses are estimates of mercury uptake rate in ug Hg Daphnia^{-1} hr^{-1} based on the body burden at one minute postexposure and the duration of exposure in hours.

[d]The estimate of body weight used in these calculations, .09 x 10^{-3} g dry weight Daphnia^{-1}, was made from the mean body length, using the length-dry weight relationship determined by Schindler (1968).

was so slow that the amount of mercury lost was negligibly small during exposure of 13 hours or less.

These results demonstrate that mercury clearance rates from both fast and slow compartments were proportional to the mercury content of the compartment, and mercury uptake rates and fractional clearance rates of both fast- and slow-clearing mercury compartments were independent of the exposure duration.

On the basis of the above observation it can be concluded that experimental determination of methylmercury accumulation rate constants requires determination of only the following:

(1) the amount of methylmercury accumulated over only a single exposure period; and

(2) the relative sizes and fractional clearance rates for fast- and slow-clearing compartments.

Separation of the total body burden into fast- and slow-clearing compartments permits a redefinition of the single uptake vector into two corresponding subvectors representing mercury uptake into each compartment. Results in Table 4 demonstrate that the amount of mercury in the fast compartment after 2.2 hours of exposure was $.22 \times 10^{-3}$ ug Hg Daphnia^{-1} for a mean net uptake rate of $.11 \times 10^{-3}$ ug Hg Daphnia^{-1} hr^{-1} during the first two hours of exposure. Because of the high fractional clearance rate from the fast compartment, however, this is an

underestimate of true uptake rate. The actual fast compartment uptake rate can be estimated by making use of a single-compartment first-order accumulation model which appears to adequately describe the methylmercury kinetics of the fast compartment in this case. Using this model, the mercury content of the fast compartment can be related to the uptake rate and clearance rate constants by

$$Y(t) = \frac{U}{k_{cl}}(1-e^{-kt})$$

where Y(t) is the mercury content of the compartment after t hours of exposure to methylmercury in water, U is the uptake rate in ug Hg Daphnia^{-1} hr^{-1}, and k_{cl} is the fractional clearance rate in hr^{-1}. As the duration of exposure increases, the value of Y(t) approaches U/k_{cl}, so that when the fast compartment has effectively reached steady state, the mercury content of the fast compartment is related to rates of uptake and loss by

$$Y(t) = Yss = \frac{U}{k_{cl}}$$

where Yss is the mercury content of the fast compartment at steady state. By rearranging the above expression an expression for uptake rate can be obtained

$$U = Yss \cdot k_{cl} .$$

In the present study the fast compartment appears to have reached steady state by the eighth hour of exposure. Using a value for the steady state mercury content of the fast compartment of 0.61×10^{-3} ug Hg Daphnia^{-1} and fractional clearance rate value of .35 hr^{-1} (Table 4), the uptake rate for methylmercury uptake

into the fast compartment was about $.21 \times 10^{-3}$ ug Hg Daphnia^{-1} hr^{-1}. Hence, even with exposures as short as two hours, the net uptake rate underestimates the value of fast compartment uptake rate by 50 to 100%.

Results in Table 4 demonstrate that mercury content of the slow compartment increases at a constant rate of approximately $.06 \times 10^{-3}$ ug Hg Daphnia^{-1} hr^{-1} throughout the 13 hours of exposure. As explained above, this value reflects the true uptake rate into the slow compartment.

Results in Fig. 4 and Table 4 show that after two hours of exposure mercury content of the fast compartment was greater than that of the slow compartment. However, as duration of exposure increased, the slow compartment slowly increased in relative size. This was consistent with the observation that uptake rate into the fast compartment was initially faster than that into the slow compartment, but after eight hours of exposure net uptake into the fast compartment was reduced to zero as the compartment reached steady state, while net uptake of mercury into slow compartment continued unchanged. It would follow that, if exposure were continued beyond 13 hours, the slow compartment would soon make up a much larger proportion of total body burden than would the fast compartment. The fast compartment would, consequently, contribute a minor proportion of the mercury content of Daphnia under conditions of continuous exposure over the life time of the animal,

despite the fact that the rate of uptake into the fast compartment was two to three times faster than the rate of uptake into the slow compartment.

In Table 1 the uptake of mercury from water to Daphnia was expressed as a transfer coefficient, representing the amount of water, in ml or g, that was completely emptied of its mercury content in one hour by one gram (dry weight) of Daphnia. Its units are $g\ g^{-1}\ hr^{-1}$. Use of this unit facilitates comparisons of experimental results between experiments, since uptake rates are normalized for differences in Daphnia biomass and concentrations of mercury in the water. The transfer coefficient, TC, was defined as follows:

$$TC = \frac{Y(t)}{W \cdot t \cdot C}$$

where Y(t) was the mercury content of the whole body or the fast or slow compartment of Daphnia of dry weight W after t hours of exposure to water containing mercury at concentration C. Since the expression contains no terms for mercury release from Daphnia, the transfer coefficient as used in Table 1 or 4 represents only an index of the uptake rate rather than a true uptake rate for either whole body or fast compartment uptake studies for the reasons given above. However, the transfer coefficient for uptake into the slow compartment calculated in this way and reported in Table 4 is approximately equal to the true transfer coefficient for uptake into the slow compartment, since short-term uptake experiments

require no correction for the loss of Hg from the slow compartment. In Table 1 and 4 transfer coefficients for whole body uptake and fast compartment uptake of Hg are shown to decline with increasing duration of exposure, reflecting the effect of rapid mercury loss from the fast compartment. Transfer coefficient values for uptake into the slow compartment were independent of the duration of exposure, as predicted on the basis of the clearance coefficients reported in Table 4.

Effect of the Concentration of Methylmercury in Water on Uptake and Clearance of Methylmercury.

To determine if accumulation of methylmercury by Daphnia was influenced by the concentration of methylmercury in water, methylmercury accumulation was determined by exposing animals to five concentrations of methylmercury in water ranging from $.03 \times 10^{-3}$ to 4.2×10^{-3} ug Hg ml^{-1}. Clearance of mercury accumulated at these concentrations was then determined in the usual way.

The results in Table 5 show that the amount of Hg taken up by Daphnia increased with increasing ambient mercury concentration. At the lowest mercury concentration, 0.027×10^{-3} mg Hg ml^{-1}, Daphnia accumulated $.073 \times 10^{-3}$ mg Hg Daphnia^{-1} in or on its tissues after eight hours of exposure. A 160-fold increase in ambient mercury concentration to 4.2×10^{-3} ug Hg ml^{-1} resulted in a 180-fold increase in the amount of mercury accumulated. At intermediate concentrations the proportionality between mercury uptake and ambient mercury concentrations was reasonably similar. This clearly demonstrates that the uptake rate of methylmercury was directly related to the concentration of mercury in the water. The transfer coefficients, also seen in Table 5, increased steadily by about 50% from 2900 to 4500 hr^{-1}, with increasing ambient mercury concentration from $.027 \times 10^{-3}$ to $.84 \times 10^{-3}$ ug Hg ml^{-1}, demonstrating that the increase in Hg uptake was proportionately greater than the increase in the ambient Hg concentration. A

Table 5. The uptake of methyl mercury from water by Daphnia at various concentrations of methyl mercury in the water. Animals of 2.5 - 3.0 mm body length were exposed for approximately eight hours to various concentrations of methyl mercury in water at 20°C.

Mean Hg[a] concentration in exposure medium, ug Hg ml^{-1}	Numbers of Daphnia[b]	Duration of exposure, hours	Hg content[c] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Transfer coefficient,[d] hr^{-1}
$.027 \times 10^{-3}$ (<-2)	1 x 20	8.8	$.073 \times 10^{-3}$ ($.008 \times 10^{-3}$)	2900
$.092 \times 10^{-3}$ (-9)	3 x 5	8.9	$.32 \pm .04 \times 10^{-3}$ ($.036 \pm .005 \times 10^{-3}$)	3700±300
$.41 \times 10^{-3}$ (-10)	4 x 5	8.9	$1.54 \pm .11 \times 10^{-3}$ ($.173 \pm .002 \times 10^{-3}$)	4000±300
$.84 \times 10^{-3}$ (-6)	2 x 5	8.9	3.39×10^{-3} ($.381 \times 10^{-3}$)	4500
4.2×10^{-3} (-5)	4 x 5	8.9	$13.36 \pm .58 \times 10^{-3}$ ($1.50 \pm .07 \times 10^{-3}$)	3400±200

[a] Hg concentrations based on measured radioactivity concentrations and a specific activity of 10.5×10^{-4} cpm ug Hg^{-1}. Values in parentheses refer to per cent changes in Hg concentrations in exposure medium during the eight hour exposure period.

[b] Numbers of groups x numbers of animals per group.

[c] Hg content was based on measured radioactivity content. Values in parentheses represent the net uptake rate of mercury in ug Hg Daphnia^{-1} hr^{-1}. Values are mean ± S.E. between groups.

[d] The estimate of body weight used in these calculations, $.105 \times 10^{-3}$ g Daphnia^{-1}, was made from the mean body length, using the length-dry weight relationship determined by Schindler (1968).

further increase in the ambient Hg concentration to 4.2×10^{-3} ug Hg ml^{-1} resulted in a decline in the transfer coefficient to 3400 hr^{-1}.

The results of the clearance study, given in Fig. 4, showed that methylmercury clearance was biphasic in all cases. Assuming that first-order kinetics obtain in the clearance of methylmercury, the ^{203}Hg body burden data were analysed in terms of the general exponential equation

$$Yt = ae^{-bt}$$

where Yt was the amount of mercury in Daphnia at any time, t was the time in hours, b was the fractional clearance rate and a was the amount of mercury in Daphnia at time zero of clearance. The fractional clearance rates for methylmercury clearance from the slow compartment were similar for all groups and therefore were independent of the methylmercury concentration in Daphnia. These fractional clearance rates ranged from 1.2 to 1.5% per hour.

The results in Table 6 show that, although the mercury content of the slow compartment increased with increasing ambient mercury concentration, the transfer coefficient declined slightly from 1300 hr^{-1} in Daphnia exposed to $.092 \times 10^{-3}$ ug Hg ml^{-1} to 900 hr^{-1} in animals exposed to 4.2×10^{-3} ug Hg ml^{-1}. This suggests that the efficiency of methylmercury uptake from water into the slow compartment declines very slightly with increasing ambient methylmercury concentration. Methylmercury accumulation

Fig. 4. Whole body clearance of methylmercury in Daphnia magna containing different concentrations of methylmercury. In all cases mercury body burdens were accumulated during eight hours of exposure to ^{203}Hg-labelled CH_3HgCl at different concentrations in water at 20°C. The initial body content is the body burden at one minute postexposure. The data are presented as radioactivity in cpm and specific activity was 10.5×10^4 cpm ug Hg ml^{-1}.

○ Exposure medium contained $.09 \times 10^{-3}$ ug Hg ml^{-1}

$Y(t) = 11.8e^{-.012t}$*

▲ Exposure medium contained $.41 \times 10^{-3}$ ug Hg ml^{-1}

$Y(t) = 52e^{-.012t}$

■ Exposure medium contained $.84 \times 10^{-3}$ ug Hg ml^{-1}

$Y(t) = 82e^{-.012t}$

● Exposure medium contained 4.2×10^{-3} ug Hg ml^{-1}

$Y(t) = 381e^{-.015t}$

The curves represent the "best fitting" exponential curves defined by the equations where Y(t) is the body burden (cpm per Daphnia) retained by Daphnia and t is the clearance time in hours. Note that the curves were fitted to slow compartment data only. Each point is the mean ± S.E. of two to four groups.

*Experiment terminated after 24 hours due to low levels of ^{203}Hg in Daphnia. A value of .012 was assumed for the fractional clearance rate to estimate the size of the slow-clearing compartment.

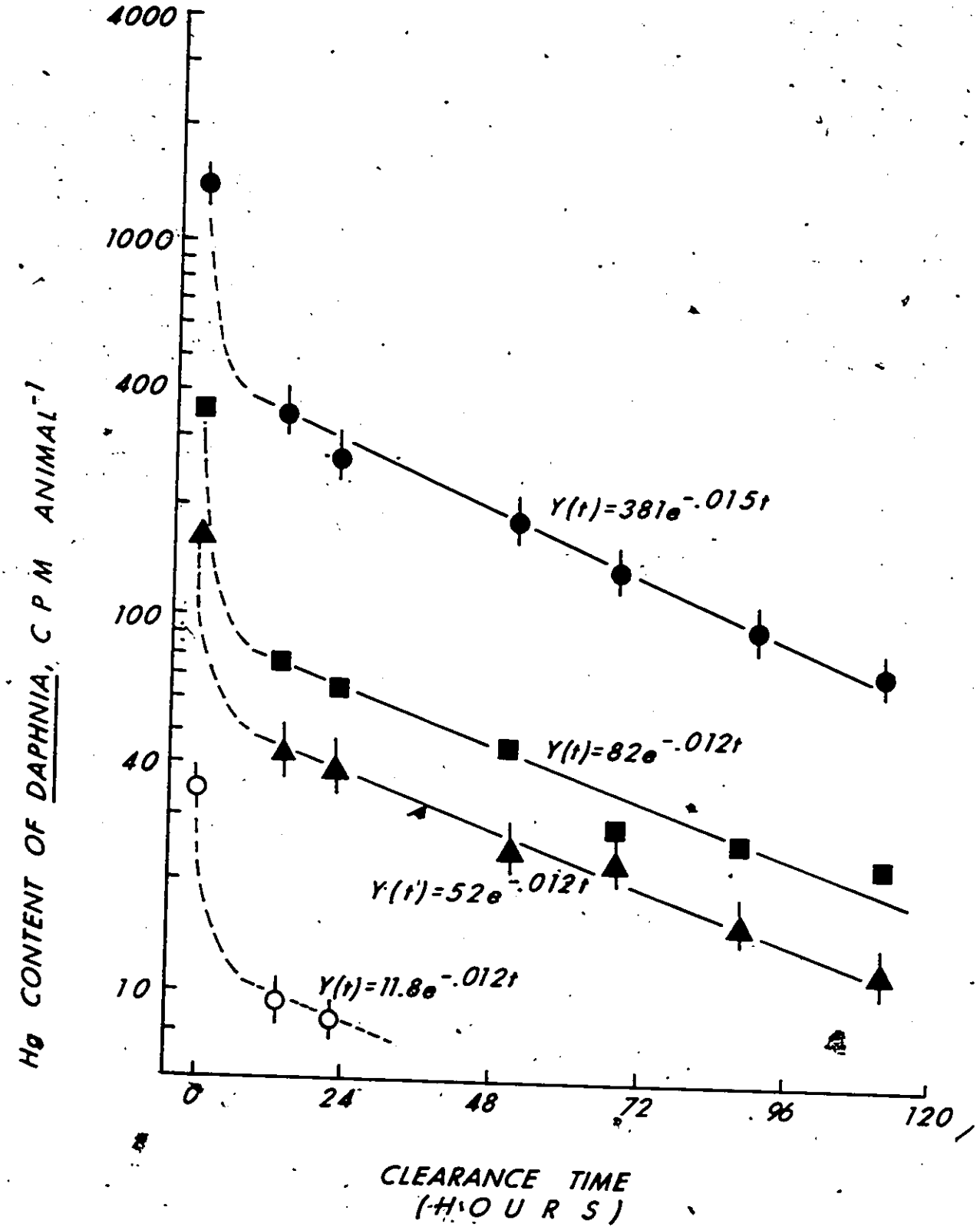

4000
1000
400
100
40
10
Hg CONTENT OF DAPHNIA, C P M ANIMAL^{-1}
Y(t)=381e$^{-.015t}$
Y(t)=82e$^{-.012t}$
Y(t)=52e$^{-.012t}$
Y(t)=11.8e$^{-.012t}$
0
24
48
72
96
120
CLEARANCE TIME
(HOURS)

FIG 4

Table 6. The effect of varying the concentration of methyl mercury in the water on compartmental kinetics parameter values for methyl mercury accumulation from water.

Mean Hg concentration[a] in exposure medium, ug Hg ml^{-1}	Hg content[a] Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Fast-clearing compartment[b]			Slow-clearing compartment[b]		
		Compartment[c] size, ug Hg Daphnia^{-1}	Hg uptake,[d] transfer coefficient, hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}	Compartment[c] size, ug Hg Daphnia^{-1}	Hg uptake,[d] transfer coefficient, hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}
$.027 \times 10^{-3}$ (<-2)	$.07 \times 10^{-3}$	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
$.092 \times 10^{-3}$ (-9)	$.32 \pm .04 \times 10^{-3}$	$.215 \pm .036 \times 10^{-3}$[e] (68±4)	2400±400[e]	<.20[f]	$.112 \pm .023 \times 10^{-3}$[e] (32±4)	1300±300[e]	.012[e]
$.41 \times 10^{-3}$ (-10)	$1.54 \pm .11 \times 10^{-3}$	$1.071 \pm .081 \times 10^{-3}$ (70±2)	2800±200	<.20[f]	$.463 \pm .044 \times 10^{-3}$ (30±2)	1200±100	.012±.001
$.84 \times 10^{-3}$ (-6)	3.39×10^{-3}	2.66×10^{-3} (78)	3300	<.20[f]	$.751 \times 10^{-3}$ (22)	900	.012
4.2×10^{-3} (-5)	$13.36 \pm .58 \times 10^{-3}$	$9.72 \pm .25 \times 10^{-3}$ (73±1)	2400±100	<.20[f]	$3.63 \pm .57 \times 10^{-3}$ (27±1)	900±150	.015±.001

[a] Amounts of Hg were determined from measured radioactivity using a specific activity value of 10.5×10^{4} cpm ug Hg^{-1}.

[b] "Best-fitting" exponential curves were determined from each group. Values are means ± S.E. between groups, where applicable.

[c] Values in parentheses are compartment size as a per cent of whole body Hg content at one minute postexposure.

[d] The estimated body weight, $.105 \times 10^{-3}$ g Daphnia^{-1}, based on mean body length and length-dry weight relationship of Schindler (1966).

[e] Based on an assumed slow compartment fractional clearance rate of .012 hr^{-1}.

[f] Based on the assumption that 12 hours post exposure 5% of the one minute postexposure body burden remained in the fast compartment.

studies conducted at ambient methylmercury concentrations used in the present study may therefore underestimate uptake rates into the slow-clearing compartment. However, this effect appears to be small.

Loss of Methylmercury at the Moult.

To explore the effect of moulting on the retention of methylmercury, Daphnia ranging in size from 2.0 to 3.3 mm body length were allowed to accumulate methylmercury from water by exposing them for two hours to ^{203}Hg-labelled methylmercury in water at a concentration of $.5 \times 10^{-3}$ ug Hg ml^{-1}, and were then transferred to clean water for 24 hours to allow the complete loss of the fast-clearing compartment. Animals were then placed into separate containers so that the loss of mercury from individuals could be followed. The mercury body burden of each animal was determined at roughly 24-hour intervals for the next 96 hours. Careful note was made about whether or not moulting occurred during each 24-hour interval.

The results in Table 7 show that when moulting occurred the fractional clearance rate of methylmercury was slightly faster than during intervals when no moulting occurred. This difference was not significant at the 95% level of confidence, however, using Student's t-test. The rate of loss of methylmercury from the slow-clearing compartment is, therefore, neither increased nor decreased by moulting.

Table 7. Clearance of methylmercury in moulting and non-moulting Daphnia following uptake of methylmercury from water.

Treatment	Numbers of animals	Fractional clearance rate,[a] day^{-1}
Moulted	12	.123±.015
Non-moulted	15	.085±.015

[a]Fractional clearance rate defined as

$$k_{cl}(\text{days}) = \frac{\ln Yx - \ln Yx+1}{tx+1 - tx} \times 24$$

where Yx and Yx+1 are mercury body burdens at the xth and x+1st measurements postexposure and tx and tx+1 are the times in hours of the xth and x+1st measurements postexposure. Values are presented as mean ± S.E.

Uptake from Water and Clearance of Inorganic Mercury.

The accumulation of inorganic mercury from water by Daphnia was determined by exposing four replicate groups of 20 - 30 Daphnia to ^{203}Hg-labelled inorganic mercury ($HgCl_2$) in water at a concentration of 2×10^{-3} ug Hg ml^{-1} for up to 12 hours. Subgroups of 8 - 10 animals were removed from each group after 15 minutes and 2, 4, 8, and 12 hours of exposure to determine the amount of Hg accumulated. Animals exposed for two hours or longer contained sufficient mercury to permit the study of mercury clearance. These animals were transferred to uncontaminated water, where clearance of inorganic mercury was determined over the next 10 days.

The results in Fig. 5 and Table 8 show the accumulation of inorganic mercury by Daphnia during 12 hours of exposure. The mercury content of Daphnia increased rapidly during the first two hours of exposure and then declined sharply. Daphnia continued to accumulate mercury at a diminished rate for the next 10 hours. The mercury uptake rate, over the first 15 minutes, was 1.6×10^{-3} ug Hg Daphnia hr^{-1}, and uptake continued at that rate for the next 1.75 hours. The rate of net uptake declined rapidly between the second and fourth hours of exposure and appeared to remain constant between the fourth and twelfth hours of exposure at a rate of 0.8×10^{-3} ug Hg Daphnia^{-1} hr^{-1}. The change in the net uptake rate over the first 12 hours of exposure suggests that inorganic mercury accumulation involves at least two compartments.

Fig. 5. Uptake of inorganic mercury from water by Daphnia magna during 12 hours of exposure to ^{203}Hg-labelled $HgCl_2$ at a concentration of 2.23×10^{-3} ug Hg ml^{-1} at 20°C. Solid circles are the body burdens of inorganic mercury in Daphnia in cpm; open squares are the mean net rates of mercury uptake between sampling times in ug Hg Daphnia^{-1} hr^{-1}; and triangles are the mercury content in the exposure medium in ug Hg ml^{-1}. Each point represents the mean ± S.E. of four groups.

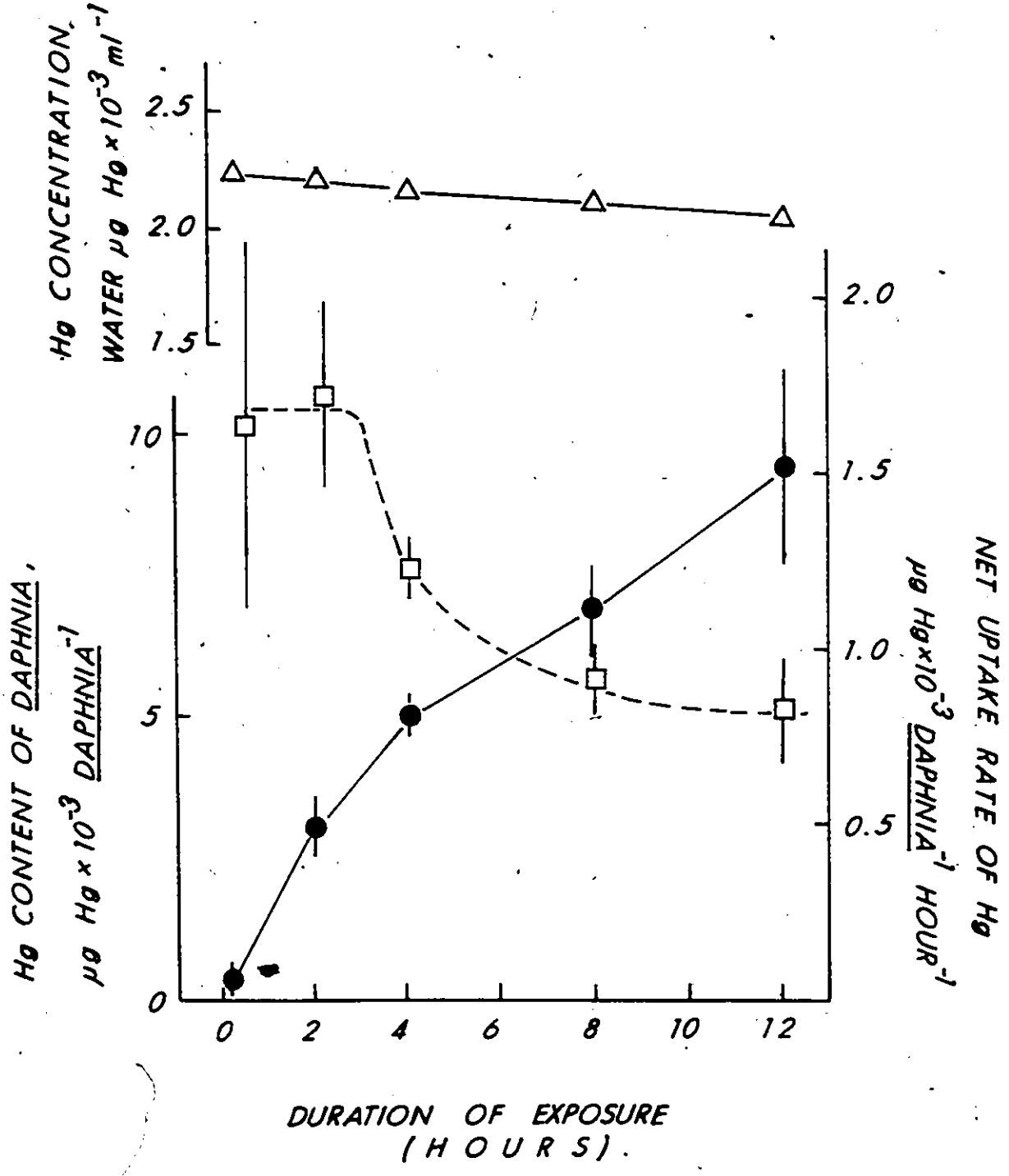

Hg CONCENTRATION, WATER µg Hg × 10⁻³ ml⁻¹
2.5
2.0
1.5
Hg CONTENT OF DAPHNIA, µg Hg × 10⁻³ DAPHNIA⁻¹
10
5
0
NET UPTAKE RATE OF Hg µg Hg×10⁻³ DAPHNIA⁻¹ HOUR⁻¹
2.0
1.5
1.0
0.5
0
2
4
6
8
10
12
DURATION OF EXPOSURE (HOURS).

FIG 5

Table 8. The uptake of inorganic mercury from water by Daphnia magna during periods of exposure varying in length from 15 minutes to 12 hours. Animals ranging in body length from 2.5 to 3.0 mm were exposed to ^{203}Hg-labelled $HgCl_2$, a concentration of 2.23×10^{-3} ug Hg ml^{-1} at 20°C.

Duration of exposure, hours	Numbers[a] of Daphnia	Mean Hg concentration[b] in exposure medium, ug Hg ml^{-1}	Hg content[c] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Transfer coefficient,[d] hr^{-1}
.25	10	2.2×10^{-3} (<5)	$.403 \pm .045 \times 10^{-3}$ ($1.61 \pm .18 \times 10^{-3}$)	7000±800 (7000±800)
2	4 x 6	2.2×10^{-3} (<5)	$3.37 \pm .32 \times 10^{-3}$ (1.70×10^{-3})	7300±700 (7400)
4	4 x 6	2.2×10^{-3} (<5)	$4.93 \pm .14 \times 10^{-3}$ ($.78 \times 10^{-3}$)	5300±150 (3400)
8	4 x 6	2.2×10^{-3} (<5)	$7.12 \pm .45 \times 10^{-3}$ ($.55 \times 10^{-3}$)	3900±250 (2400)
12	4 x 7	2.2×10^{-3} (5)	$9.62 \pm .97 \times 10^{-3}$ ($.63 \times 10^{-3}$)	3600±360 (2700)

[a] Numbers of groups x numbers of animals per group.

[b] Mercury concentrations were calculated from measured radioactivity content of samples using a value of 72.3×10^4 cpm ug Hg^{-1} as the specific activity.

[c] Mercury content based on measured radioactivity content. Values represent mean ± S.E. for groups except for animals exposed for .25 hr where values represent mean ± S.E. for individuals. Values in parentheses represent mercury uptake rates over intervals between measurements.

[d] The estimate of body weight used in these calculations, $.105 \times 10^{-3}$ g Daphnia^{-1}, was made from the mean body length using the length-dry weight relationship determined by Schindler (1968). Values in parentheses represent the transfer coefficient calculated on the basis of the rate of uptake of mercury over the interval between body burden measurements.

Fig. 6. Whole body clearance of inorganic mercury from Daphnia magna after exposures of varying duration to ^{203}Hg-labelled $HgCl_2$ in water at 20°C. The specific activity of the ^{203}Hg-labelled mercury was 3.5×10^4 cpm ug Hg^{-1}.

□ Duration of exposure was 2 hours

$$Y(t) = 2469e^{-1.34t} + 83e^{-.44t} + 145e^{-.047t} + 36e^{-.005t}$$

△ Duration of exposure was 4 hours

$$Y(t) = 2925e^{-1.27t} + 279e^{-.42t} + 256e^{-.049t} + 79e^{-.004t}$$

○ Duration of exposure was 8 hours

$$Y(t) = 4366e^{-1.29t} + 910e^{-.39t} + 387e^{-.049t} + 141e^{-.004t}$$

■ Duration of exposure was 12 hours

$$Y(t) = 7199e^{-1.40t} + 812e^{-.43t} + 797e^{-.053t} + 185e^{-.005t}$$

The curves represent the "best fitting" exponential curves defined by the above equations where Y(t) is the mercury body burden of Daphnia (in cpm per Daphnia) retained by Daphnia and t is the clearance time in hours. Each point is the mean ± S.E. of four groups of animals.

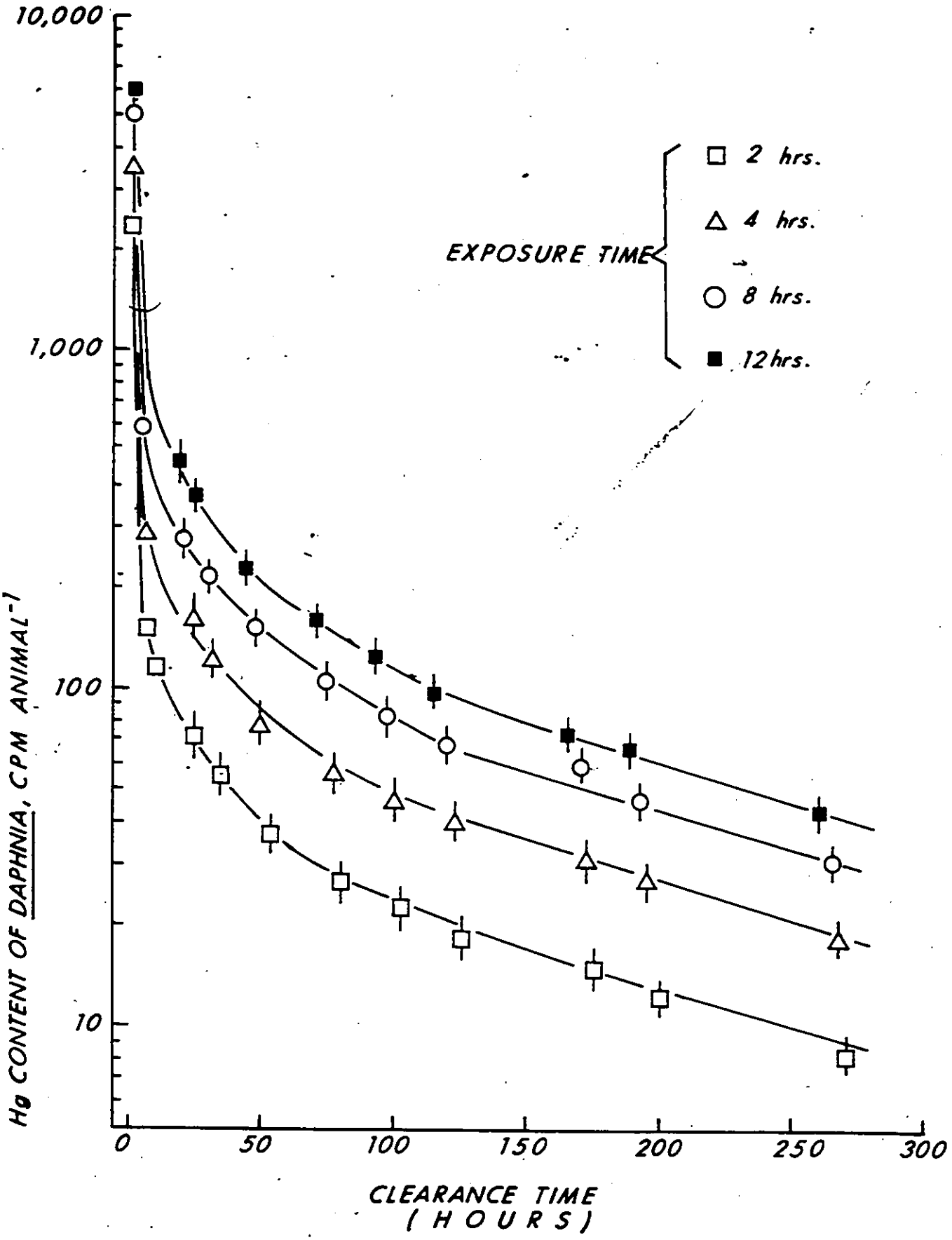

10,000
1,000
100
10
Hg CONTENT OF DAPHNIA, CPM ANIMAL⁻¹
EXPOSURE TIME
2 hrs.
4 hrs.
8 hrs.
12 hrs.
0
50
100
150
200
250
300
CLEARANCE TIME
(HOURS)

FIG 6

The scatter in the results with inorganic mercury will make interpretation of this curve difficult. However, these data have been subjected to detailed compartmental analysis as shown in Table 9.

When Daphnia were transferred to clean water, mercury loss was initially very rapid but declined with time, as with methylmercury. These clearance data were analysed using the curve-fitting procedure described previously for methylmercury. When curves were fitted to data for each subgroup it was found that the "best-fitting" curves were defined by the sum of four exponentials, although data for some groups were fitted adequately by sums of three exponentials. This suggests that loss of inorganic mercury from Daphnia involves at least four compartments. A simple four compartment model is illustrated in Fig. 7. Failure to resolve the fourth exponential term in some cases was probably due to the inaccuracies associated with the mercury body burden measurements in the initial, rapidly changing segment of the clearance curve. From the results of work with methylmercury in the present study, the accumulation of methylmercury involved two non-interconnecting compartments with mercury uptake into each compartment at a constant rate and clearance of mercury from each compartment at a rate proportional to the amount of Hg present in the compartment. In order to determine if the same held true for the accumulation of inorganic mercury, the curve-fitting procedure was repeated with curves being fitted simultaneously to data of all four subgroups of

Table 9. The effect of varying the duration of the dosing period on compartmental kinetics parameter values for accumulation of inorganic mercury from water. Animals of 2.5 - 3.0 mm body length were exposed to inorganic mercury in water at a concentration of 2.2×10^{-3} ug Hg ml^{-1} for periods of 2, 4, 8, and 12 hours at 20°C.

	Compartment A[a]		Compartment B		Compartment C		Compartment D	
Duration of exposure, hours	Compartment[b] size, ug Hg *Daphnia*$^{-1}$	Hg loss, fractional clearance rate, hr^{-1}	Compartment size, ug Hg *Daphnia*$^{-1}$	Hg loss, fractional clearance rate, hr^{-1}	Compartment size, ug Hg *Daphnia*$^{-1}$	Hg loss, fractional clearance rate, hr^{-1}	Compartment size, ug Hg *Daphnia*$^{-1}$	Hg loss, fractional clearance rate, hr^{-1}
2	3.01×10^{-3}	1.58±.074 (.43)	0.11×10^{-3}	.355±.051 (1.95)	$.19 \times 10^{-3}$	.050±.003 (14)	$.05 \times 10^{-3}$	.0049±.0002 (141)
4	3.58×10^{-3}	1.58±.074 (.43)	0.35×10^{-3}	.355±.051 (1.95)	$.31 \times 10^{-3}$	.050±.003 (14)	$.10 \times 10^{-3}$	.0049±.0002 (141)
8	5.37×10^{-3}	1.58±.074 (.43)	1.15×10^{-3}	.355±.051 (1.95)	$.39 \times 10^{-3}$	.050±.003 (14)	$.20 \times 10^{-3}$	.0049±.0002 (141)
12	8.60×10^{-3}	1.58±.074 (.43)	2.15×10^{-3}	.355±.051 (1.95)	1.08×10^{-3}	.050±.003 (14)	$.25 \times 10^{-3}$	.0049±.0002 (141)

[a] "Best-fitting" exponential curves were determined from each group. Values are means ± S.E. between groups.

[b] Mercury concentrations were calculated from measured radioactivity content of samples using a value of 72.3×10^{4} cpm ug Hg^{-1} as the specific activity.

[c] Value in parentheses is biological half-time in hours.

each group. In this analysis, however, the number of compartments was fixed at four and all subgroups were to have common exponents for each compartment although the values of the exponents were not specified. If the mercury uptake rate of each compartment was constant and clearance was first order, the change in the net uptake rate of each compartment should be consistent with the observed clearance rate from each compartment; i.e., if the value of the fractional clearance rate of a compartment suggested that the compartment should be approaching steady state with respect to mercury accumulation by the second hour of exposure, then the change in the mercury content should be consistent with this fact. For the sake of simplicity, the results of only one group are presented (Table 9).

The coefficient of compartment D, the compartment with the slowest fractional clearance rate, appears to increase at a constant rate over the full 13 hours of exposure (Table 9). This indicates that this mathematical compartment may reflect a real compartment in which the net uptake rate is constant over the first 13 hours of exposure. This is consistent with the estimated fractional clearance rate of mercury from this compartment of .0049 hr^{-1}. Having a fractional clearance rate this small, the amount of Hg lost during the exposure period would be negligibly small and net uptake rate would appear to be constant.

On the other hand, the mercury content of compartment A

Fig. 7. Schematic representation of the four-compartment model used to fit data on accumulation of inorganic mercury from water by Daphnia magna. A(t), B(t), C(t), and D(t) are the mercury content of each compartment at any time t; K_u^a, K_u^b, K_u^c, and K_u^d are uptake rate constants for the respective compartments in ml Daphnia^{-1} hr^{-1}; K_{cl}^a, K_{cl}^b, K_{cl}^c, and K_{cl}^d are fractional clearance rates for the respective compartments; and P_w is the concentration of mercury in water.

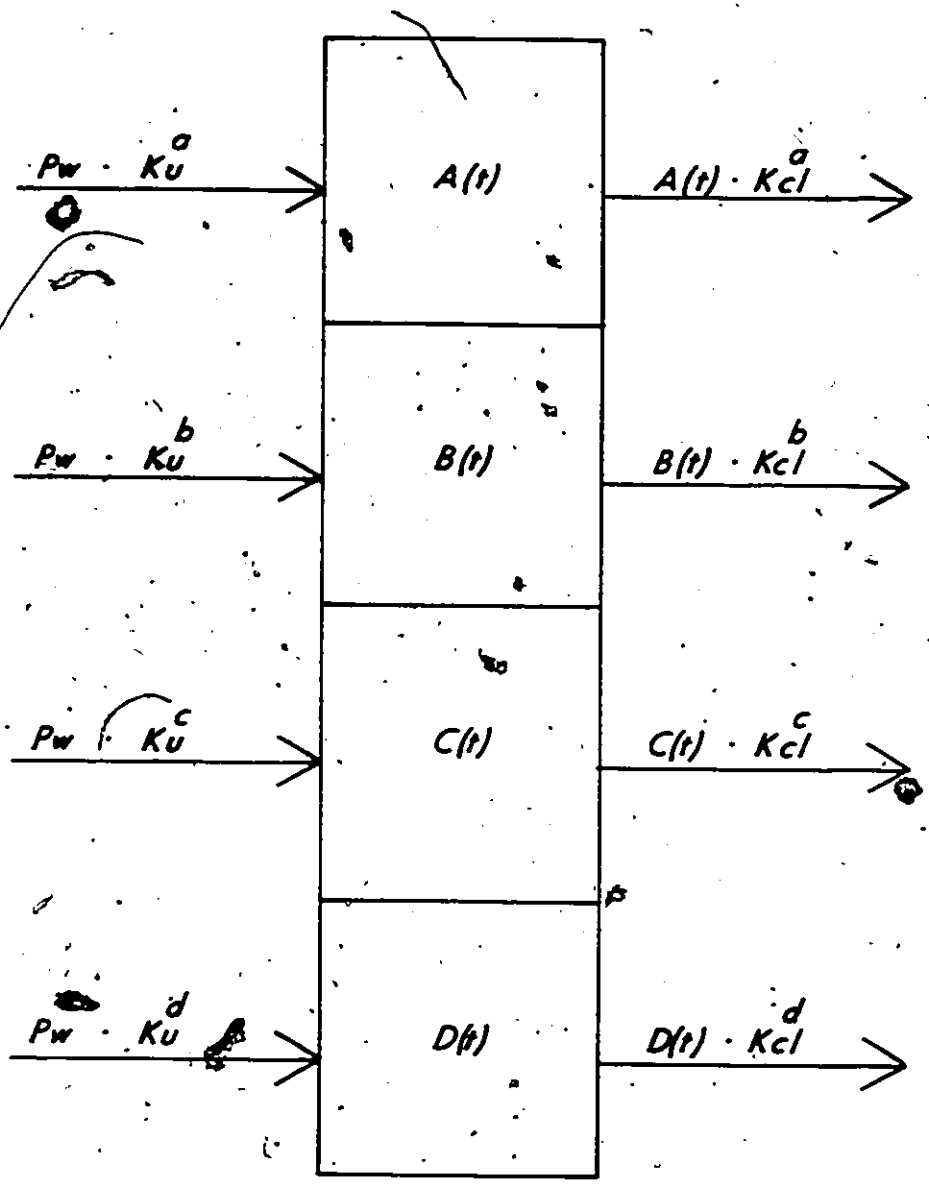

$Pw \cdot Ku^a$
$A(t)$
$A(t) \cdot Kcl^a$
$Pw \cdot Ku^b$
$B(t)$
$B(t) \cdot Kcl^b$
$Pw \cdot Ku^c$
$C(t)$
$C(t) \cdot Kcl^c$
$Pw \cdot Ku^d$
$D(t)$
$D(t) \cdot Kcl^d$

Fig. 7

increased, though at an ever-decreasing rate, over the 12 hours of exposure even though the value of the exponent for this compartment, 1.58 hr^{-1}, suggested that mercury clearance was very rapid and should have approached steady state within three hours of exposure. The lack of internal consistency of the results of applying this accumulation model suggests that a four compartment model involving non-interconnecting compartments is inadequate to describe the accumulation of inorganic mercury from water. No attempt was made to fit to the data other multi-compartmental models with interconnecting compartments, as such exercises are very difficult and do not yield unique parameter values. Such models would be of little use in predicting the accumulation of inorganic mercury no matter how well they fit these experimental results. Further experimental work will be necessary to elucidate the details of the processing of inorganic mercury accumulation by Daphnia.

The mercury uptake rate during the first 15 minutes of exposure can be corrected for the amount of Hg lost during that interval to yield an estimate of the rate of uptake of inorganic mercury by Daphnia. Even over this short interval, some mercury was undoubtedly lost by Daphnia due to the very large fractional clearance rate of mercury from compartment A. Assuming that all Hg taken up during that period was taken up into compartment A, an essentially valid assumption, the uptake rate, U, could be calculated using the following expression

$$U = \frac{Y(t) \cdot k_{cl}}{1 - e^{-k_{cl} \cdot t}}$$

By substituting for k_{cl} the value of the fractional clearance rate of compartment A in Table 9 and allowing Y(t) to equal the body burden after 15 minutes of exposure, the corrected value for inorganic mercury uptake was 2×10^{-3} ug Hg Daphnia for a transfer coefficient value of 8500 hr^{-1}. This value is very similar to the value of the transfer coefficient for uptake of methylmercury estimated above.

Effect of Body Size on Uptake and Clearance of Methylmercury.

The effect of body size on the uptake and clearance of methylmercury was determined by exposing Daphnia of different sizes to water containing ^{203}Hg-labelled methylmercury at a concentration of $.8 \times 10^{-3}$ ug Hg ml^{-1} for eight hours and then removing them, determining the amount of mercury they had taken up, and transferring them to clean water to determine mercury clearance. Daphnia used in the study were from the size ranges 1 - 1.3, 1.9 - 2.3, and 3 - 3.3 mm, which included Daphnia from the second instar to the largest and oldest animals commonly occurring in the cultures.

The results in Table 10 show that the larger animals took up methylmercury more quickly than did the smaller ones. However, on a per unit weight basis smaller animals took up methylmercury faster than the larger ones as indicated by the inverse

Table 10. The uptake of methyl mercury from water by Daphnia magna of various body sizes. The experiment was conducted at 20±1°C.

Body length,[a] (mm)	Numbers[b] of Daphnia	Duration of exposure, hours	Mean Hg concentration[c] in exposure medium, ug Hg ml^{-1}	Hg content[d] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Transfer coefficient, hr^{-1}
1.1 ($.0075 \times 10^{-3}$)	4 x 10	8.3	$.82 \times 10^{-3}$ (-1)	$.13 \pm .01 \times 10^{-3}$ ($.016 \pm .001 \times 10^{-3}$)	2500±200
2.1 ($.05 \times 10^{-3}$)	5 x 6	8.7	$.79 \times 10^{-3}$ (-1)	$.40 \pm .06 \times 10^{-3}$ ($.046 \pm .007 \times 10^{-3}$)	1200±170
3.1 ($.15 \times 10^{-3}$)	5 x 6	8.7	$.80 \times 10^{-3}$ (-1)	$1.15 \pm .17 \times 10^{-3}$ ($.13 \pm .02 \times 10^{-3}$)	1100±160

[a] Body length values are mid-points of ranges used. Values in parentheses are corresponding dry body weights in grams determined from length-dry weight relationship of Schindler (1968).

[b] Numbers of groups x numbers of Daphnia per group.

[c] Hg content based on measured radioactivity and specific activity value of 14.6×10^4 cpm ug Hg^{-1}. Values in parentheses are the change in Hg concentration of the exposure medium during exposure in per cent.

[d] Hg content based on measured radioactivity. Values in parentheses are net uptake rates in ug Hg Daphnia^{-1} hr^{-1}. Values are reported as means ± S.E. between groups.

relationship between body weight and transfer coefficient in Table 10. Over the range of body weights studied the smallest Daphnia studied contained concentrations of methylmercury in their tissues about twice as great as in adults.

The results in Fig. 8 show that when these animals were transferred to clean water, mercury clearance was biphasic in animals of all sizes. Body size had a significant effect on clearance from the fast-clearing compartment, where biological half-lives for the smallest animals were longer, 4 - 7 hours, than those for the largest animals, 2 - 3 hours. The biological half-life for mercury clearance from the slow compartment ranged from 87 - 99 hours and the biological half-life was independent of body size.

The transfer coefficient for methylmercury uptake into the slow compartment declined significantly with increasing body weight (Fig. 9). Since the loss of mercury from the slow compartment was negligible during the eight-hour uptake period, it is safe to conclude that the observed relationship between body weight and the transfer coefficient reflects, quantitatively, the effect of body size on methylmercury uptake from water. These data were fitted well by the power function

$$TC_s = 18W^{-0.37}$$

where TC_s is the transfer coefficient for the slow compartment and W is the dry weight of Daphnia in grams. These results suggest

Fig. 8. Whole body clearance of methylmercury by Daphnia magna of three body weight ranges. Mercury body burden was accumulated during eight hours of exposure to ^{203}Hg-labelled CH_3HgCl in water at a concentration of $.8 \times 10^{-3}$ ug Hg ml^{-1} at 20°C. The initial mercury content is the body burden at one minute postexposure. The data are presented as radioactivity in cpm and specific activity was 14.6×10^4 cpm ug Hg^{-1}.

- ● Initial body length was 3.3 - 3.5 mm
  $Y(t) = 94e^{-.27t} + 71e^{-.008t}$
- ■ Initial body length was 1.9 - 2.3 mm
  $Y(t) = 28e^{-.20t} + 30e^{-.008t}$
- ▼ Initial body length was 1.0 - 1.3 mm
  $Y(t) = 8.2e^{-.14t} + 10.6e^{-.007t}$

The curves represent the "best fitting" exponential curves defined by these equations where (t) is the body burden (cpm per Daphnia), retained by Daphnia and t is the clearance time in hours. Each point is the mean $\pm$ S.E. of five groups.

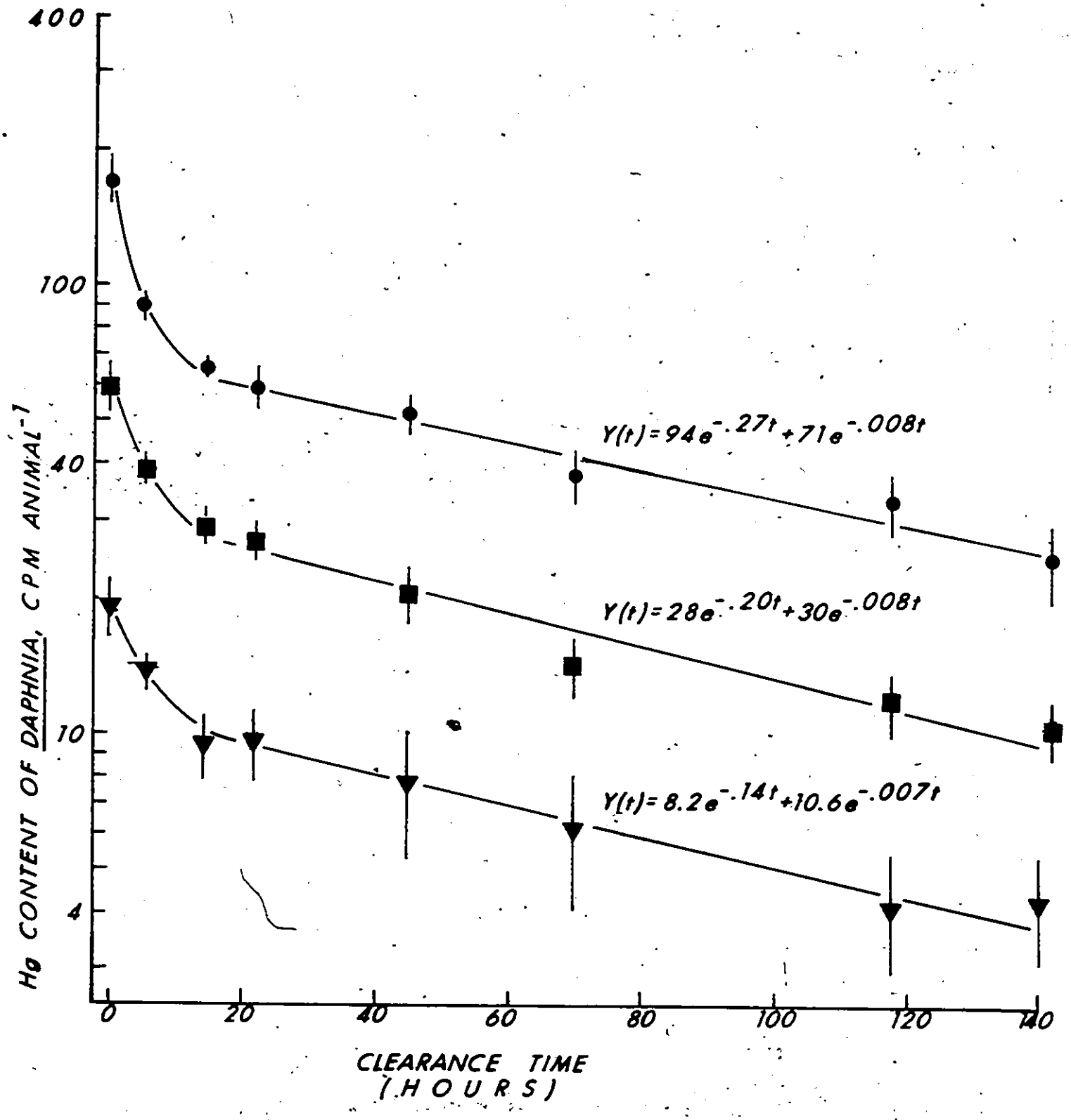

400
100
40
10
4
Ho CONTENT OF DAPHNIA, CPM ANIMAL⁻¹
Y(t)=94e⁻.27t+71e⁻.008t
Y(t)=28e⁻.20t+30e⁻.008t
Y(t)=8.2e⁻.14t+10.6e⁻.007t
0
20
40
60
80
100
120
140
CLEARANCE TIME
(HOURS)

FIG 8

Fig. 9. Relationships between body size and whole body uptake and uptake into the slow compartment as transfer coefficients for methylmercury. Determinations were made at 20°C.

Whole body transfer coefficient is the sum of slow compartment transfer coefficient and fast compartment transfer coefficient corrected for clearance.

□ $TC'_W = 330W^{-.19}$

Slow compartment transfer coefficient

○ $TC_S = 18W^{-.37}$

where TC'_W and TC_S are whole body and slow compartment transfer coefficients and W is the dry weight in grams. Best fitting curves were determined by the least-squares method. Each point is the mean $\pm$ S.E. of five groups.

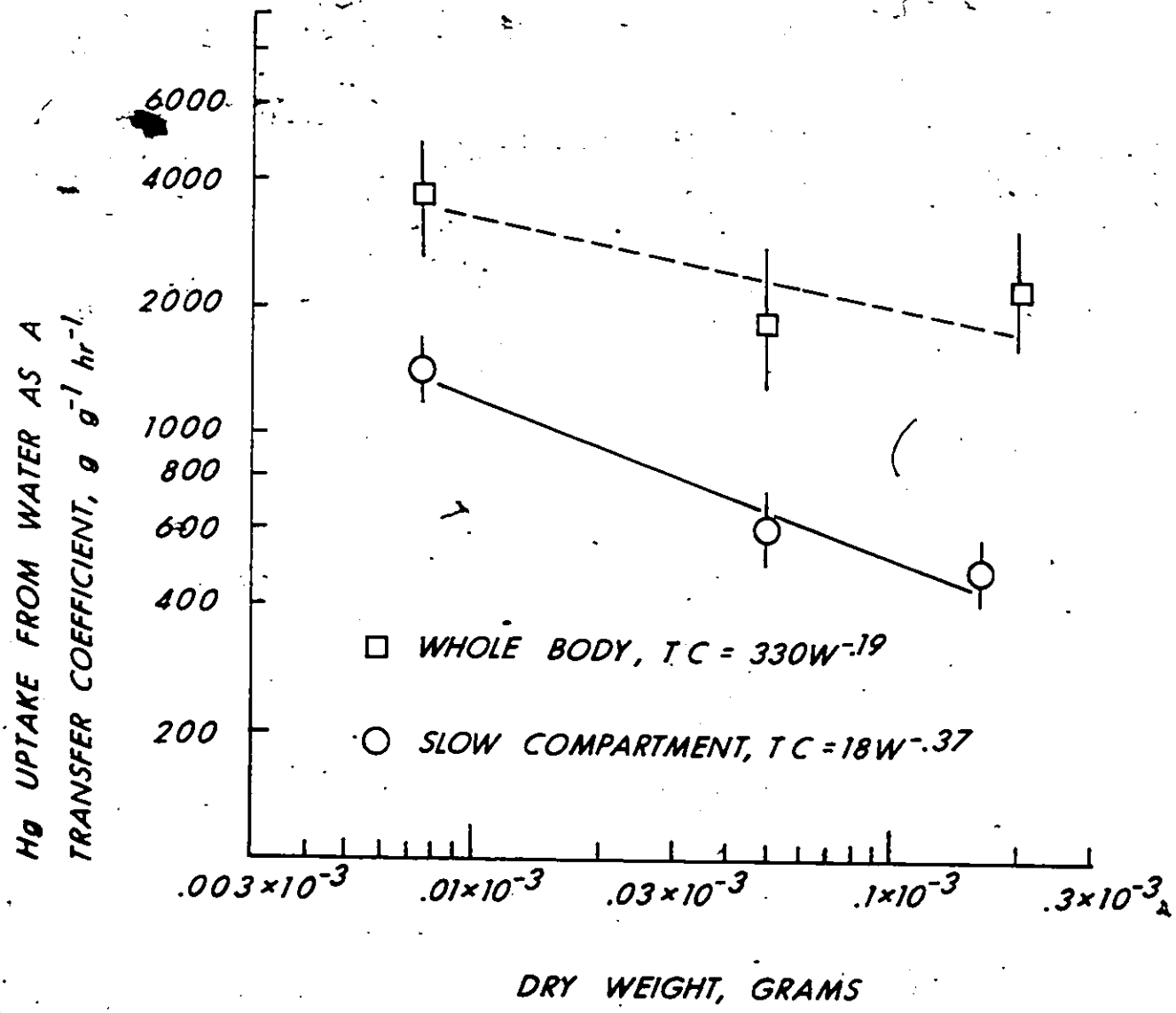

Hg UPTAKE FROM WATER AS A
TRANSFER COEFFICIENT, g g⁻¹ hr⁻¹
6000
4000
2000
1000
800
600
400
200
WHOLE BODY, TC = 330W^-.19
SLOW COMPARTMENT, TC = 18W^-.37
.003×10⁻³
.01×10⁻³
.03×10⁻³
.1×10⁻³
.3×10⁻³
DRY WEIGHT, GRAMS

FIG 9

that small, young Daphnia take up methylmercury from water into the slow-clearing compartment almost three times as quickly as adults on a per unit weight basis.

When the net uptake rate for methylmercury uptake into the fast-clearing compartment was corrected for clearance as above (page 41), this value for the uptake rate can be used to estimate the true value of the fast compartment transfer coefficient using the expression

$$TC' = \frac{U}{C_w \cdot W}$$

where TC' is the transfer coefficient corrected for mercury loss, C_w is the mercury concentration in water, and W is the dry weight of Daphnia in grams. When the transfer coefficient for the slow compartment and the corrected transfer coefficient for the fast compartment are summed they yield an estimate of the transfer coefficient for the whole body uptake of methylmercury from the water. The relationship between body weight and the transfer coefficient for whole body uptake rate can be defined as

$$TC'_w = 330W^{-.191}$$

where TC'_w is the whole body transfer coefficient.

The whole body uptake rate of methylmercury from water is therefore only about 1.5 times greater in a very young animal on a per unit basis than in an adult. Therefore, when mercury-free Daphnia are exposed to methylmercury in water the initial net uptake rate is about 1.5 times faster in small animals than in

Table 11. The effect of body size on the compartmental kinetic parameter values for methyl mercury accumulation from water. All animals were exposed for eight hours to ^{203}Hg-labelled methyl mercury in water at a concentration of .8 x 10^{-3} ug Hg[a] ml^{-1} at 20°C.

Body size,[b] mm	Numbers[c] of Daphnia.	Hg content[a] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Fast-clearing compartment[d]			Slow-clearing compartment[d]		
			Compartment[e] size, ug Hg Daphnia^{-1}	Hg uptake, transfer coefficient, hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}	Compartment size, ug Hg Daphnia^{-1}	Hg uptake, transfer coefficient, hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}
1.1 (.0075 x 10^{-3})	5 x 10	.13±.01 x 10^{-3}	.056±.002 x 10^{-3} (43±12)	1100±50	.11±.04	.073±.001 x 10^{-3} (57±12)	1400±50	.007±.002
2.1 (.05 x 10^{-3})	5 x 6	.40±.04 x 10^{-3}	.19±.05 x 10^{-3} (47±5)	700±150	.20±.09	.21±.02 x 10^{-3} (53±5)	600±50	.008±.002
3.1 (.145 x 10^{-3})	5 x 7	1.15±.09 x 10^{-3}	.64±.20 x 10^{-3} (56±8)	700±200	.27±.04	.48±.03 x 10^{-3} (44±8)	500±50	.008±.002

[a] Amounts of mercury were calculated from measured radioactivity content of samples using a value of 14.6 x 10^{4} cpm ug Hg^{-1} as the specific activity.

[b] Values in parentheses are the dry weight of Daphnia in grams determined from mean measured body length of Daphnia and length-dry weight relationships determined by Schindler (1968).

[c] Number of groups x number of animals per group.

[d] The "best-fitting" exponential curves were determined for clearance data of each group. Values presented are means ± S.E. of groups having the same body size.

[e] Values in parentheses represent the estimated mercury content of the compartment relative to the whole body content of Hg at one minute postexposure in per cent.

large animals, but as the fast compartment approaches steady state the net uptake rate will become more steeply related to body size as the uptake rate into the slow compartment makes up an ever-increasing proportion of the whole body net uptake rate.

Effect of Temperature on Uptake and Clearance of Methylmercury.

To determine whether temperature had an influence on the accumulation of methyl mercury from water, the uptake of methylmercury by Daphnia was determined at 5.5, 10.5, 15 and 20°C. Daphnia were exposed for eight hours to ^{203}Hg-labelled methylmercury in water at a concentration of 2×10^{-3} ug Hg ml^{-1}. The clearance of mercury accumulated at these temperatures was then determined at these same temperatures in the usual way. The animals used in tests at 10.5, 15, and 20°C were produced at those temperatures by females that had been reared at those temperatures. Animals tested at 5.5°C had been produced at 10.5°C and had been held at 5.5°C for at least four weeks.

The results in Table 12 show that ambient temperature had a pronounced effect on mercury accumulation. The amount of mercury taken up was least at 5.5°C and increased three fold to a peak value at 15°C before declining slightly with a further increase in temperature to 20°C.

Mercury clearance was biphasic at all temperatures (Fig. 10). Fractional clearance rates for both fast- and

Table 12. The effect of temperature on the uptake of methyl mercury from water by Daphnia magna during an eight-hour period of exposure.

Water temperature, °C	Numbers[a] of Daphnia	Duration of exposure, hours	Mean Hg concentration[b] in exposure medium, ug Hg ml^{-1}	Hg content[c] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Transfer coefficient,[d] hr^{-1}
5.5	1 x 13	8.1	2.0×10^{-3} (-10)	$.5 \times 10^{-3}$	220
10.5	3 x 9	8.0	2.1×10^{-3} (-2)	$1.2 \pm .04 \times 10^{-3}$	520±10
15	1 x 14	8.0	2.1×10^{-3} (<-1)	1.6×10^{-3}	660
20	3 x 12	8.1	2.2×10^{-3} (<-1)	$1.2 \pm .1 \times 10^{-3}$	500±30

[a] Number of groups x number of animals per group.

[b] Hg concentrations based on measured radioactivity concentrations and a specific activity of 3.46×10^{4} cpm ug Hg^{-1}. See Table for details. Values in parentheses refer to per cent changes in Hg concentration in exposure medium during exposure.

[c] Hg content based on measured radioactivity content.

[d] The estimate of body weight used in these calculations, $.135 \times 10^{-3}$ g Daphnia^{-1}, was made from the mean body length, using the length-dry weight relationship determined by Schindler (1968).

Fig. 10. Whole body clearance of methylmercury from Daphnia magna at various temperatures following uptake from water during eight hours of exposure at a concentration of 2.0×10^{-3} ug Hg ml^{-1}. The data are presented as radioactivity in cpm and specific activity of the ^{203}Hg-labelled mercury was 3.46×10^{4} cpm ug Hg^{-1}.

○ Water temperature was 20°C

$$Y(t) = 12.8e^{-.21t} + 33.4e^{-.009t}$$

● Water temperature was 15°C

$$Y(t) = 18.7e^{-.24t} + 39.0e^{-.009t}$$

□ Water temperature was 10.5°C

$$Y(t) = 27.2e^{-.22t} + 17.4e^{-.010t}$$

■ Water temperature was 5.5°C

$$Y(t) = 5.6e^{-.16t} + 12.4e^{-.010t}$$

Best-fitting curves were determined by least-squares method. Y(t) is the mercury body burden in cpm after t hours of clearance. Points marked with vertical bars represent means $\pm$ S.

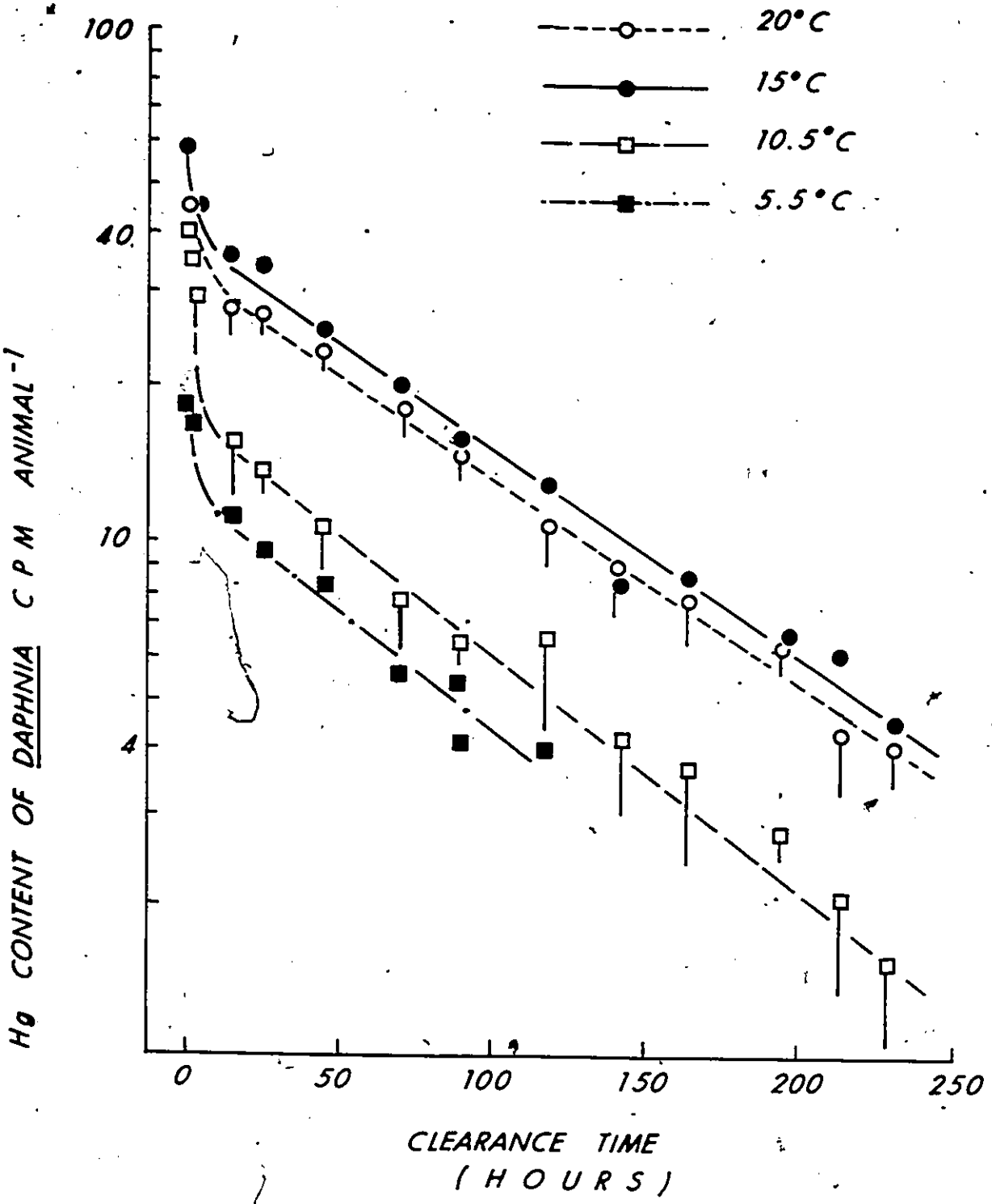

20°C
15°C
10.5°C
5.5°C
100
40
10
4
0
50
100
150
200
250
Hg CONTENT OF DAPHNIA C P M ANIMAL⁻¹
CLEARANCE TIME
(H O U R S)

FIG. 10

slow-clearing compartments were independent of temperature. Biological half-lives for mercury clearance from the fast compartment were in the range of 3 - 4 hours and biological half-lives for mercury clearance from the slow compartment were in the range of 67 - 77 hours.

Temperature appears to have had a significant effect on the relative sizes of the fast and slow compartments. The slow compartment made up 65 to 75% of the initial body burden at 5.5, 15, and 20°C, but at 10.5°C the slow compartment made up only about 40% (Table 13). This suggests that temperature influenced accumulation into fast and slow compartments in different ways. Since mercury clearance from neither compartment was influenced by temperature, the effect of temperature on the acquired body burden of mercury must be mediated through an effect on mercury uptake.

Temperature had a less pronounced effect on the uptake rate of methylmercury into the slow compartment (Fig. 11). The uptake rate into the slow compartment increased steadily from a minimum value of 150 g g^{-1} hr^{-1} at 5.5°C to a peak value of 490 g g^{-1} hr^{-1} at 15°C. A further increase in temperature to 20°C resulted in a slight decline in uptake to 400 g g^{-1} hr^{-1}.

The effect of temperature on the whole body transfer rate for methylmercury uptake from water was determined by summing the uptake rate of mercury into the slow compartment and the

Table 13. The effect of temperature on compartmental kinetics parameter values for methyl mercury accumulation from water. Animals of 3 - 3.3 mm body length were exposed to ^{203}Hg-labelled methyl mercury at a concentration[a] of 2.1×10^{-3} ug Hg ml^{-1} for eight hours.

Water temperature, °C	Hg content[a] of Daphnia at one minute postexposure, ug Hg Daphnia^{-1}	Fast-clearing compartment[b]			Slow-clearing compartment[b]		
		Compartment[c] size, ug Hg Daphnia^{-1}	Hg uptake,[d] transfer coefficient, hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}	Compartment[c] size, ug Hg Daphnia^{-1}	Hg uptake,[d] transfer coefficient, hr^{-1}	Hg loss, fractional clearance rate, hr^{-1}
5.5	$.5 \times 10^{-3}$	$.17 \times 10^{-3}$ (33)	80	.16	$.34 \times 10^{-3}$ (67)	150	.011
10.5	$1.2 \pm .04 \times 10^{-3}$	$.75 \pm .07 \times 10^{-3}$ (60 ± 6)	330 ± 30	$.22 \pm .04$	$.50 \times 10^{-3}$ (40 ± 6)	220 ± 40	$.010 \pm .001$
15	1.6×10^{-3}	$.52 \times 10^{-3}$ (32)	230	.24	1.1×10^{-3} (68)	490	.009
20	$1.2 \pm .1 \times 10^{-3}$	$.33 \pm .06 \times 10^{-3}$ (26 ± 5)	140 ± 30	$.24 \pm .04$	$.95 \pm .09 \times 10^{-3}$ (74 ± 5)	400 ± 40	$.010 \pm .002$

[a]Amounts of mercury were calculated from measured radioactivity content of samples using a value of 3.46×10^{4} cpm ug Hg^{-1} as the specific activity.

[b]The "best-fitting" exponential curves were determined for clearance data of each group. Values presented are means ± S.E. of groups from the same temperature.

[c]Values in parentheses represent the estimated mercury content of the compartment relative to the whole body content at one minute postexposure in per cent.

[d]The estimate of body weight used in these calculations, $.135 \times 10^{-3}$ g dry weight Daphnia^{-1}, was made from the mean body length, using the length-dry weight relationship determined by Schindler (1968).

Fig. 11. Relationships between temperature and transfer coefficients for whole body uptake (○) and slow compartment uptake (□) of methylmercury from water. Transfer coefficient values for methylmercury uptake into the whole body are the sum of slow compartment uptake and fast compartment uptake rates corrected for clearance. Each point represents the mean ± S.E.

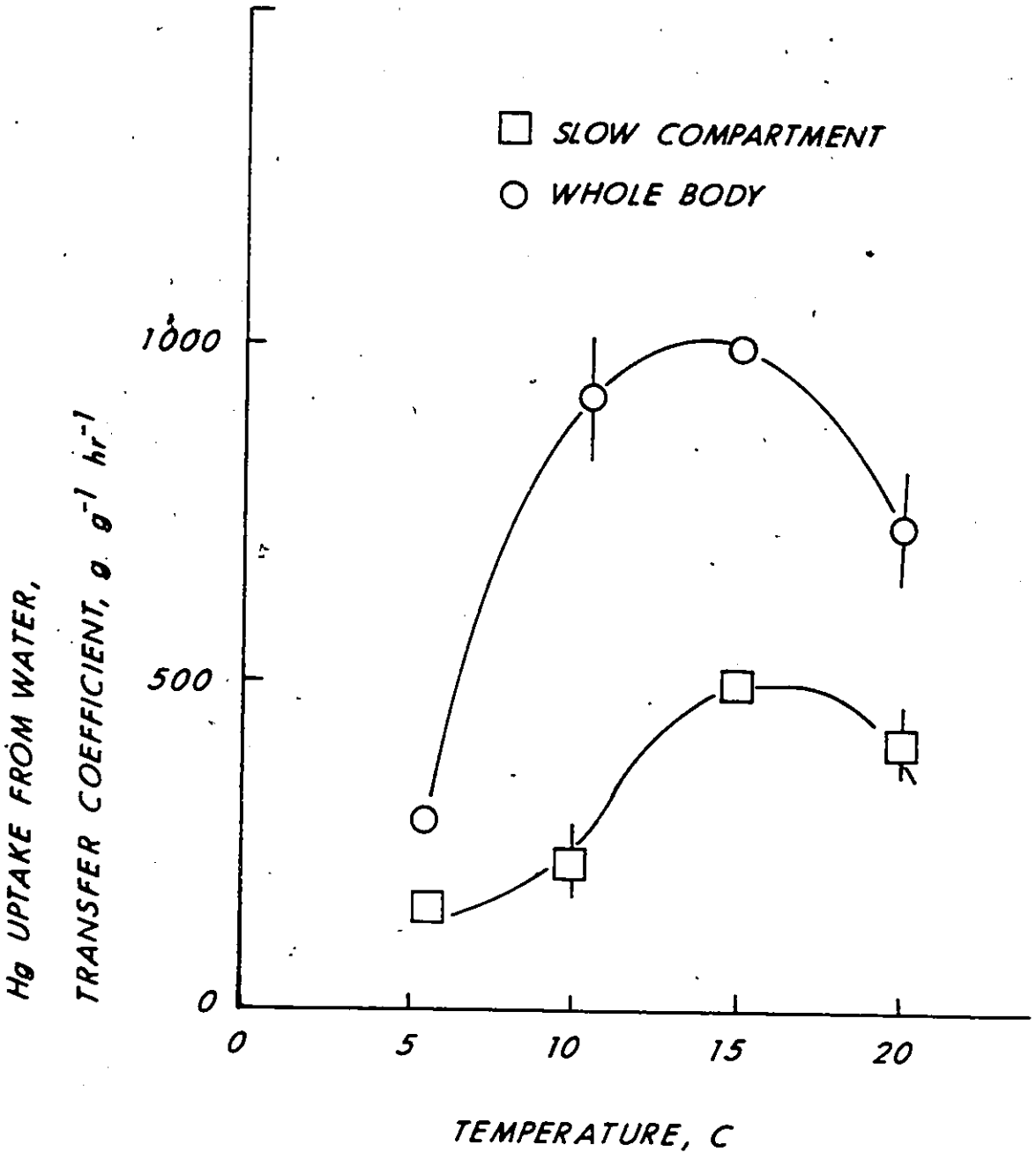

SLOW COMPARTMENT
WHOLE BODY
1000
500
0
0
5
10
15
20
Hg UPTAKE FROM WATER,
TRANSFER COEFFICIENT, ø g⁻¹ hr⁻¹
TEMPERATURE, C

FIG 11

clearance-corrected uptake rate into the fast compartment for animals tested at each temperature. These results, shown in Fig. 11, demonstrate that the whole body transfer coefficient was least, 330 g g^{-1} hr^{-1}, at 5.5°C and increased sharply to 920 g g^{-1} hr^{-1} at 10.5°C. There was little difference between transfer coefficients at 10.5°C and 15°C but between 15°C and 20°C the transfer coefficient declined slightly to 730 g g^{-1} hr^{-1}.

Uptake and Clearance of Methylmercury with Uptake via the Food Vector.

To determine the efficiency with which Daphnia assimilate methylmercury from the diet, Daphnia were fed on methylmercury-contaminated food by exposing them for 25 minutes to a suspension of ^{203}Hg-contaminated yeast with a cell density of $.1 \times 10^{6}$ cells ml^{-1}. Daphnia were then transferred to a suspension of uncontaminated yeast cells. Daphnia were maintained in the uncontaminated medium for six days, and the clearance of the ingested mercury was determined from measurements of the radioactivity retained. The amount of mercury that was assimilated by Daphnia was determined from the mercury retained by Daphnia after the unassimilated Hg had been lost from the gut.

When Daphnia were exposed for 25 minutes to a suspension of ^{203}Hg-laden yeast cells containing 2×10^{-3} ug Hg per million yeast cells, Daphnia accumulated 1.43×10^{-3} ug Hg Daphnia^{-1}. If the Hg content of Daphnia was due solely to the Hg content of ingested yeast cells, the feeding rate of Daphnia was $.16 \times 10^{6}$ cells Daphnia^{-1} hr^{-1}. This is consistent with the values reported by McMahon (1965) for Daphnia feeding on yeast cells under similar conditions. The presence of mercury in the yeast suspension, therefore, did not adversely influence the feeding behaviour of Daphnia.

The results in Fig. 12 show that when Daphnia were transferred to an uncontaminated yeast suspension, the loss of Hg from Daphnia occurred in two phases. The fast-clearing phase, which amounted to approximately 200 cpm Daphnia^{-1}, or about 40% of the ingested Hg, was lost with a biological half-life of one hour and appears to have been lost completely by the eighth hour of clearance. The remaining 300 cpm Daphnia^{-1}, or 60% of the ingested dose, was cleared much more slowly with a biological half-life of 140 hours. Clearance from the latter compartment was monitored for almost two biological half-lives and no evidence of additional compartments was observed.

The fast-clearing phase undoubtedly represents the loss of ingested mercury which was not assimilated by Daphnia and which was lost as the gut contents were voided. The slow-clearing phase appears to represent the mercury which was assimilated into the tissues of Daphnia.

The amount of mercury that was assimilated into the slow compartment was estimated by extrapolating to zero time the curve that best fit the slow-clearance phase of the clearance curve. The ratio of the amount of mercury assimilated and the amount of mercury ingested is an estimate of the efficiency with which Daphnia assimilated ingested Hg. The size of the slow-clearing phase, in the present case, was estimated to be 314 cpm and the total amount

Fig. 12. Clearance of ingested methylmercury by Daphnia magna. The experiment was conducted at 20°C in yeast cell suspensions containing 0.1 x 10^6 cells ml^{-1}. Mercury loss from the slow compartment could be described by the expression

$$Yt = .314e^{-.0052t}$$

where Yt is mercury content of the slow-clearing compartment and t is clearance time in hours. Points represent the mean $\pm$ S.E. estimates for Hg body burden of Daphnia in counts per minute per Daphnia. The specific activity of ^{203}Hg-labelled CH_3HgCl was 35.7 x 10^4 cpm ug Hg^{-1}.

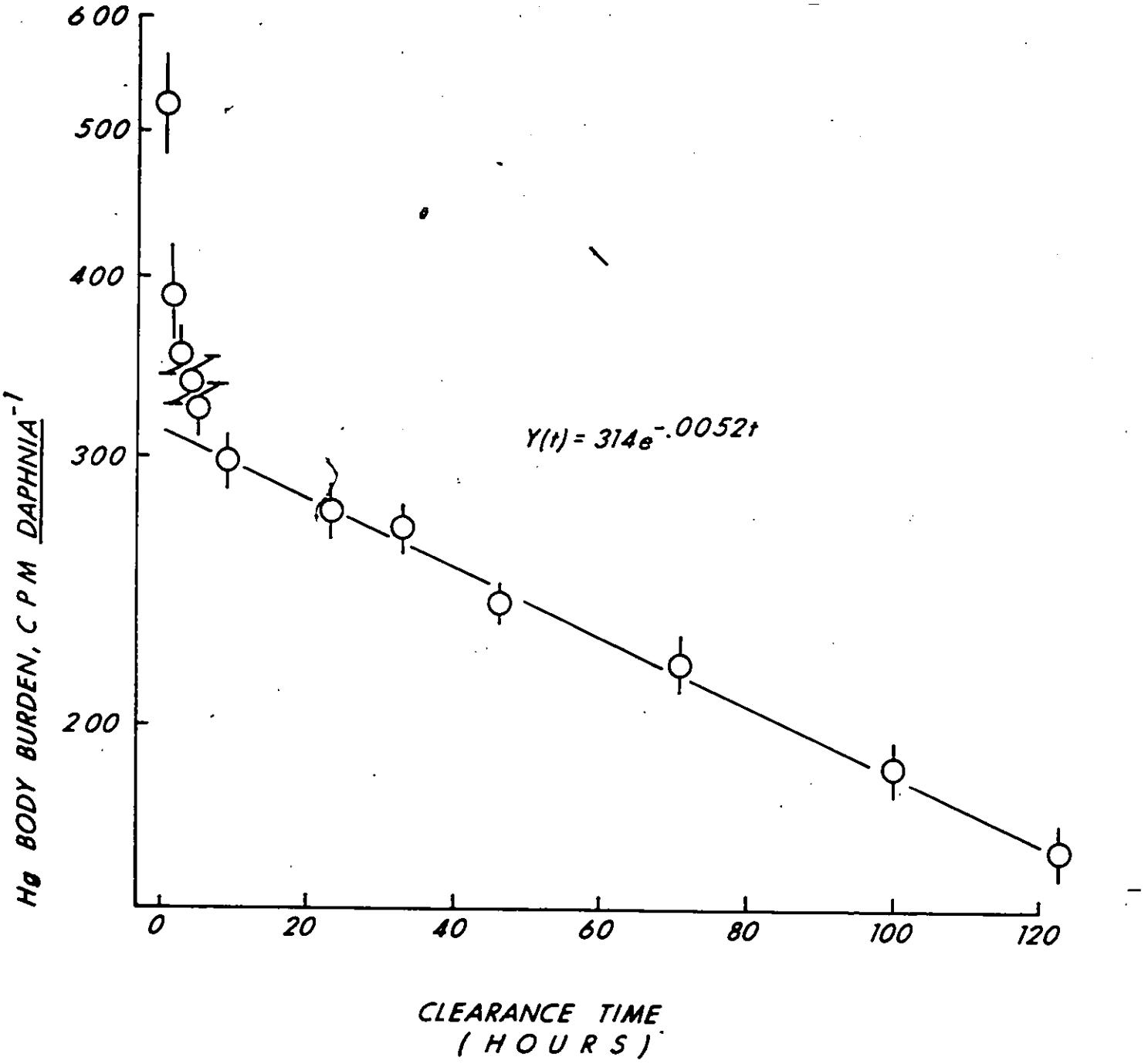

600
500
400
300
200
0
20
40
60
80
100
120
Hg BODY BURDEN, CPM DAPHNIA^{-1}
$Y(t) = 314e^{-.0052t}$
CLEARANCE TIME
(HOURS)

FIG. 12.

of Hg ingested was 515 cpm, making the assimilation efficiency for methylmercury approximately 61%.

It is important, at this point, to consider two potentially important sources of error in this technique. First, it is important to demonstrate that the estimate of "ingested mercury" includes only Hg ingested with the diet and includes no ^{203}Hg that had been lost from the yeast to the water and was in turn taken up from the water by Daphnia. Second, it must be demonstrated that the duration of the exposure period was less than the voidance time of ingested food. If the estimate of "ingested mercury" includes some mercury taken up from the water, the "ingested mercury" would be overestimated and the assimilation efficiency might be underestimated. On the other hand, if some of the ingested mercury had passed completely through the gut prior to measurement of initial body burden (= amount of ingested ^{203}Hg), then the assimilation efficiency would be overestimated.

To determine if Daphnia might be taking up ^{203}Hg from the water during exposure to the ^{203}Hg-contaminated food, 10 Daphnia were exposed for 30 minutes to the filtrate (Whatman GF/C) of a ^{203}Hg-contaminated feeding suspension in which a group of 10 Daphnia had already grazed for 30 minutes. The results in Table 14 show that more than 98% of the radioactivity was removed from the feeding suspension by filtration, indicating that 98% of the

Table 14. Uptake of mercury via food and water vectors during exposure to ^{203}Hg-labelled methyl mercury in feeding experiments. Feeding suspension used had a cell density of .1 x 10^6 cells per ml containing 1.8 ug Hg g^{-1} yeast cells.[a]

Condition	Numbers of *Daphnia*	Hg concentration[b] in exposure medium, ug Hg ml^{-1}	Duration of exposure, hours	Hg content[c] of *Daphnia* at one minute postexposure, ug Hg *Daphnia*
Feeding suspension	10	2.95 x 10^{-3}	0.5	1.33 x 10^{-3}
Filtrate of feeding[d] suspension	10	0.04 x 10^{-3}	0.5	.02 x 10^{-3}

[a]Calculated on the basis of an assumed dry weight of yeast of .0165 x 10^{-3} g per million cells.

[b]Calculated from measured radioactivity of exposure medium and specific activity of 29.7 x 10^4 cpm ug Hg^{-1}.

[c]Calculated from measured radioactivity of *Daphnia*.

[d]Prepared from feeding suspension that had been prepared as described above (page 9) and to which *Daphnia* had been exposed for 30 minutes. Suspension was filtered through a Whatman GF/C filter.

^{203}Hg in the suspension was associated with the cells and only 2% may have been in the aqueous phase. Daphnia exposed for 30 minutes to the filtrate accumulated only about $.02 \times 10^{-3}$ ug Hg Daphnia^{-1}, or only about 2% as much mercury as was taken up by animals exposed to the yeast suspension prior to filtration. Using the estimates of transfer coefficients for uptake of methylmercury from water to Daphnia made above, Daphnia could be expected to take up only .005 to $.02 \times 10^{-3}$ ug Hg Daphnia^{-1} from the aqueous phase if all Hg in the GF/C filtrate was bioavailable. This source of error was so small that no attempt was made to correct the values for ingested mercury for it.

To determine whether Daphnia lost any mercury during the exposure period either due to egestion of unassimilated mercury or the excretion of assimilated Hg, Daphnia were exposed to a ^{203}Hg-contaminated feeding suspension for varying periods from 10 to 40 minutes and mercury clearance in uncontaminated yeast suspension was followed for 48 hours. If a significant fraction of the ingested mercury was lost during the dosing period, the rate of Hg accumulation would be expected to decline with increasing exposure time and the estimated assimilation efficiency would increase. The results in Fig. 13 show that the uptake of Hg increased linearly with the duration of exposure over a forty-minute period. This suggests that Daphnia fed at a constant rate and that none of the ingested mercury was lost within the

Fig. 13. Uptake of methylmercury by Daphnia during exposures varying in duration from 10 to 40 minutes to a yeast suspension of .1 x 10^6 cells ml^{-1} containing 1.4 mg Hg g^{-1} dry weight of yeast. The least squares regression line was

$$Yt = .042 \times 10^{-3}t$$

where Yt is the mercury body burden in ug Hg Daphnia^{-1} and t is the exposure time in minutes. Each point is a group of five Daphnia.

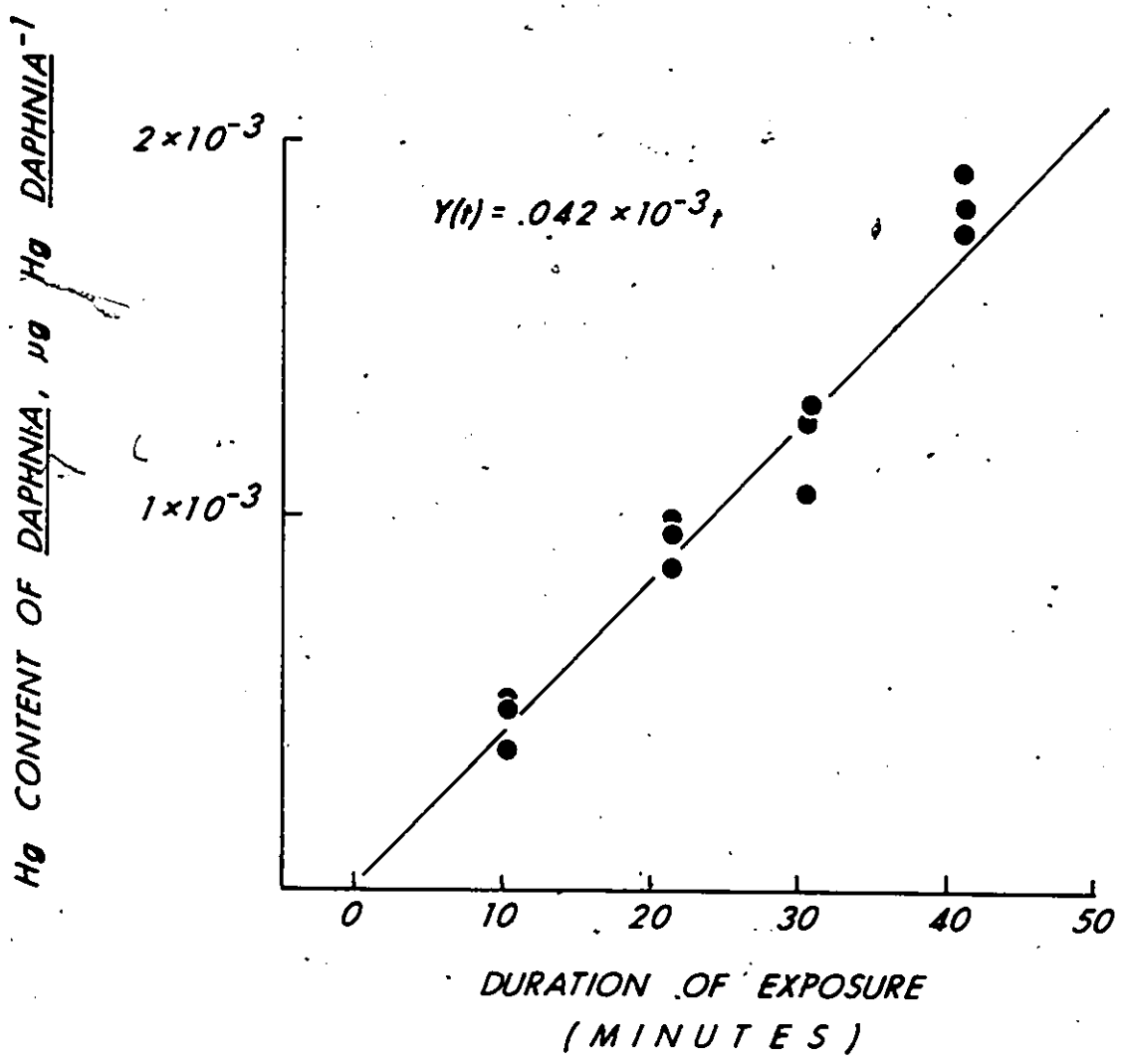

Hg CONTENT OF DAPHNIA, μg Hg DAPHNIA⁻¹
2×10⁻³
1×10⁻³
Y(t) = .042 ×10⁻³ t
0
10
20
30
40
50
DURATION OF EXPOSURE
(MINUTES)

FIG 13

forty-minute dosing period. The results of the clearance phase of this experiment, given in Table 15, showed that the net amount of mercury that was assimilated also increased with the duration of exposure. The ratio of assimilated mercury to ingested mercury showed some slight variation but overall remained constant at between 79 and 86% efficiency. The net assimilation efficiency was therefore independent of dose duration for dosing periods of up to 40 minutes.

It is possible to calculate a transfer coefficient for mercury uptake from food that is analogous to the transfer coefficient for mercury uptake from water. This unit is useful, since it normalizes uptake rates for effects of mercury concentration in the diet and body size, thereby facilitating comparison between experiments. In addition, the dose rate can be calculated using the transfer coefficient, requiring only an estimate of the concentration of mercury in the diet. The transfer coefficient for the uptake of mercury into the slow compartment from the diet, TC_S^D, which represents the rate of uptake into body tissues, can be expressed as follows:

$$TC_S^D = \frac{Y(t)}{P_F . W . t}$$

where Y(t) is the estimated mercury content of the slow compartment at time zero of clearance in ug Hg Daphnia^{-1}, P_F is the mercury concentration in the diet in ug Hg g yeast cells, W is the dry weight of Daphnia in grams, and t is the duration of exposure to

Table 15. The effect of duration of exposure on efficiency of assimilation of ingested mercury by Daphnia magna. This experiment was conducted at 20°C using Daphnia of 3 - 3.3 m body length. Animals were exposed to ^{203}Hg in a yeast suspension of $.25 \times 10^6$ cells ml^{-1} containing 0.6 mg Hg g^{-1} dry weight of yeast cells.[a]

Duration of exposure, minutes	Number of Daphnia[b]	Ingestion rate,[c] cells Daphnia^{-1} hr^{-1}	Amount of Hg[d] ingested, ug Hg Daphnia^{-1}	Net amount of Hg[e] assimilated, ug Hg Daphnia^{-1}	Net efficiency of Hg assimilation	Transfer[f] coefficient, hr^{-1}
10	3 x 5	$.27 \pm .03 \times 10^6$ ($.032 \pm .003$)	$.45 \pm .05 \times 10^{-3}$	$.37 \pm .09 \times 10^{-3}$	$.82 \pm .05$	$.027 \pm .003$
22	3 x 5	$.26 \pm .01 \times 10^6$ ($.031 \pm .001$)	$.93 \pm .05 \times 10^{-3}$	$.74 \pm .01 \times 10^{-3}$	$.79 \pm .03$	$.024 \pm .001$
30	3 x 5	$.25 \pm .02 \times 10^6$ ($.029 \pm .002$)	$1.21 \pm .09 \times 10^{-3}$	$1.05 \pm .07 \times 10^{-3}$	$.86 \pm .01$	$.025 \pm .002$
41	3 x 5	$.26 \pm .01 \times 10^6$ ($.031 \pm .001$)	$1.78 \pm .09 \times 10^{-3}$	$1.48 \pm .08 \times 10^{-3}$	$.83 \pm .02$	$.026 \pm .001$

[a] Based on an assumed dry weight of yeast of $.0165 \times 10^{-3}$ g per million cells.

[b] Number of groups x number of animals per group.

[c] Value in parentheses is the ingestion rate expressed as grams of yeast ingested per gram of Daphnia per hour.

[d] Amount of Hg calculated from measured radioactivity of Daphnia using specific activity of 29.4×10^4 cpm ug Hg^{-1}.

[e] Amount of Hg calculated from estimates of assimilated radioactivity.

[f] Dry weight of Daphnia, $.14 \times 10^{-3}$ g Daphnia $^{-1}$, determined from mean measured body length and length-dry weight relationship determined by Schindler (1968).

the contaminated yeast suspension in hours. The meaning of the term is probably more easily grasped by considering a more general form of the equation

$$TC_S^D = i.e_D$$

where i is the ingestion rate in terms of grams of food ingested per gram of Daphnia per hour and e_D is the efficiency of assimilation of mercury from the diet. In the present study Daphnia ingested yeast cells at a rate of .016 grams (dry weight) of yeast per gram (dry weight) of Daphnia per hour and assimilated 60% of the methylmercury that the food contained. The value of the transfer coefficient was therefore .011 hr^{-1}, that is, one gram of Daphnia will take up into its tissues the equivalent of the mercury content of .011 grams of food each hour. Hence if the diet contained 1 ug Hg g^{-1}, Daphnia feeding on that material would be taking up methylmercury at a rate of .011 ug Hg g^{-1} of Daphnia hr^{-1}.

Effect of Ambient Food Density on Feeding Rate and Assimilation Efficiency of Ingested Methylmercury.

To determine the effect of ambient food density on the uptake rate of methylmercury, Daphnia were fed on radioactive yeast suspensions of different cell densities: $.01 \times 10^6$; 0.1×10^6; and 0.5×10^6 cells ml^{-1}. All Daphnia were fed for 30 minutes. The yeast cells of all suspensions contained the same concentration of methylmercury, 1.1 mg Hg g^{-1} dry weight of food. At the end of the feeding period Daphnia were transferred to non-radioactive yeast suspensions with the same cell densities as the radioactive suspensions, for 24 hours to allow complete voidance of unassimilated ^{203}Hg from the gut. Whole body clearance of assimilated Hg was determined from radioactivity determination over the postexposure period.

The results in Fig. 14 show that the amount of Hg ingested by Daphnia increased with increasing ambient cell concentration but that the increase was not linear. This reflected the effect of ambient food concentration on ingestion rate by Daphnia. Rigler (1961) demonstrated that when a filter-feeding crustacean encounters low concentration of food, feeding rate is limited by the animal's ability to filter water and feeding rate is therefore proportional to the concentration of food. Above a critical concentration of food feeding rate is constant and limited by the ability of the animal to ingest the food cells. The critical food

Fig. 14. Amounts of methylmercury ingested (○) and assimilated (□) by Daphnia feeding in yeast suspensions of various cell densities. Animals were exposed for 30 minutes to suspensions of $.03 \times 10^6$, $.1 \times 10^6$, and $.5 \times 10^6$ cells ml^{-1} each of which contained ^{203}Hg-labelled methylmercury at a concentration of 1.1 mg Hg g^{-1} dry weight. Each point represents the mean $\pm$ S.E. of three determinations. Curves were drawn by eye.

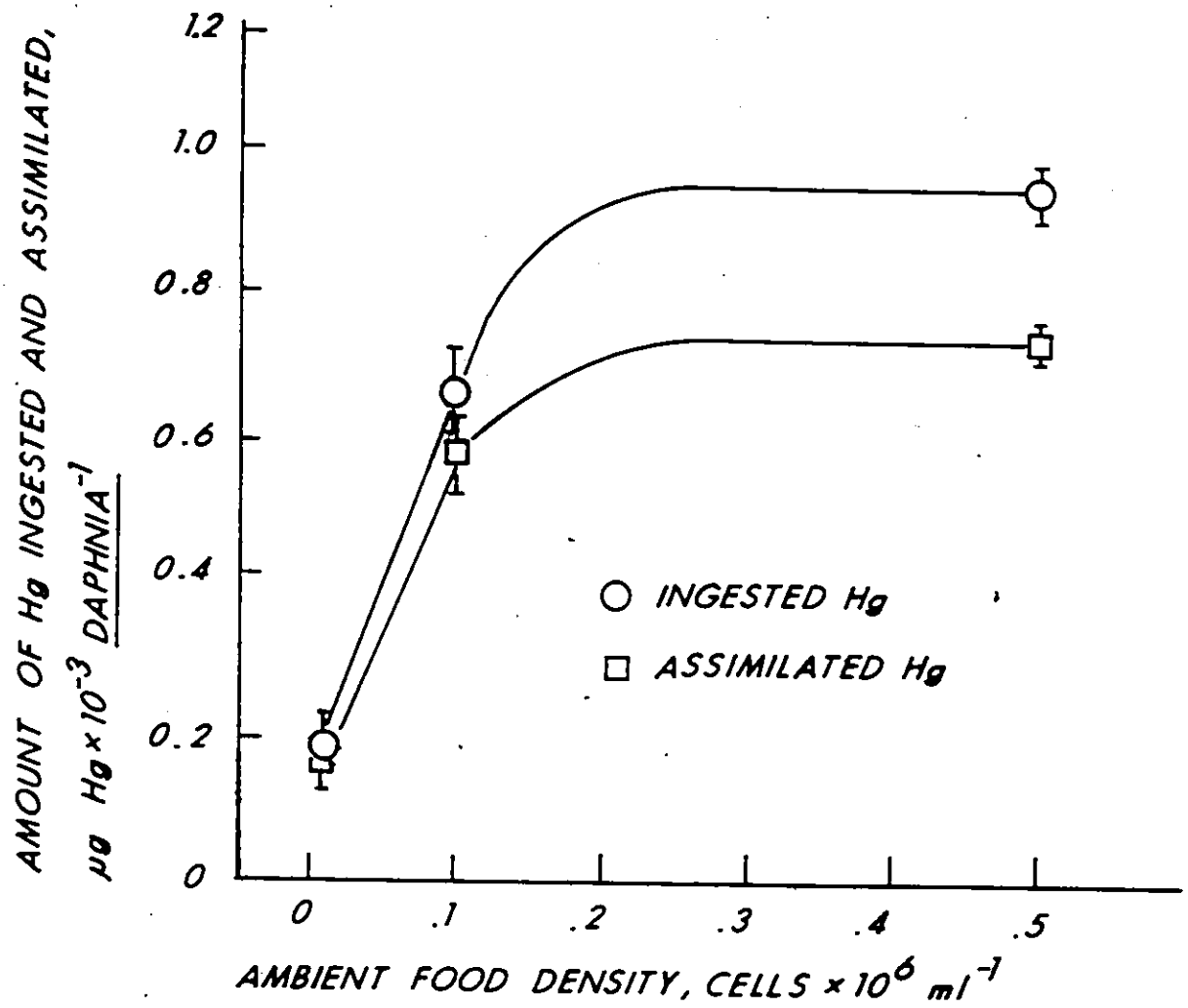

AMOUNT OF Hg INGESTED AND ASSIMILATED, μg Hg × 10⁻³ DAPHNIA⁻¹
1.2
1.0
0.8
0.6
0.4
0.2
0
0
.1
.2
.3
.4
.5
AMBIENT FOOD DENSITY, CELLS × 10⁶ ml⁻¹
INGESTED Hg
ASSIMILATED Hg

FIG 14

density can be called the "incipient limiting food density". From the results of the present study, "incipient limiting food density" lies between .1 x 10^6 and .5 x 10^6 cells ml^{-1}, which was consistent with the findings of Rigler (1961).

It is important to note that the maximum feeding rate of Daphnia observed in this study, when expressed in terms of volume of food consumed per Daphnia per hour, .007 mm^3 Daphnia hr^{-1},[1] was similar to that observed by McMahon and Rigler (1965) when the values were corrected for body size differences. This further supported the contention that neither the mercury content of the food nor any changes in the food caused by mercury contamination had any effect on the feeding behaviour of Daphnia.

The results in Table 16 show that the assimilation efficiency of methylmercury from the diet declined from 96% at a feeding rate of .03 x 10^6 cells Daphnia^{-1} hr^{-1} to 78% at a feeding rate of .11 x 10^6 cells Daphnia^{-1} hr^{-1}. However the plot of results in Fig. 14 shows that, despite the reduction in assimilation efficiency of methylmercury with increasing ambient food density, the rate of uptake of methylmercury into the tissues (= the rate of methylmercury assimilation) increases almost as

---

[1]Based on an assumed cell volume of 66 u^3 (McMahon and Rigler, 1965).

Table 16. The effect of ambient food concentration on the uptake of methyl mercury by Daphnia magna. This experiment was conducted at 20°C using Daphnia of 2.5 - 3.0 mm body length. Animals were exposed to ^{203}Hg during a 30 minute exposure to yeast suspensions containing 1.1 mg Hg g dry weight of yeast.[a]

Initial food density,[b] cells ml^{-1}	Number of Daphnia[c]	Ingestion rate,[d] cells Daphnia^{-1} hr^{-1}	Amount of Hg[e] ingested, ug Hg Daphnia^{-1}	Amount of Hg[f] assimilated, ug Hg Daphnia^{-1}	Net efficiency of Hg assimilation, %	Transfer coefficient,[g] hr^{-1}
$.01 \times 10^6$ (-60)	4 x 5	$.03 \pm .01 \times 10^{-3}$ ($.004 \pm .001$)	$1.78 \pm .02 \times 10^{-3}$	$1.74 \pm .05 \times 10^{-3}$	98 ± 2	$.004 \pm .001$
$.10 \times 10^6$ (-25)	4 x 5	$.09 \pm .01 \times 10^{-3}$ ($.013 \pm .002$)	$6.62 \pm .65 \times 10^{-3}$	$5.87 \pm .74 \times 10^{-3}$	88 ± 4	$.011 \pm .001$
$.5 \times 10^6$ (-12)	3 x 6	$.11 \pm .02 \times 10^{-3}$ ($.016 \pm .003$)	$9.50 \pm .34 \times 10^{-3}$	$7.38 \pm .13 \times 10^{-3}$	78 ± 2	$.012 \pm .002$

[a] Based on an assumed dry weight of yeast of $.0165 \times 10^{-3}$ g per million cells.

[b] Based on the number of cells added. Values in parentheses are the per cent reduction of cell densities during exposure as determined by the decline in radioactivity of the exposure medium.

[c] Numbers of groups x numbers of animals per group.

[d] Values in parentheses are the ingestion rates expressed as grams of yeast ingested per gram of Daphnia per hour.

[e] Amount of Hg was calculated from measured radioactivity of Daphnia using specific activity of 59.8×10^4 cpm ug Hg^{-1}.

[f] Amount of Hg was calculated from estimates of assimilated radioactivity.

[g] Dry weight of Daphnia, $.115 \times 10^{-3}$ g Daphnia^{-1}, determined from mean measured body length and length-dry weight relationship determined by Schindler (1968).

dramatically with increasing ambient food density as does the ingestion rate. Ambient food density has a pronounced effect on the rate of uptake of methylmercury into Daphnia tissues. This effect is primarily due to an effect on the ingestion rate with a lesser effect on assimilation efficiency.

Effect of Body Size on Assimilation and Clearance of Ingested Methylmercury.

To determine the effect of body size on uptake of methylmercury from food and its subsequent clearance, Daphnia of sizes 1 - 1.3, 1.7 - 2.3, and 3.0 - 3.7 mm body length were fed on yeast containing ^{203}Hg-labelled methylmercury and then were transferred to non-radioactive yeast suspensions for determination of methylmercury assimilation efficiencies and clearance rates. Feeding suspensions contained .1 x 10^6 cells ml^{-1}. Daphnia ingested ^{203}Hg-labelled methylmercury during 25 minutes of exposure to yeast containing 1.4 mg Hg g^{-1} dry weight.

Mercury ingestion increased with increasing body size (Table 17), reflecting the influence of body size on feeding rate that has been reported previously by several authors (Burns, 1969; McMahon, 1965). Sushchenya (1970) reported that the relationship between feeding rates and body sizes of various crustaceans was of the form

$$I_F = a\, W^b$$

Table 17. The effect of body size on the uptake of methyl mercury via the food vector by Daphnia magna. Animals were exposed to ^{203}Hg for 25 minutes to yeast suspensions of .1 x 10^6 cells ml^{-1} containing 1.4 mg Hg g dry weight[a] of yeast at 20°C.

Body size,[b] mm	Number of Daphnia[c]	Ingestion rate,[d] cells Daphnia hr^{-1}	Amount of Hg[e] ingested, ug Hg Daphnia^{-1}	Amount of Hg[f] assimilated, ug Hg Daphnia^{-1}	Net efficiency of Hg assimilation, %	Transfer coefficient, hr^{-1}
1.15 (.0075)	3 x 17	.015±.002 x 10^6 (.032±.011)	.13±.035 x 10^{-3} (17.4±4.7)	.07±.02 x 10^{-3}	57±1	.018±.003
2.0 (.045)	3 x 10	.580±.002 x 10^6 (.021±.001)	.52±.01 x 10^{-3} (11.56±.22)	.33±.01 x 10^{-3}	63±6	.013±.001
3.1 (.16)	3 x 7	.156±.09 x 10^6 (.016±.009)	1.43±.08 x 10^{-3} (8.9±.5)	.90±.08 x 10^{-3}	63±3	.010±.001

[a]Based on an assumed dry weight of yeast of .0165 x 10^{-3} g per million cells.

[b]Values in parentheses are dry weights in mg estimated using the length-dry weight relationship of Schindler (1968).

[c]Number of groups x number of animals per group.

[d]Value in parentheses is the ingestion rate expressed as grams of yeast ingested per gram of Daphnia per hour.

[e]Amount of Hg calculated from measured radioactivity of Daphnia using specific activity of ^{203}Hg was 35.7 x 10^4 cpm ug Hg^{-1}.

[f]Amount of Hg calculated from estimates of assimilated radioactivity.

where I_F was the rate of ingestion in grams of food per animal per hour, W was the body weight, a was the coefficient defining the level of food consumption per unit time when W = 1 and b defined the rate of change of food consumption with body weight. This model yielded a good fit to the data giving the following relationship between ingestion and body size:

$$I_{Hg} = 4.14\ W^{.81}$$

where I_{Hg} was the ingestion rate of mercury in ug Hg Daphnia^{-1} hr^{-1} and W was the dry weight of Daphnia in grams (Fig. 15). The value of the coefficient, in this case 4.14 ug Hg Daphnia^{-1} hr^{-1}, will vary with the ambient food concentration and with the concentration of mercury in the diet. The value of the body weight exponent in this study, .81, agrees well with values reported by Sushchenya (1970) for the body weight exponent for ingestion rate by D. magna and D. pulex of .81 to .88.

The results in Table 17 showed that both the amount of methylmercury assimilated and the assimilation efficiency increase with body size. The effect of body size on assimilation efficiency was, however, very slight. Hence, the relationship between body size on methylmercury assimilation rate is very similar to that of body size and ingestion rate, demonstrating clearly that the effect of body size on ingestion rate was far more important in determining the rate of assimilation of methylmercury than was the effect of body size on assimilation efficiency (Fig. 15).

Fig. 15. Relationship of ingestion and assimilation of methylmercury to body size of Daphnia when Daphnia were exposed to a yeast suspension of .1 x 10^{-3} cells ml^{-1} containing 1.4 mg Hg g^{-1} dry weight of yeast.

○ Rate of mercury ingestion

$$I_{Hg} = 4.14W^{.806}$$

● Rate of mercury assimilation

$$A_{Hg} = 2.82W^{.822}$$

where I_{Hg} was the rate of ingestion of mercury in ug Hg Daphnia^{-1} hr^{-1}; A_{Hg} was the rate of mercury assimilation in ug Hg Daphnia hr^{-1}; and W was the dry weight of Daphnia in grams. The "best-fitting" regression lines were determined by method of least squares. Each point represents one group of 5 - 20 Daphnia.

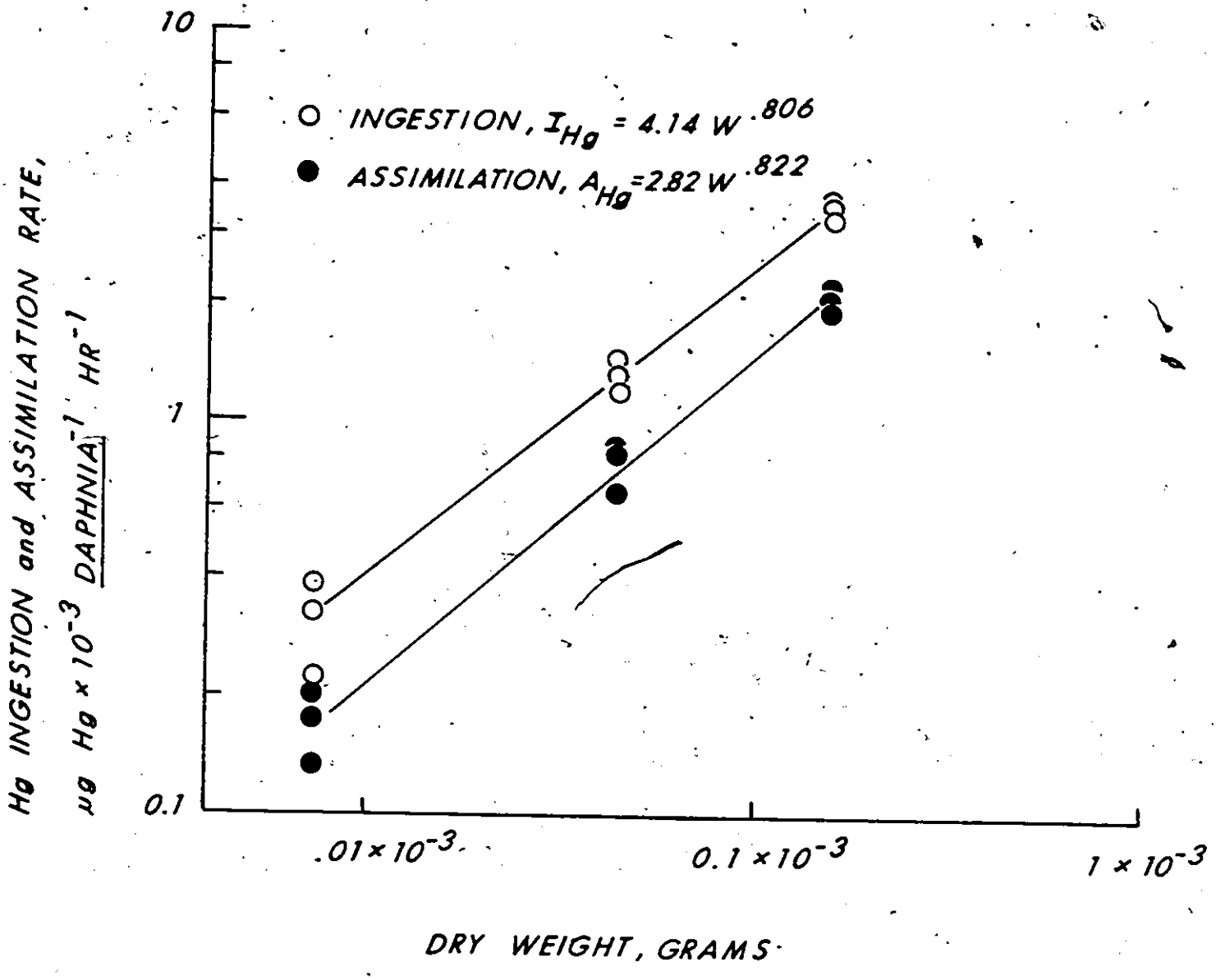

10
1
0.1
INGESTION, $I_{Hg} = 4.14\ W^{.806}$
ASSIMILATION, $A_{Hg} = 2.82\ W^{.822}$
Hg INGESTION and ASSIMILATION RATE,
$\mu g\ Hg \times 10^{-3}$ DAPHNIA^{-1} HR^{-1}
$.01 \times 10^{-3}$
0.1×10^{-3}
1×10^{-3}
DRY WEIGHT, GRAMS

FIG 15

The results in Fig. 16 show that the fractional clearance rate of assimilated mercury declined slightly with increasing body size. The biological half-time for the group containing the smallest animals was 110 hours and this increased with increasing body size to 133 hours acording to the relationship

$$BT_{\frac{1}{2}} = 223\ W^{.06}$$

where $BT_{\frac{1}{2}}$ was the biological half-time in hours. This effect is so small that the change in fractional clearance rate with increasing body size over the life history of the animal would not have an appreciable influence on methylmercury accumulation.

Effect of Temperature on Uptake and Clearance of Ingested Methylmercury.

To determine the effect of temperature on uptake rate and clearance of methylmercury taken up via the food vector, rates of ingestion, assimilation, and clearance of methylmercury were determined at temperatures of 10, 20, and 28°C. Daphnia were given an ingested dose of ^{203}Hg-labelled methylmercury by allowing them to graze for 30 minutes in a yeast suspension of 0.1×10^{6} cells ml^{-1} containing 1.7 mg Hg g^{-1} of yeast. Daphnia were then transferred to a suspension of uncontaminated yeast cells where they cleared their guts of unassimilated mercury. The amount of Hg assimilated was determined in the usual way from the Hg content of Daphnia following egestion of unassimilated mercury.

Fig. 16. Clearance of an ingested dose of methylmercury by Daphnia magna of three body size ranges. Experiment was conducted at 20°C. Curves fitted to slow compartment data only.

● Initial weight of Daphnia approximately .16 x 10^{-3} g dry weight
$Yt = 314e^{-.0052t}$

□ Initial weight of Daphnia approximately .045 x 10^{-3} g dry weight
$Yt = 116e^{-.0055t}$

○ Initial weight of Daphnia approximately .0075 x 10^{-3} g dry weight
$Yt = 26e^{-.0062t}$

The curves represent "best fit" exponential curves defined by equations, where Yt = Hg body content retained by Daphnia and t = clearance time in hours. Points represent mean ± S.E. estimates for Hg body burden of Daphnia in counts per minute per Daphnia.

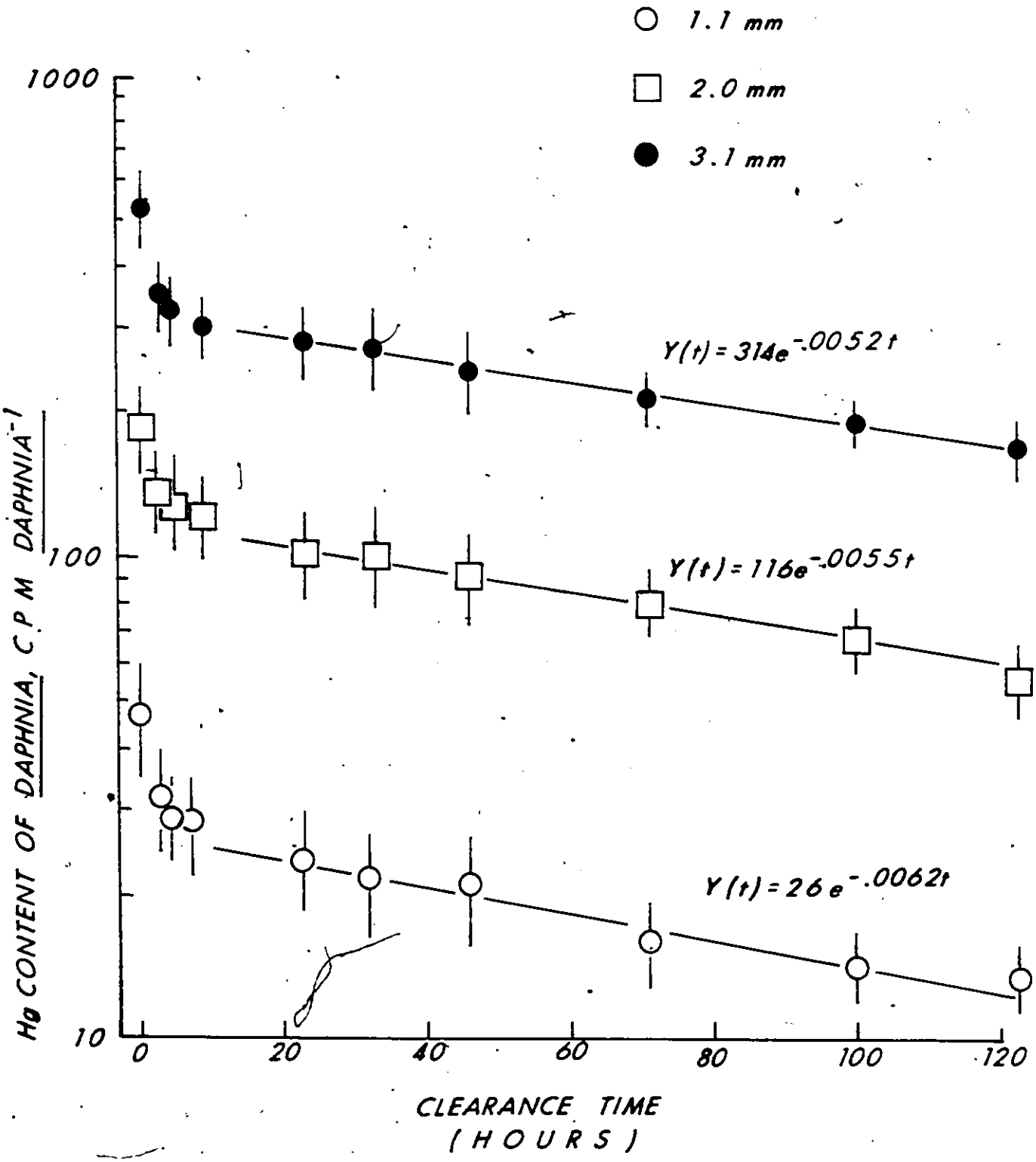

1.1 mm
2.0 mm
3.1 mm
1000
100
10
Y(t) = 314e^-.0052t
Y(t) = 116e^-.0055t
Y(t) = 26e^-.0062t
Hg CONTENT OF DAPHNIA, C P M DAPHNIA^-1
0
20
40
60
80
100
120
CLEARANCE TIME
(H O U R S)

FIG 16

The rate of methylmercury clearance at each temperature was determined in the usual way from daily measurements of the amount of mercury retained by Daphnia taken over the first four days. All animals used in these tests were offspring of females that had been reared at experimental temperatures and hence were thoroughly acclimated to experimental conditions.

Because of limitations of space and equipment only two groups of animals could be tested simultaneously at each temperature, instead of three or more groups per test condition as were used in other studies. Although the variation between groups was occasionally large, the trends in the data were repeatable.

Temperature had little effect on ingestion rate. The results in Table 18 show that there was no difference between the ingestion rates observed at 10° and 20°C, and that the ingestion rate at 28°C was only about 25% lower than it was at 20°C. Temperature did, however, have a pronounced influence on assimilation efficiency. Assimilation efficiency was lowest, about 40%, at 10°C and increased to a maximum, 74%, at 20°C. At 28°C assimilation efficiency was lower, 60%, than it had been at 20°C. Due to the combined effects of temperature on ingestion rate and assimilation efficiency, the rate of methylmercury assimilation (i.e., the rate of uptake of methylmercury into the

Table 18. The effect of temperature on uptake of methyl mercury from food by Daphnia magna. Animals of 2.5 - 3.0 mm body length were exposed to ^{203}Hg laden food for 30 minutes. Yeast suspensions of $.1 \times 10^6$ cells ml^{-1} contained 1.7 mg Hg g dry weight of yeast[a].

Temperature, C	Number of Daphnia[b]	Ingestion rate,[c] cells Daphnia^{-1} hr^{-1}	Amount of Hg[d] ingested, ug Hg Daphnia^{-1}	Amount of Hg[e] assimilated, ug Hg Daphnia^{-1}	Net efficiency of Hg assimilation, %	Transfer coefficient,[f] hr^{-1}
10	2 x 6	$.12 \times 10^6$ ($.10-.15 \times 10^6$)	1.78×10^{-3}	$.71 \times 10^{-3}$	.40 (.32-.50)	.007 (.007-.007)
20	2 x 6	$.12 \times 10^6$ ($.11-.12 \times 10^6$)	1.70×10^{-3}	1.30×10^{-3}	.74 (.72-.77)	.013 (.012-.014)
28	2 x 6	$.09 \times 10^6$ ($.05-.12 \times 10^6$)	1.25×10^{-3}	$.75 \times 10^{-3}$	.60 (.59-.61)	.008 (.005-.011)

[a]Based on an assumed dry weight of yeast of $.0165 \times 10^{-3}$ g per million cells.

[b]Number of groups x number of animals per group.

[c]Values in parentheses are the observed ingestion rates for each group.

[d]Amount of Hg calculated from measured radioactivity of Daphnia using specific activity of 25.5×10^4 cpm ug Hg^{-1}.

[e]Amount of Hg calculated from estimates of assimilated radioactivity.

[f]Dry weight of Daphnia, $.115 \times 10^{-3}$ g of Daphnia^{-1}, determined from mean measured body length and length-dry weight relationship determined by Schindler (1968).

tissues) is almost twice as great at 20°C as at either 10 or 28°C.

The fractional clearance rates of methylmercury that had been assimilated from the diet were the same at all temperatures as had earlier been observed when uptake was via the water vector. Biological half-times of methylmercury loss were in the range of 135 - 150 hours (Fig. 17).

Assimilation Efficiency and Clearance of Ingested Inorganic Mercury.

To determine the assimilation efficiency of inorganic mercury from the diet and its subsequent clearance, Daphnia ranging in size from 2.3 - 2.7 mm body length were exposed to a yeast suspension of $.1 \times 10^6$ cells ml^{-1} containing ^{203}Hg-labelled $HgCl_2$ at a concentration of 1.6 ug Hg g^{-1} dry weight of yeast. Following exposure to the ^{203}Hg contaminated food Daphnia were transferred to non-radioactive medium, where Hg loss was monitored in the usual way for 52 hours to determine the amounts of mercury ingested and assimilated and the rate at which the assimilated mercury was lost.

The results in Table 19 show that Daphnia took up $.81 \times 10^{-3}$ ug Hg Daphnia^{-1} during 30 minutes of exposure to the contaminated yeast suspension. The estimate of ingestion rate $.059 \times 10^6$ cells Daphnia^{-1} hr^{-1}, based on the observed Hg uptake, was slightly lower than estimates of ingestion rate of food made

Table 19. The assimilation efficiencies of methyl and inorganic mercury from the diet by Daphnia magna. Animals were exposed to yeast suspensions of .1 x 10^6 cells ml containing ^{203}Hg-labelled CH_3HgCl or $HgCl_2$ at 20°C.

Chemical form of Hg	Number of Daphnia[a]	Body size,[b] mm	Concentration of Hg in yeast,[c] ng Hg g^{-1}	Ingestion rate,[d] cells Daphnia^{-1} hr^{-1}	Amount of Hg ingested,[e] ug Hg Daphnia^{-1}	Amount of Hg assimilated,[f] ug Hg Daphnia^{-1}	Net efficiency of Hg assimilation, %	Transfer coefficient, hr^{-1}
$HgCl_2$	5 x 5	2.5 (.095)	1.6	.059±.04 x 10^6 (.010±.007)	.81 x 10^{-3}	.04±.01 x 10^{-3}	5±1	.0005±.0002
CH_3HgCl	3 x 10	2.0 (.045)	1.4	.058±.002 x 10^6 (.021±.001)	.52±.01 x 10^{-3}	.33±.03 x 10^{-3}	63±6	.013±.001

[a] Numbers of groups x numbers of animals per group.

[b] The value in parentheses is the dry weight in grams x 10^{-3} which was made from the mean body length using the length-dry weight relationship determined by Schindler (1968).

[c] Mercury concentrations based on measured radioactivity concentrations using a value of 35.7 x 10^4 cpm ug Hg^{-1} for methylmercury and 101.4 x 10^4 cpm ug Hg^{-1} for inorganic mercury.

[d] Value in parentheses is ingestion rate expressed as grams of yeast ingested per gram of Daphnia per hour.

[e] Amount of Hg ingested is computed from measured radioactivity of Daphnia.

[f] Amount of Hg assimilated is computed from estimated assimilated radioactivity.

Fig. 17. Clearance of an ingested dose of methylmercury at three temperatures. The experiment was conducted at yeast cell density of .1 x 10^6 cells ml^{-1} using Daphnia of 2.5 - 3.0 mm body length. Curves were fitted to slow-clearing compartment data only.

● 10°C, $Y(t) = 40e^{-.0050t}$

■ 20°C, $Y(t) = 74e^{-.0044t}$

▲ 28°C, $Y(t) = 60e^{-.0045t}$

The curves represent the "best fit" exponential curves defined by the equations, where Y(t) = the per cent of the initial Hg content remaining in the slow-clearing compartment in Daphnia and t = clearance time in hours. Points represent mean estimates for the per cent retained of the one minute postexposure body burden.

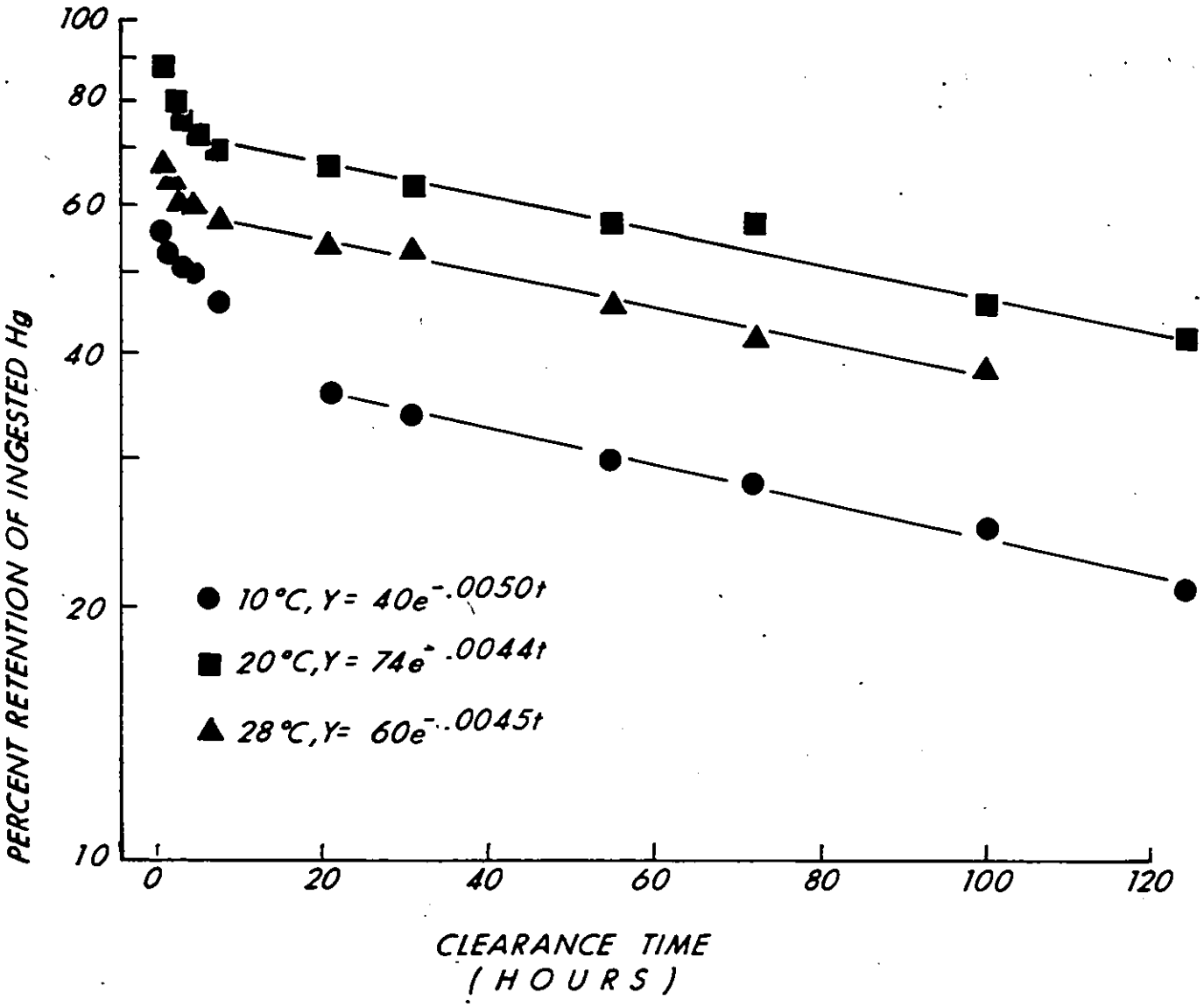

100
80
60
40
20
10
PERCENT RETENTION OF INGESTED Hg
0
20
40
60
80
100
120
CLEARANCE TIME
(H O U R S)
10 °C, Y = 40e^-.0050t
20 °C, Y = 74e^-.0044t
28 °C, Y = 60e^-.0045t

Fig 1+

in methyl Hg uptake studies, even allowing for the smaller size of the Daphnia used in the present study. This suggested that the yeast containing inorganic Hg might have been less palatable to Daphnia than yeast contaminated with methylmercury. If feeding inhibition persisted when Daphnia were transferred to non-radioactive food, the effect would be to increase assimilation efficiency by increasing residence time of food in the gut. In order to ensure that the reduced estimate of feeding rate was not due to the loss of mercury from the yeast in the feeding suspension prior to ingestion, aliquots of the feeding suspension were filtered using a GF/C filter. The filtrate contained only 4.5% of the total ^{203}Hg in the feeding suspension, demonstrating that little of the ^{203}Hg had been lost from the yeast prior to ingestion.

Although the uptake of ^{203}Hg-labelled inorganic Hg by Daphnia from the filtrate was not determined directly, the amount of inorganic Hg taken up directly from the water during the exposure period can be calculated from uptake rates for inorganic mercury from water determined in water vector uptake studies reported in the present work. Using a value of 1 ml of water cleared of Hg per Daphnia per hour, Daphnia may have taken up $.06 \times 10^{-3}$ ug Hg Daphnia^{-1} from the exposure medium in 30 minutes of exposure. This amounts to only about 7% of the total amount of Hg taken up. Studies of clearance of inorganic mercury taken up

via the water vector suggest that this inorganic mercury taken up from water would be lost very quickly and hence might result in an overestimation of the amount of Hg ingested by 7% or less but would not influence the estimate of the amount of Hg assimilated. This source of error will result in a slight underestimation of the assimilation efficiency of inorganic Hg by this technique.

The loss of ^{203}Hg-labelled inorganic mercury by Daphnia, when transferred to a non-radioactive feeding suspension, is shown in Fig. 18 where clearance of ingested inorganic mercury is contrasted to the loss of a dose of ^{203}Hg-labelled methylmercury administered under similar conditions. The results show that clearance of ingested inorganic mercury is biphasic, as had been the case with methylmercury. About 95% of the ingested inorganic mercury was lost in the fast-clearing phase which appeared to be totally cleared by about five hours postexposure. This phase represented the egestion of unassimilated mercury. The slow-clearing phase; which represented the assimilated mercury, made up only about 5% of the ingested inorganic mercury, compared to 60 - 90% with methylmercury. The slow-clearing inorganic mercury phase was cleared with a biological half-time of 13 hours, which was approximately 10 times faster than the clearance of methylmercury. The experiment was terminated after only 52 hours, because after that time the radioactivity content of Daphnia was too small to measure accurately.

Fig. 18. Clearance of ingested inorganic mercury and methylmercury by Daphnia magna. Animals ingested the ^{203}Hg-labelled compounds during a 25-minute exposure to contaminated yeast cells at a concentration of 0.1×10^6 cells ml^{-1}. Mercury loss from the slow compartments in each case could be described as follows:

○ methylmercury, $Y(t) = 62.0e^{-.005t}$

□ inorganic mercury, $Y(t) = 5.4e^{-.052t}$

where $Y(t)$ = the per cent of the ingested dose remaining in the slow compartment after t hours of clearance. Points represent the mean $\pm$ S.E. between groups of Daphnia.

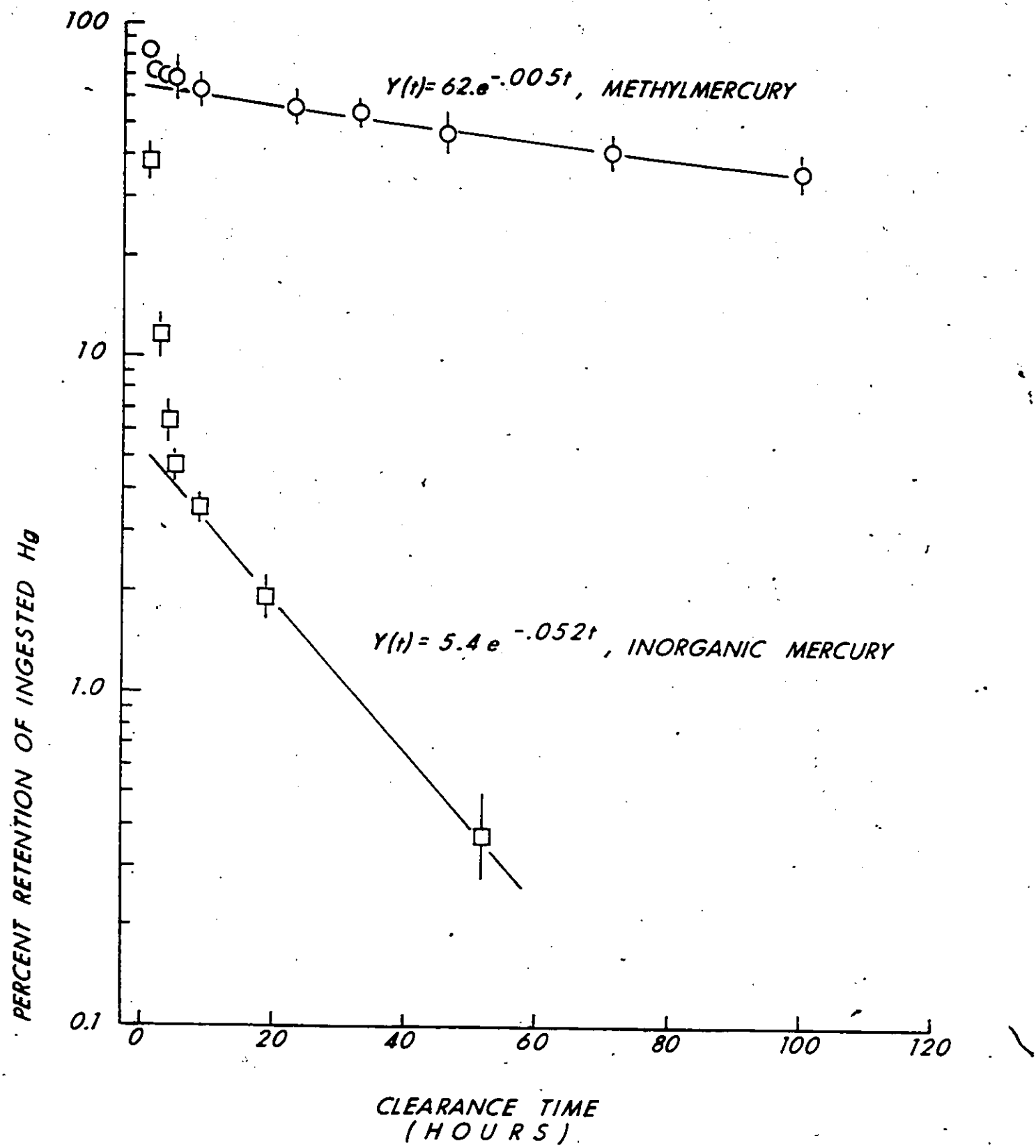

100
10
1.0
0.1
Y(t)= 62.e^-.005t , METHYLMERCURY
Y(t) = 5.4 e^-.052t , INORGANIC MERCURY
PERCENT RETENTION OF INGESTED Hg
0
20
40
60
80
100
120
CLEARANCE TIME
(HOURS)

FIG 18

To determine if the assimilation efficiency of inorganic mercury was influenced by the concentration of inorganic mercury in the diet, Daphnia were fed on yeast suspensions containing three different concentrations of mercury: .5; 1.9; and 5.9 mg Hg g^{-1} dry weight of yeast. Following exposure to the ^{203}Hg contaminated food, Daphnia were transferred to non-radioactive medium, where ^{203}Hg loss was monitored in the usual way for 30 hours.

The results in Table 20 show that Daphnia fed at a rate of .53 x 10^6 cells Daphnia^{-1} hr^{-1} on yeast containing the lowest concentration of inorganic mercury. This feeding rate was lower than that observed when Daphnia fed on yeast containing methyl-mercury. In addition, the feeding rate declined as the inorganic mercury concentration of the yeast was increased.

The results in Fig. 19 show that the size of the slow compartment relative to the total amount of Hg ingested increased with increasing concentration of mercury in the yeast, indicating that assimilation efficiency of inorganic mercury was increasing with increasing inorganic mercury concentration in the yeast. This relationship between assimilation efficiency and the concentration of mercury in the food was probably due to an indirect effect resulting from the effect of inorganic mercury on ingestion rate since ingestion rate declined with increasing inorganic mercury concentration in yeast. The reduced feeding rate appears to have persisted long enough into the postexposure period to have caused a significant difference in the residence times of the ingested

Table 20. The effect of the concentration of inorganic mercury in the food on the assimilation efficiency of inorganic mercury by Daphnia magna. Animals in the range of 2.3 - 2.7 mm body length were exposed for 30 minutes to a yeast suspension of .1 x 10^6 cells ml^{-1} containing ^{203}Hg-labelled $HgCl_2$ at 20°C.

Hg concentration in yeast,[a] mg Hg g^{-1}	Number of Daphnia,[b]	Ingestion rate,[c] cells Daphnia^{-1} hr^{-1}	Amount of Hg ingested,[d] ug Hg Daphnia^{-1}	Amount of Hg assimilated,[e] ug Hg Daphnia^{-1}	Net efficiency of Hg assimilation, %	Transfer coefficient,[f] hr^{-1}
.5	3 x 5	.53±.06 x 10^6	.22±.02 x 10^{-3}	.022±.005 x 10^{-3}	9.5±1.7	.0009±.0002
1.9	3 x 5	.39±.02 x 10^6	.59±.03 x 10^{-3}	.074±.008 x 10^{-3}	12.7±1.4	.0008±.0002
5.9	3 x 5	.33±.02 x 10^6	1.60±.08 x 10^{-3}	.343±.014 x 10^{-3}	21.6±2.7	.0006±.0002

[a] Hg concentration of mercury is based on measured radioactivity of samples of measured numbers of cells using a value for the specific activity of mercury of 1.014 x 10^4 cpm ug Hg^{-1} and an assumed value for the dry weight of yeast of .0165 x 10^{-3} g per million cells.

[b] Numbers of groups x numbers of animals per group.

[c] Based on the amount of radioactivity ingested.

[d] Amount of Hg ingested was calculated from the amount of radioactivity ingested.

[e] Amount of Hg assimilated was calculated from the estimated amount of radioactivity assimilated.

doses in the guts of Daphnia resulting in differences in assimilation efficiencies. Since all animals in the present study of inorganic mercury uptake from food appear to have been feeding at lower rates than were observed in methylmercury feeding studies, assimilation efficiencies estimated in all of these studies may overestimate the true assimilation efficiency for inorganic mercury. This point is elaborated in the discussion. It should be noted that fractional clearance rates from the slow compartment are independent of the amount of inorganic mercury in the compartment over a range of concentrations of more than an order of magnitude (Fig. 19).

Fig. 19. Clearance of ingested inorganic mercury from Daphnia magna. Animals ingested the ^{203}Hg-labelled $HgCl_2$-contaminated food during a 25-minute exposure to yeast suspensions containing different concentrations of inorganic mercury in yeast. Curves were fitted to the slow compartment only.

□ 0.5 mg Hg g^{-1} dry weight of yeast cells

$Y(t) = 10e^{-.046t}$

△ 1.9 mg Hg g^{-1} dry weight of yeast cells

$Y(t) = 13e^{-.044t}$

○ 5.9 mg Hg g^{-1} dry weight of yeast cells

$Y(t) = 22e^{-.053t}$

where Y(t) = the per cent of the ingested inorganic mercury remaining in the slow compartment after t hours of clearance. Points represent the mean $\pm$ between groups.

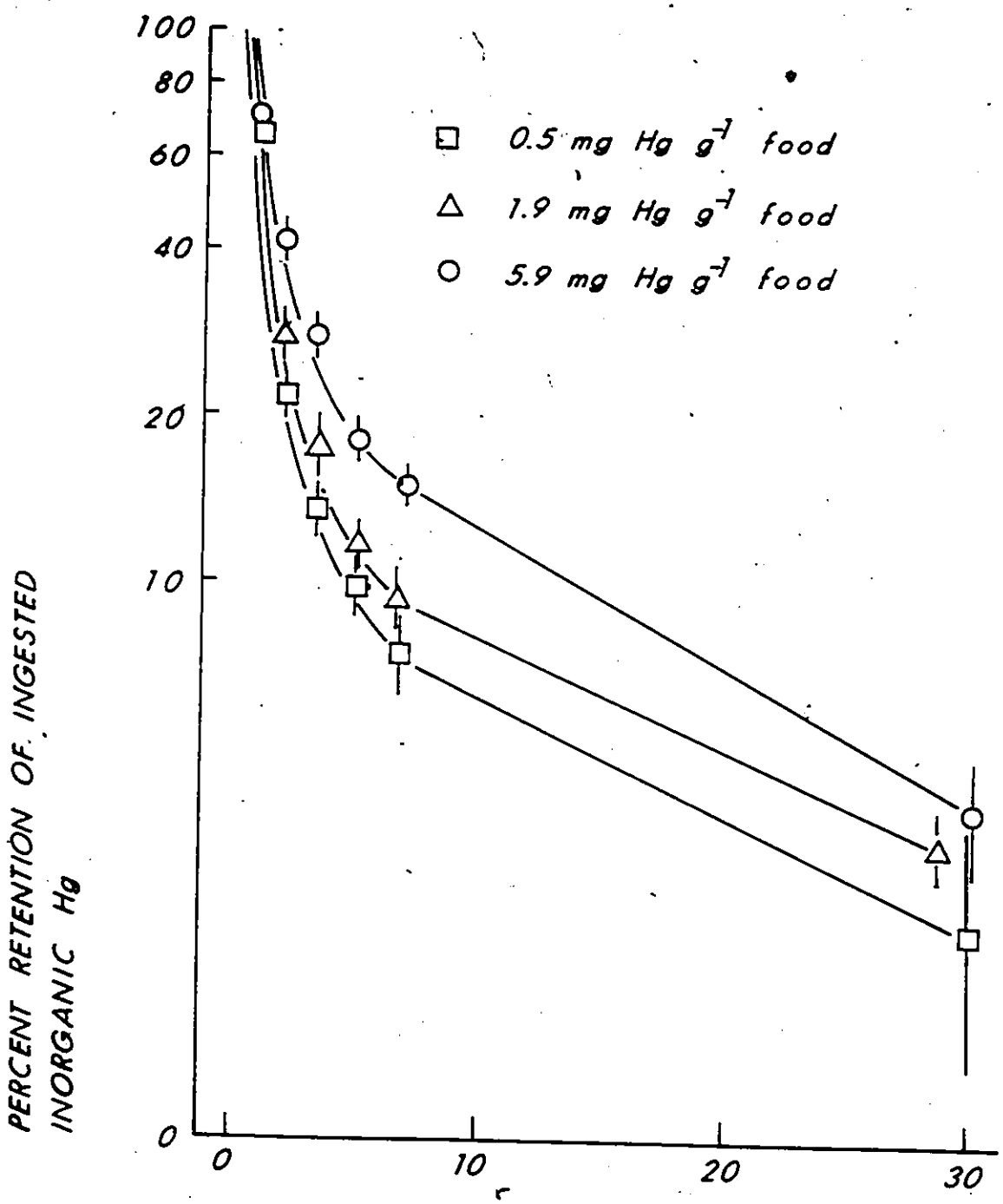

100
80
60
40
20
10
0
0
10
20
30
0.5 mg Hg g⁻¹ food
1.9 mg Hg g⁻¹ food
5.9 mg Hg g⁻¹ food
PERCENT RETENTION OF INGESTED INORGANIC Hg
CLEARANCE TIME (HOURS)

FIG. 19

DISCUSSION

This section is divided into four parts. In the first part, the bioaccumulation models used in the work are briefly outlined. Next, the relationships of bioaccumulation kinetic processes to biological control processes are discussed. Data from the present work are then used in the consideration of interspecific variations in pollutant accumulation. Finally, a mercury bioaccumulation model is presented in which the present methylmercury accumulation information is coupled with published information on Daphnia energetics to estimate age-specific exposure/dose relationships.

Mercury Bioaccumulation Models.

In radiotracer investigations suitable models of tracer uptake and elimination must be developed in order to use the experimental data. In this context, a "suitable" model may be any that fits the data, with no particular regard to biological realism or to experimental feasibility of subsequent model testing and application to real world situations. Alternatively, a model may be developed prior to data gathering, its form being based only on its potential for resolving specific aspects of a problem within specified limits of accuracy. The latter approach places the problem in a highly simplified context from the outset and

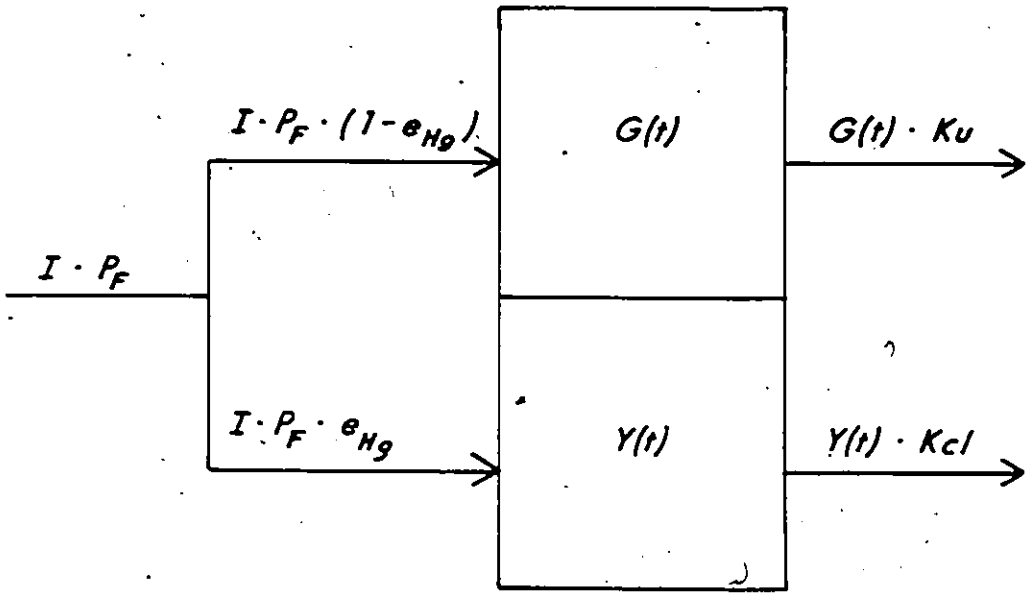
generally results in information that has little generality. The approach taken in the present study incorporates elements of these two positions, in that we construct mathematical models of mercury dynamics in Daphnia without regard to biological realism, and then identify relationships between the behaviour of model parameters and the known behaviour of potential biological control factors.

Uptake of mercury via the food vector was conceptualized as illustrated in Fig. 20A. Daphnia were assumed to feed constantly on mercury-contaminated food. The uptake rate of mercury into Daphnia was therefore determined by the ingestion rate of food and the mercury concentration of food. Uptake of mercury into Daphnia tissues was assumed to be determined by the ingestion rate of mercury as modified by a coefficient of assimilation efficiency. Mercury clearance from the tissues was assumed to be first order. Clearance of unassimilated mercury from the gut is ignored, since the mercury content of the gut is external to the body and may be unimportant from the point of view of bioaccumulation.

The direct uptake of methylmercury from water is conceptualized as a two phase process (Fig. 20B). Each compartment took up methylmercury directly from the water at a rate that was directly proportional to the methylmercury concentration in the water. Each compartment lost mercury directly to the water at a rate proportional to the mercury content of the compartment.

Fig. 20. Schematic representation of models used to describe the accumulation of mercury from food and water. (A) Accumulation of methylmercury and inorganic mercury from food, where the mercury content of the gut, G(t), and the tissues, Y(t), are determined by the ingestion rate of food, I, the mercury content of food, P_F, and the assimilation efficiency of ingested mercury, e_{Hg}, and the fractional clearance rates of gut, K_u, and tissue compartments, K_{cl}. (B) Accumulation of methylmercury from water, where A(t) and B(t) are the mercury content of each compartment, K_u^a and K_u^b are uptake rate constants, K_{cl}^a and K_{cl}^b are fractional clearance rates and P_w is the concentration of mercury in water.

A.


$I \cdot P_F \cdot (1 - e_{Hg})$
$G(t)$
$G(t) \cdot Ku$
$I \cdot P_F$
$I \cdot P_F \cdot e_{Hg}$
$Y(t)$
$Y(t) \cdot Kcl$

B.

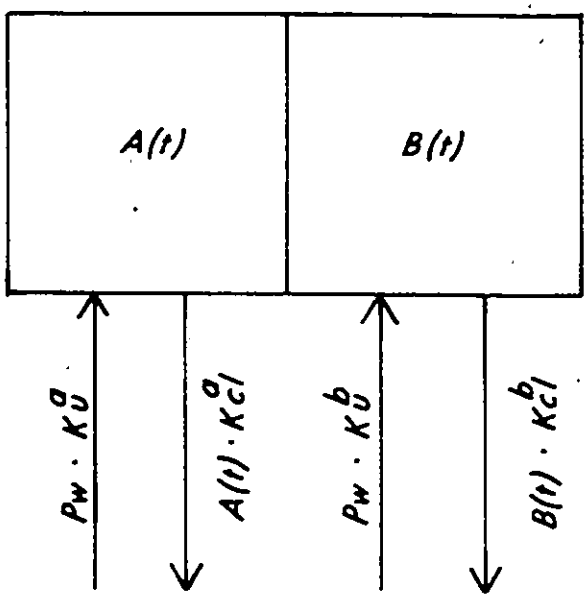

$A(t)$
$B(t)$
$Pw \cdot K_U^a$
$A(t) \cdot K_{Cl}^a$
$Pw \cdot K_U^b$
$B(t) \cdot K_{Cl}^b$

FIG 20

As pointed out earlier, the results for accumulation of inorganic mercury from the water indicated that accumulation was a multicompartmental process which was non-linear in the form described in Fig. 7. Since non-linear models are computationally very difficult and since no satisfactory linear model could be justified on the basis of the available data, no attempt was made to carry the investigation of inorganic mercury accumulation from water further.

It is important to consider whether exposure and dose conditions experienced by animals in these experiments reflect those in nature. During these studies animals were exposed briefly to higher ambient mercury concentrations in water (.1 - 1.0 x 10^{-3} ug Hg ml^{-1}) than those experienced by natural populations (<.05 x 10^{-3} ug Hg ml^{-1}; Sherbin, 1979). They also experienced higher doses of mercury in their tissues (1 - 20 ug Hg g^{-1}, d.w.) than either natural populations (.01 to .1 ug Hg g^{-1}, d.w.; Sherbin, 1979) or Daphnia under culture conditions (1 ug Hg g^{-1}, d.w.; Biesinger et al, 1980). Doses of methylmercury routinely experienced by experimental animals were lower than those causing elevated mortality rates in Daphnia under chronic methylmercury exposure via the water vector (>75 ug Hg g^{-1}, d.w.), but often exceeded those causing reproduction impairment (>16 ug Hg g^{-1}, d.w.; Biesinger et al, 1980). There was, however, no indication that these somewhat unnatural exposure and dose conditions have resulted in artifactual observations in either mercury accumulation patterns or estimates of rate constants.

Bioaccumulation Processes : Food Vector.

The accumulation of mercury by Daphnia is determined by the rates of uptake and loss of mercury via food and water vectors. Below, bioaccumulation is broken down into its various processes and each is considered separately. When mercury is taken up via the food vector, the concentration of mercury in Daphnia tissues is determined by at least the following states and processes:

a) the rate of ingestion of mercury-contaminated food;

b) the concentration of mercury in the diet;

c) the efficiency of assimilation of mercury from the diet;

d) the intraorganismic distribution of mercury to kinetic compartments of different turnover rates;

e) the loss of mercury; and

f) the growth dilution of accumulated mercury.

The involvement of these states and processes in mercury accumulation from food is dealt with here.

Ingestion Rates.

The ingestion rate of mercury by Daphnia is a function of the ingestion rate of food and the concentration of mercury in the ingested food. Although it was not the intention of this study to investigate the control of feeding in Daphnia, the control of pollutant uptake is so obviously fundamentally related to feeding that the control of feeding rate should be discussed briefly here.

Therefore, data from the present work as well as published information on Daphnia feeding are used to assess Daphnia feeding rates, maximum and minimum feeding rates, feeding rates under natural conditions, and effects of body size and temperature on feeding rates.

In the present work Daphnia adults of 2.5 - 3.0 mm body length, and weighing approximately .1 - .15 mg dry weight, ingested yeast cells at rates ranging from .03 to .26 x 10^6 cells hr^{-1} at 20°C. This corresponds to ingestion rates of .004 to .03 grams of food per gram body weight of Daphnia per hour on a dry weight basis. Assuming Daphnia feed constantly at these rates throughout the day, Daphnia will ingest from 10 to 75 per cent of their body weight per day. Other authors have reported similar feeding rates for adult D. magna feeding under laboratory conditions. Schindler (1968) fed D. magna on Chlorella and observed ingestion rates of .014 to .05 grams per gram per hour, or 35 to 120 per cent of the body weight per day, at ambient food concentrations ranging from 1 to 12 mg of Chlorella per litre. Calculations using the results of Kersting (1978) yielded similar estimates of ingestion rates ranging from .01 to .06 grams per gram per hour for D. magna feeding on Chlorella at cell densities similar to those used by Schindler (1968).

In 1961 Rigler demonstrated that Daphnia feeding rates

were related to ambient food concentration. Below a critical ambient food concentration feeding rate was directly proportional to ambient food concentration, but at or above the critical ambient food concentration feeding rate was constant. Later McMahon and Rigler (1963) found that the maximum feeding rate was probably related to the volume of food being ingested, since Daphnia feeding at its maximum rate on both Chlorella and yeast ingested approximately the same volume of food per hour, .016 mm^3 per hour per .28 mg dry weight animal. Assuming these two species of food have densities similar to water and have a dry weight to wet weight ratio of about .1, this maximum ingestion rate corresponds to a value of about .06 grams per gram per hour. The upper limit to the ingestion rate of mercury by Daphnia via the food vector should be the mercury content of a mass of food equal to six per cent of the animals' weight per hour.

The minimum value for mercury ingestion rate in a viable adult can be taken to be the mercury content of the amount of food required by Daphnia daily in order to maintain its body weight. Smith (1963) showed that starved adult D. magna lost weight at a rate of about 10% per day at 20°C. Daphnia must ingest enough food to make up for this weight loss. Schindler (1968) reported values of approximately 4.9 calories per mg dry weight for the energy content of Daphnia tissues. An adult weighing 0.1 mg dry weight would require .05 assimilated calories per day for maintenance based on Smith (1963). Assuming an assimilation efficiency

of 55%, as determined by Bourne (1959) for the assimilation efficiency of carbon from Chlorella, Daphnia would require 0.09 ingested calories per day to maintain body weight. Using a value of 5.2 calories per mg as the calorie content of Chlorella (Schindler, 1968), Daphnia of body weight 0.1 mg would require .017 mg dry weight of food per day (17% of body weight) or .007 mg per hour (0.7% of body weight) to maintain its body weight at 20°C. This value can be calculated a second way using the estimated rate of carbon turnover in Daphnia. Bourne (1959) determined that the biological half-time for the loss of assimilated ^{14}C from Daphnia was about 52 hours. This corresponds to a rate of loss of carbon from Daphnia of about 1.3 per cent per hour or about .0013 mg per hour from an animal weighing 0.1 mg dry weight, which in turn corresponds to an ingestion rate of 2.4% of body weight per hour to maintain body weight. This value is about three times as great as that determined by the former method. The discrepancy between the results of these two methods is probably due to the fact that the lower value was determined under starvation conditions and represents the minimum amount of energy required to maintain body weight, while the higher value includes energy expenditure for growth and reproduction. The lower value is therefore a better estimate of the minimum ingestion rate for food that will allow Daphnia to maintain energy equilibrium. The minimum daily rate of mercury ingestion by Daphnia is the mercury content of a mass of food equal to 17 per cent of the animal's weight.

The rates of uptake of mercury, or any pollutant, via the food vector by Daphnia lie within clearly definable limits. The upper limit, determined by the capacity of the feeding mechanism, is the mercury content of a mass of food equal to about six per cent of the body weight per hour or 140 per cent per day. The lower limit is the mercury content of about 17 per cent of the body weight per day. A reasonable estimate of the average daily intake rate under natural conditions lies midway between these extremes at 50 to 70 per cent of body weight per day, as reported by Bell and Ward (1971). The energetic demands of growth clearly determine the rate of mercury ingestion, and measurements of growth such as the production/biomass ratio could be used to drive the Hg ingestion component of a mercury bioaccumulation model.

In the present work, the effect of body size on ingestion was determined at ambient food concentrations below the incipient limiting ambient food concentration of yeast cells of approximately $.2 \times 10^6$ cells per ml. The filtration rate under these conditions of $.1 \times 10^6$ cells per ml was related to body weight according to the following:

$$F = 1238\, W^{.81}$$

where F is the filtration rate in ml per Daphnia of 1 g dry weight per hour and W is the dry weight in grams. A similar analysis of the data of McMahon (1965) for Daphnia magna of different sizes feeding on Chlorella yielded a body weight exponent of .80, which

is similar to that observed here. The value of the coefficient in the McMahon study was, however, much larger, 3800 ml per gram per hour, than that observed here. Daphnia in McMahon's study filtered on average three times faster than Daphnia in the present study, but McMahon (1968) showed that large variations between experiments in filtering rate activity may often be related to differences in water quality.

When ambient food concentrations exceed the incipient limiting concentration, the relationship between maximum ingestion rate and body size can be expressed as

$$I_{max} = aW^{.77}$$

where I_{max} is the maximum ingestion rate in grams of food per animal per hour, a is a coefficient with units of grams of food per animal of 1 gram body weight per hour, and W is the dry weight in grams. The body weight exponent has a value of .77 based on the data of McMahon (1965). From the similarity of body weight exponents for ingestion rates both below and above the incipient limiting level, it is evident that the effects of changes in body weight on the ingestion rate of food are the same regardless of the ambient food concentration; i.e., for Daphnia growing from neonate to adulthood the food ingestion rate on a per unit weight basis will decline by 50%, and if the mercury content of the food remains constant over this time the mercury uptake rate on a per unit body weight basis can be expected to decline in a similar way.

In the present study, the effect of temperature on the ingestion rate of food was determined over the temperature range from 10 to 28°C using thermally acclimated animals. Our results show that ingestion rates were the same at 10° and 20°C, but at 28°C ingestion rates were about 25% lower than at either of the other temperatures. Since the ambient food concentration used in this study was $.1 \times 10^6$ cells per ml, well below the incipient limiting level of $.2 \times 10^6$ cells per ml, the observed reduction in feeding rates of Daphnia acclimated to 28°C must have resulted from a temperature induced reduction in filtration rate. However, McMahon (1965) has shown that, when 20°C-acclimated animals were transferred to temperatures between 10°C and 28°C, ingestion rates increased five fold as temperatures were increased from 10 to 28°C. The discrepancy between the present work and that of McMahon (1965) probably results from differences in acclimation temperature on feeding rate, since our observations were obtained from organisms temperature-acclimated for at least one generation and tested at their acclimation temperatures. Kibby (1971) demonstrated the pronounced effect of acclimation temperature on feeding rates at a range of temperatures in Daphnia. Our supposition of the importance of temperature acclimation in the present case is borne out by Schindler's (1968) observation, in agreement with our results, that Daphnia magna acclimated to 10° and 20° and tested at their acclimation temperatures showed no

significant difference in filtration rates.

Most metabolically related processes, however, demonstrate temperature dependence, with rates increasing with increasing temperatures and then decreasing with further increases in temperature. The filtering rate of Daphnia magna probably follows this general rule and detailed studies of filtration rate using a wider range of temperatures, for example, 5°C. to 30°C, might be able to demonstrate this phenomenon with thermally acclimated Daphnia tested at their acclimation temperatures. Hence, with acclimated D. magna maximum filtration rates may occur at temperatures of 15 to 18°C, in which case measurements of filtration rates at 10°C and 20°C could be similar but less than maximum.

This explanation appears to create a problem in relation to information on growth at various temperatures (MacArthur and Baillie, 1929). These authors showed that animals grew faster at 18°C than at 10°C. In light of the above growth rate-temperature relationship, it would appear that Daphnia can grow faster at 18° than at 10°C even though their filtration rate would have been similar at the two temperatures. Information reported by Schindler (1968) may resolve this problem in that he found that Daphnia magna assimilated carbon more efficiently at 20°C than at 10°C, so that even though ingestion rates at the two temperatures were similar the amount of assimilated calories at 20°c

would be greater than at 10°C. The present work on mercury uptake supports this explanation since methylmercury was assimilated about twice as efficiently at 20°C (75%) as at 10°C (40%).

Assimilation of Mercury from Food.

Our results demonstrate that adult Daphnia feeding at an ambient food concentration of 0.1 x 10^6 yeast cells per ml assimilated about 80% of the ingested methylmercury. Assimilation efficiency of methylmercury was related to feeding rate with assimilation efficiencies declining as ingestion rate was increased. Such an inverse correlation between assimilation and ingestion had been observed by Schindler (1968) for carbon assimilation in D. magna. Apparently as feeding rates increase, the residence time of food in the gut declines, as was shown by Bourne (1959). Less time is therefore available for digestion of food and the absorption of nutrients and/or pollutants. If this assessment of the cause effect relationship between ingestion rate and assimilation efficiency is correct, then the assimilation efficiency of mercury should be lowest when Daphnia is feeding at maximum rates. The results for Daphnia feeding at their maximum rate at 20°C (food concentrations .5 x 10^6 cells per ml) demonstrated assimilation efficiencies for methylmercury of about 78% (Table 16), which can then be considered one of the lower values for assimilation efficiency for methylmercury at this temperature. Assimilation efficiencies of about 85% measured at slightly lower feeding rates, like those of Daphnia feeding at

ambient food concentrations of 0.1×10^6 cells per ml, probably more closely reflect those found under natural conditions. It is clear from the graph in Fig. 14 that over the range of concentrations of food organisms found in nature, variation in feeding rate would exert a much greater influence on the rate of methylmercury uptake into tissues than feeding-rate-induced variation in assimilation efficiency. Operationally then, in both laboratory and field contexts feeding rate-assimilation efficiency interaction can be ignored and assimilation efficiency can be assumed to be about 80%. In this context, it is important to recognize that values for assimilation efficiency observed in this study, for adults at 20°C feeding at an ambient food density of 0.1×10^6 cells ml^{-1}, ranged from 60 - 90% but the modal value was about 80%. At least some of this variation in assimilation efficiency between experiments may be caused by small differences in digestibility of yeast but there is no evidence to support this conclusion.

In the present study, there was no detectable effect of body size on assimilation efficiency, as animals of all sizes assimilated methylmercury with similar efficiencies when feeding in a yeast suspension of 0.1×10^6 cells per ml (Table 18). This appears to be inconsistent with the study of Daphnia bioenergetics by Schindler (1968), who reported that "Assimilation rates (for carbon) (cal/mg of animal/hr) decreased slightly (by 10%) as body

size increased. Feeding rates were unaffected (by body sizes)", implying that assimilation efficiency of carbon from the diet was body size dependent. Since Burns (1969) and McMahon (1965) have demonstrated that filtration rates on a per unit body weight basis decline with increased body weight, as was also observed in the present study, it may be that Schindler's results on ingestion rate were in error. The effect of body size on the rate of uptake of methylmercury into the tissues reflects only the effect of body size on ingestion rate since assimilation efficiency is independent of body weight. If adequate growth models exist for Daphnia in which feeding rate and assimilation efficiency of calories are defined as functions of environmental variables, the coupling of estimates of methylmercury concentrations in food to food ingestion rate values should, while holding assimilation efficiency for methylmercury constant, result in valid estimates of instantaneous dose rates of methylmercury from food.

The data in Table 18 show that the uptake rate of methylmercury into the tissues from food (ug Hg per gram of body mass per hour) by neonates weighing approximately .007 mg dry weight would be about 1.6 times as great as that of adults weighing 0.15 mg. Since the fractional clearance rates for ingested methylmercury have been shown in the present study to be almost independent of body weight, these neonates should have higher concentrations of methylmercury in their tissues than adults. However, the food conversion efficiency for growth in immatures is so much greater

than for adults (Crabtree, 1975), that the more rapid growth rate in immatures should more than compensate for their higher mercury uptake rates. This question will be dealt with later in the discussion. Temperature had a pronounced effect on assimilation efficiency, with values increasing from about 40% at 10°C to about 75% at 20°C and then declining to about 60% at 28°C. Feeding rate, which has already been shown to influence assimilation efficiency, was not responsible for these changes, since ingestion rates were the same at 10 and 20°C, while assimilation efficiencies differed. Also, assimilation efficiency was lower at 28°C than at 20°C, when a higher assimilation efficiency at 28°C might have been expected based on the slower ingestion rate at that temperature. Assimilation of substances from the diet depends in part on chemical digestive processes which no doubt involve enzymes. The rates of such processes would be expected to increase with temperature until the optimum temperature is reached, then further increases in temperature should cause a decline in reaction rates, as observed between 20 and 28°C. The results of the present study appear to conform to this pattern.

The present data demonstrate that, unlike the variables of body size and ambient food density, which influence the uptake rate of methylmercury into the tissues by exerting control over ingestion rate, the principle influence of temperature appears to be on assimilation efficiency although ingestion rate is also

influenced to a significant degree. Attempts to estimate methylmercury dose rate from food should take into account both of these temperature-related effects.

Since the effects of temperature on ingestion rate and assimilation efficiency combine to cause a rate of methylmercury uptake into Daphnia tissues that is approximately twice as fast at 20°C as at either 10°C or 28°C, and since fractional clearance at all temperatures is constant, Daphnia can be expected to contain higher concentrations of methylmercury at 20°C than at higher or lower temperatures.

With inorganic mercury an assimilation efficiency of only about 5% was observed for adults feeding in yeast suspensions of 0.1×10^6 cells per ml containing 1.6 mg Hg g^{-1}. This low value has been observed for many other species (Lloyd, 1979; DeFreitas et al, 1974). Our results also showed that ingestion rates of food contaminated with inorganic Hg were consistently lower than in the case of methylmercury-contaminated diets, and ingestion rates of food declined as the inorganic mercury concentrations in the food increased from 0.5 mg to 5.0 mg Hg g^{-1} dry weight (Table 21). Also assimilation efficiency increased with increasing concentration of inorganic mercury in the food, due presumably to the observed reduction in ingestion rates, and hence the longer residence time in the gut. These apparent effects of inorganic mercury in the diet on assimilation efficiency of mercury suggest that the

assimilation efficiency values estimated here may be higher than those that occur under natural conditions, since the concentration of inorganic mercury in natural phytoplankton should be too low to inhibit ingestion and cause the increase in assimilation efficiency observed in these experiments.

Clearance of Ingested Mercury.

Two phases were observed in the clearance of both ingested methylmercury and ingested inorganic mercury, a fast phase requiring from 5 to 8 hours to complete for both forms of mercury and a slow phase with a biological half-time for methylmercury of about 100 hours and only about 12 hours for inorganic mercury. The fast-clearing phase probably represents mainly that portion of the ingested mercury that is not assimilated into the tissues of the organism and is lost from the gut with the feces. The slow-clearing phase represents the mercury that is assimilated into the tissues. For both forms of mercury all of the assimilated mercury appears to behave as a single compartment, since no evidence of slower clearing compartments was found even though mercury clearance was followed until over 85% of the slow compartment had been lost.

The fast-clearing phase took considerably longer to complete (5 - 8 hours) than might have been expected if mercury loss from that compartment represented only egestion of unassimilated

mercury, which should have been completely voided in three hours or fewer, as reported by McMahon (1970) and other workers (Bourne, 1959; Hayward and Gallup, 1976; Rigler, 1961). The longer voidance times for both methyl and inorganic mercury suggest the existence of a fast-clearing tissue compartment with a clearance rate slightly slower than, but indistinguishable from, the rate of egestion of gut contents as measured in the present work. Although this slightly slower-clearing compartment may be real, its turnover time is so short, relative to that of the slow-clearing phase, that it can be treated as a non-assimilated fraction from the point of view of bioaccumulation. It should be noted, however, that in toxicological terms fast-clearing tissue compartments must be taken into account since, regardless of their short retention times, these compartments could make up a large proportion of the total assimilated dose.

In studies with methylmercury, the content of slow-clearing compartments declined exponentially with time, demonstrating that methylmercury clearance was a first order process. Fractional clearance rates of ingested methylmercury under the various test conditions used in this study ranged from 0.4 to 0.7 per cent per hour for adults at 20°C. This rate is slightly slower than for clearance of methylmercury taken up via the water vector of 0.6 - 1.2 per cent per hour, but both of these values are consistent with the fractional clearance rate of 1.2% hr^{-1}.

reported by Huckabee et al (1975) for methylmercury clearance from D. pulex following prolonged exposure to methylmercury via both food and water vectors simultaneously.

The present results on clearance of methylmercury (Fig. 17) demonstrate that fractional clearance was independent of temperature. This is rather remarkable, in view of the control exerted by temperature over rates of many physiological functions, including rates of mercury uptake via both food and water vectors. These results suggest that the rate limiting step controlling methylmercury clearance is not related to metabolic rate. Sharpe (1975) failed to find any effect of temperature on a similar situation with methylmercury clearance in goldfish. Fowler et al (1978) observed a small positive correlation between temperature and the rate of methylmercury loss from the mussel, Mytilus galloprovincialis, but the interpretation of data is difficult since their data were gathered over too short a period of clearance and do not allow resolution of clearance compartments, making it impossible to distinguish between effects of temperature on compartment size and fractional clearance rates. This problem could be resolved by following clearance of methylmercury from mussels for a much longer period.

There is a growing body of information which suggests that turnover of several elements including arsenic and selenium may be temperature-dependent under certain conditions and

temperature-independent under others. The effect of temperature on turnover rate appears to be species-specific (Fowler and Benayoun, 1976); it is related to the route of entry of the pollutant into the organism (Fowler and Unlu, 1978); and in some cases it influences one compartment and not another (Unlu and Fowler, 1979). Fowler and Unlu (1978, 1979) have suggested that temperature-dependence vs temperature-independence may be related to chemical species, with fractional clearance of inorganic arsenic being temperature-dependent, while clearance of organoarsenic metabolites is temperature-independent. Their data, however, do not completely support this suggestion. The data from the present study may support this suggestion, however, since fractional clearance of methylmercury taken up by both food and water vectors was temperature-independent. A study of the effect of temperature on the fractional clearance of inorganic mercury was not performed in the present work, but may aid in resolving the problem.

Body weight had little effect on fractional clearance of dietary methylmercury and had no effect on clearance of the slow-clearing methylmercury accumulated from water. Since metabolic rate is body weight-dependent in Daphnia (Richman, 1958), this finding confirms the earlier conclusion that methylmercury clearance is not metabolic rate-related. There are no other published studies of effect of body size or stage of maturity in invertebrates, but in fish the fractional clearance rate of

methylmercury is strongly negatively related to body weight. Sharpe (1975) found that in goldfish the fractional clearance rate for methylmercury was inversely proportional to body weight, and more importantly found that this same function applied across species lines in bony fishes. The present results show that not only does the fractional clearance rate of Daphnia not vary with body weight as was observed with fish, but the fractional clearance rate in Daphnia is much slower than would be expected on the basis of extrapolation of the fractional clearance rate-body weight relationship in fish. The specific relationship determined for fish, therefore, does not have any general applicability to all aquatic poikilotherms, contrary to the conclusion of Trudel et al (1977). The problem of interspecific variation in fractional clearance rate-body weight relationships is discussed further below (page 140).

Inorganic mercury taken up from food was cleared much more quickly than methylmercury. The fractional clearance rate of inorganic mercury, about 5 per cent per hour, was about 10 times faster than that of methylmercury. Many authors have made similar observations for the relative clearance rates of methyl and inorganic mercury - Lloyd (1979) for Hyalella azecta, and DeFreitas et al (1974), Pentreath (1976), and Sharpe (1975) for fish. There is some evidence that the rapid loss of inorganic mercury absorbed from food is due to the fact that some inorganic

mercury becomes associated with the lining of the gut and does not penetrate into the other tissues of the test animal. Loss of inorganic mercury is due to desorption of mercury from the gut wall and/or to sloughing of gut epithelium (DeFreitas et al, 1974). If this is true for inorganic mercury absorbed from the diet by Daphnia in the present study, then estimates of assimilation efficiency are actually overestimates. An assimilation efficiency of about 5% may be an overestimate by as much as 2 or 3%, reducing the actual amount of inorganic mercury that actually reaches the internal tissues of Daphnia to less than 2% of the amount ingested.

Growth Dilution of Tissue-bound Mercury.

Growth, the elaboration of new tissue, influences the concentration of mercury in Daphnia by causing a decrease in the uptake rate (on a per unit weight basis) through the increase in body size and by diluting the mercury already present in Daphnia. If the growth rate is rapid relative to the speed at which the slowest-clearing compartment approaches steady state, then the growth rate will play an important role in determining the concentration of mercury in the tissues. It is appropriate to consider this effect here.

Few authors have studied the rate of growth in weight in

Daphnia although many have studied the rate of growth in length (Anderson, 1932; Anderson and Jenkins, 1942; MacArthur and Baillie, 1929; Richman, 1958). The rate of growth of Daphnia is clearly very rapid. MacArthur and Baillie (1929) found Daphnia magna grew from .8 to 3 mm in body length in 23 days. Using the body length-dry weight relationship of Schindler (1968) to estimate the weights of these animals, the mean daily growth rate over this period was approximately 16% of body weight per day. In order to relate the accumulation of methylmercury by Daphnia to the growth rate, consider that with a fractional clearance rate of .005 hr^{-1} for methylmercury, Daphnia would require about 13 - 15 days to achieve 90% of its steady state body burden in the absence of growth. However, over this interval Daphnia would have increased in size by about 2.5 times. Therefore, even after 13 - 15 days of exposure to methylmercury Daphnia would still be far from a steady state condition with respect to methylmercury in food. Growth rate is, however, age specific. Using the data of Anderson (1932) for growth in length during the first 17 instars of life, the data of Anderson and Jenkins (1942) for the duration of instars, and the work of Schindler (1968) for the length-dry weight relationship of D. magna, the age specific growth rate of Daphnia can be estimated. Daphnia grow from .007 mg at birth to approximately .1 mg at sexual maturity in 7 - 12 days at 20°C, depending upon the strain, for a growth rate of approximately 20 - 40% per day. Over the next

20 days of life Daphnia, now an adult, devotes part of its energy to reproduction and therefore grows at a slower rate, about 3% per day. As age increases the growth rate declines further (Crabtree, 1975). Growth can therefore be expected to play a major role in lowering the concentration of methylmercury in young Daphnia, but the effect becomes much smaller as the animals pass maturity.

Bioaccumulation Processes : Water Vector.

The present studies of direct uptake of methylmercury from water have shown that methylmercury accumulation kinetics in Daphnia can be adequately described by a two compartment model. Bioaccumulation systems involving two or more compartments are common (Ruzic, 1972) and they are much more difficult to deal with, from a computational point of view, than single compartment systems. To facilitate working with these systems it is useful to ignore small, unimportant compartments by grouping them with major compartments if the end use of the model will permit this simplification. In the case of methylmercury uptake from water into the organism, both fast and slow compartments make significant contributions to the total uptake rate of methylmercury, the fast compartment making up 50 to 80% of the total methylmercury uptake with the remainder being made up by the slow compartment. The turnover rate of methylmercury in the fast compartment is so much

more rapid than in the slow-clearing compartment that the steady state methylmercury content of the fast-clearing compartment is very small and is achieved very quickly relative to that of the slow-clearing compartment. After long term continuous exposures to methylmercury in water, the fast-clearing compartment makes up a very small proportion (< 10%) of the total body burden of methylmercury and can therefore be ignored from the point of view of methylmercury bioaccumulation. For short exposures, in toxicity tests for example, both fast- and slow-clearing compartments make up important proportions of the body burden and therefore must be considered. Also from the point of view of toxicity testing both the fast and slow compartment uptake rates are important since both contribute significantly to the methylmercury dose rate.

No attempt was made to determine directly the tissue distribution of accumulated methylmercury. The finding that moulting had no effect on the rate of loss of methylmercury from the slow-clearing compartment demonstrated that the slow-clearing compartment represents methylmercury that is bound to internal tissue and is not associated with that part of the exoskeleton that is lost during the moult. This is consistent with the observation of Fowler et al (1978) that in shrimp the slow compartment was associated with muscle (69%) and the inner, non-calcified part of the exoskeleton which is not lost during the moult (26%). The fast compartment represents, in part, surface-bound methylmercury but the mercury associated with exoskeletons shed during exposure

periods cannot account completely for the methylmercury content of the fast compartment.

Direct Uptake of Methylmercury from Water.

Smaller Daphnia accumulate methylmercury much faster than do large ones on a per unit weight basis. The relationship of whole body transfer coefficient to body weight can be defined by

$$TC = 330W^{-.19}$$

where TC is the whole body transfer coefficient and W is the dry weight in grams. This result indicates that the whole body uptake rate on a per unit weight basis of a neonate weighing .007 mg is about 1.5 times greater than that of an adult weighing .15 mg. During an acute exposure of animals of different sizes to methylmercury in water the methylmercury concentration in the tissues at the beginning of exposure will reflect this difference. The effect of body size on uptake into the slow compartment favours uptake by the smaller animals even more strongly than the whole body uptake rate as indicated by the relationship

$$TC_s = 50W^{-.37}$$

where TC_s is the transfer coefficient for uptake into the slow compartment, so that after just a few hours of exposure the mercury concentration in a neonate can be expected to be about three times as great as that of an adult as a result of only direct uptake from water.

This inverse relationship between pollutant uptake rate and body size probably accounts, at least in part, for the tendency of younger animals to be more sensitive than adults to many water-borne poisons, as was first reported for Daphnia by Breukleman (1932). The effect of body size on sensitivity of Daphnia to methylmercury poisoning due to direct uptake of methylmercury from water is not known, making it impossible to determine if there is a direct quantitative relationship between pollutant uptake rate and sensitivity in immatures and adults.

The results would suggest that under conditions of continuous exposure for several days to methylmercury in water smaller animals would have higher tissue concentrations of methylmercury than adults. The mathematical simulation of methylmercury accumulation presented below (page 154) suggests that this will be true when exposure periods do not exceed 10 days (Fig. 23C). This simulation also suggests that under continuous exposure to constant methylmercury concentrations in the water, concentrations of methylmercury in the tissues of an immature Daphnia will be only slightly lower than when the animal grows to adulthood. This appears to be due mainly to the rapid growth of young Daphnia, which functions to prevent the build-up of high concentrations of methylmercury in immatures compared to adults, through growth dilution of the accumulated mercury and by the depressing effect that increasing the body size of the neonate has on the transfer

coefficient. Therefore, despite the pronounced effect of body size on the uptake rate of methylmercury, the methylmercury concentration in Daphnia tissues, due to uptake from water, should vary only slightly over the life span of Daphnia because of the rapid growth dilution that takes place in immature Daphnia.

Temperature had a pronounced influence on the uptake of methylmercury from water into both the whole body and into the slow compartment. At temperatures between 10 and 20°C the influence on whole body uptake was small, but for the slow-clearing compartment the uptake rates at 15 and 20°C were more than double the rates at 10°C. The validity of the results at 5.5°C is questionable, since Daphnia tested at these temperatures may not have been as thoroughly acclimated as they were at other temperatures. These results suggest that under natural conditions differences in ambient temperature can cause a two-fold variation in the body burden in adults due solely to an effect on the uptake rate.

Both slow compartment and whole body methylmercury uptake rates were not linearly related to temperature but increased with increasing ambient temperature at lower temperatures and decreased with increasing temperature at higher temperatures. This behaviour suggests that methylmercury uptake is controlled by a physiological process. However, speculation on which processes might be involved would not be constructive due to the lack of suitable published information on the relationships of rates of

physiological functions to temperature in Daphnia.

It was shown below (page 142) that over a range of species varying in weight from the smallest Daphnia magna neonate (0.75 mg) to a 350 g fish, the transfer coefficient for methylmercury uptake from water into the slow-clearing compartment was related to body weight by the power function

$$TC_s = 6.9W^{-.23}$$

where TC_s is the transfer coefficient for uptake into the slow compartment on a wet weight basis and W is wet weight in grams. The slope of this function, -.23, is similar to the value that one would expect if the uptake rate was related to metabolic rate, since Hemmingsen (1960) has demonstrated a body weight-respiration rate relationship with a value for the exponent of approximately -.25 for animals ranging in size from protozoa to whales. The transfer coefficient-body weight relationship for methylmercury uptake into the slow compartment in D. magna in the present study appears not to correlate well with the body weight-metabolic rate relationship values of Richman (1958) and Sushchenya (1970), whose body weight exponents lie within the range -.1 to -.2. However, the respiration rate-body weight relationship appears to vary widely in Daphnia under the control of environmental factors, since Buikema (1972) found that body weight exponents varied from 0 to -.5 when measured under different environmental conditions. The effect of body size on the rate of methylmercury uptake may

reflect the control of uptake by a metabolic function not directly related to respiration rate.

Other explanations of this uptake rate-body size relationship are possible. For example, if methylmercury uptake from water was related to filtration rate (feeding rate) or to the surface area of the body, then methylmercury uptake would be related to body weight by a power function with a body weight exponent in the range of 0 to -.5. If uptake was related to either filtration rate or surface area alone, temperature would not be expected to influence uptake rate, but if uptake was related to O_2 consumption, uptake should double with an increase in temperature from 10 to 20°C as does O_2 consumption in Daphnia (Schindler, 1968). As observed above (Table 13), methylmercury uptake rate does increase by a factor of two between 10 and 20°C, suggesting that uptake of methylmercury is related to metabolic rate. In conclusion, the direct uptake of methylmercury from water by Daphnia appears to be related to respiration and as a result is influenced by such variables as body size and temperature which influence respiration rate. This will have important applications in both laboratory and field situations since respiration rate can be used as a "driver" in modelling the bioaccumulation of methylmercury and other pollutants.

Clearance of Methylmercury Taken Up from Water.

Fractional clearance rates for fast and slow compartments

were, with one minor exception, constant with only slight variability between experiments. The absence of any effect of body size or temperature on fractional clearance rate from the slow compartment confirms that elimination of methylmercury is independent of metabolic rate in Daphnia, as it may be in fish (Sharpe, 1975). The only important variation in fractional clearance rates occurred between the fast-clearing compartment of animals of different sizes. This effect is probably due to allometric disproportionality or possibly to differences in the condition of the body surface between younger and older animals, which might influence the rates of surface binding and release of methylmercury. The small variation between experiments probably reflects differences in the condition of animals used in the various studies, but this difference is very small and can be ignored.

The fractional clearance rate of the slowest-clearing mercury compartment following the uptake of inorganic mercury from water, .005 hr^{-1}, was very similar to the fractional clearance rate of the slow compartment of methylmercury. This is rather unusual, since most reports in the literature indicate that inorganic mercury is cleared more quickly than methylmercury (DeFreitas et al, 1974; Fowler et al, 1978; Sharpe, 1975). It should be noted that these estimates of clearance rates reported in the literature were all based on the first part of the slow

clearance curve, representing usually less than 40% of the initial body burden associated with the "slow" compartment. Kramer and Neidhart (1975) observed a small slow-clearing compartment corresponding to about 20% of the initial body burden in guppies following a brief exposure to inorganic mercury in water. The fractional clearance rate of this small slow compartment was similar to that observed for methylmercury. Their data showed, however, that the majority of the body burden ($\approx$80%) was cleared much faster than methylmercury in agreement with other published work with fish. The similarity of the fractional clearance rate for this small, slow-clearing compartment in Daphnia to the fractional clearance rate for methylmercury suggests that this compartment might represent organic mercury formed from inorganic mercury from Daphnia. If microcrustacea can methylate inorganic mercury they would represent a very important source of methylmercury production in aquatic systems. It is important, therefore, to determine if the small, slow-clearing compartment observed in inorganic Hg-dosed Daphnia represents methylmercury and if the methylmercury was synthesized from inorganic mercury by Daphnia. However, no in vivo conversion of inorganic mercury to methylmercury by metazoans has been demonstrated although this question has been examined by a number of workers, as reviewed in Jonasson and Boyle (1979).

Interspecific Variations in Methylmercury Accumulation by Aquatic Fauna.

Efforts at predicting accumulation of pollutants by acuatic organisms have been hampered by a lack of realistic and useful relationships between pollutant dynamics and measurable biological parameters. For example, valid generalizations are required to permit the prediction of uptake rates for a wide range of pollutants by a wide range of organisms. Arguments developed in the previous discussion justify the realism of coupling pollutant kinetic information to biological processes in quantitative terms. From the discussion of our results it can be concluded that both food and water uptake vectors can be quantitatively related to metabolic rates in Daphnia. Metabolic rate can be quantitatively related to body size, growth rate, and environmental temperature. Hence uptake rates of mercury and probably most aquatic pollutants should, therefore, fall within limits set by factors controlling metabolic rate and growth. Modifying environmental factors such as water temperature and food availability are of necessity important as measurable control factors in real environments and in the laboratory.

As demonstrated in the early part of the discussion, mercury uptake via the food vector is predictable on the basis of the energetic demands of the organism, availability of food,

feeding habits, and the concentration of mercury in the food. The present results for assimilation efficiency of methylmercury and inorganic mercury from the diet show that assimilation efficiency of mercury by Daphnia is similar to that of fish (DeFreitas et al, 1974) and other Crustacea (Lloyd, 1979), so that assimilation via the food vector can be assumed to be independent of species.

The graph in Fig. 21 shows that the transfer coefficient for the direct uptake of methylmercury from water in a wide variety of species is inversely related to body size. The relationship is defined by the power function

$$TC = 7.3W^{-.23}$$

where TC is the transfer coefficient in terms of grams of water cleared of pollutant per gram of animal per hour and W is the wet weight in grams. The value of the body weight exponent, -.23, is similar to the value that one would expect if uptake rate was related to O_2 consumption (Hemmingsen, 1960). The similarity of this relationship to the methylmercury uptake-body weight relationship for fish,

$$TC = 5.9W^{-.30},$$

as determined by DeFreitas and Hart (1975), supports the contention that this relationship has some general applicability to aquatic fauna.

If the reliability and generality of the relation between methylmercury uptake and body size of different species are as good

Fig. 21. The effect of body size on the transfer coefficient for uptake of methylmercury from water into the slow-clearing compartment in Crustacea and fish.


Daphnia magna (present study)


Daphnia pulex (Huckabee et al, 1975)

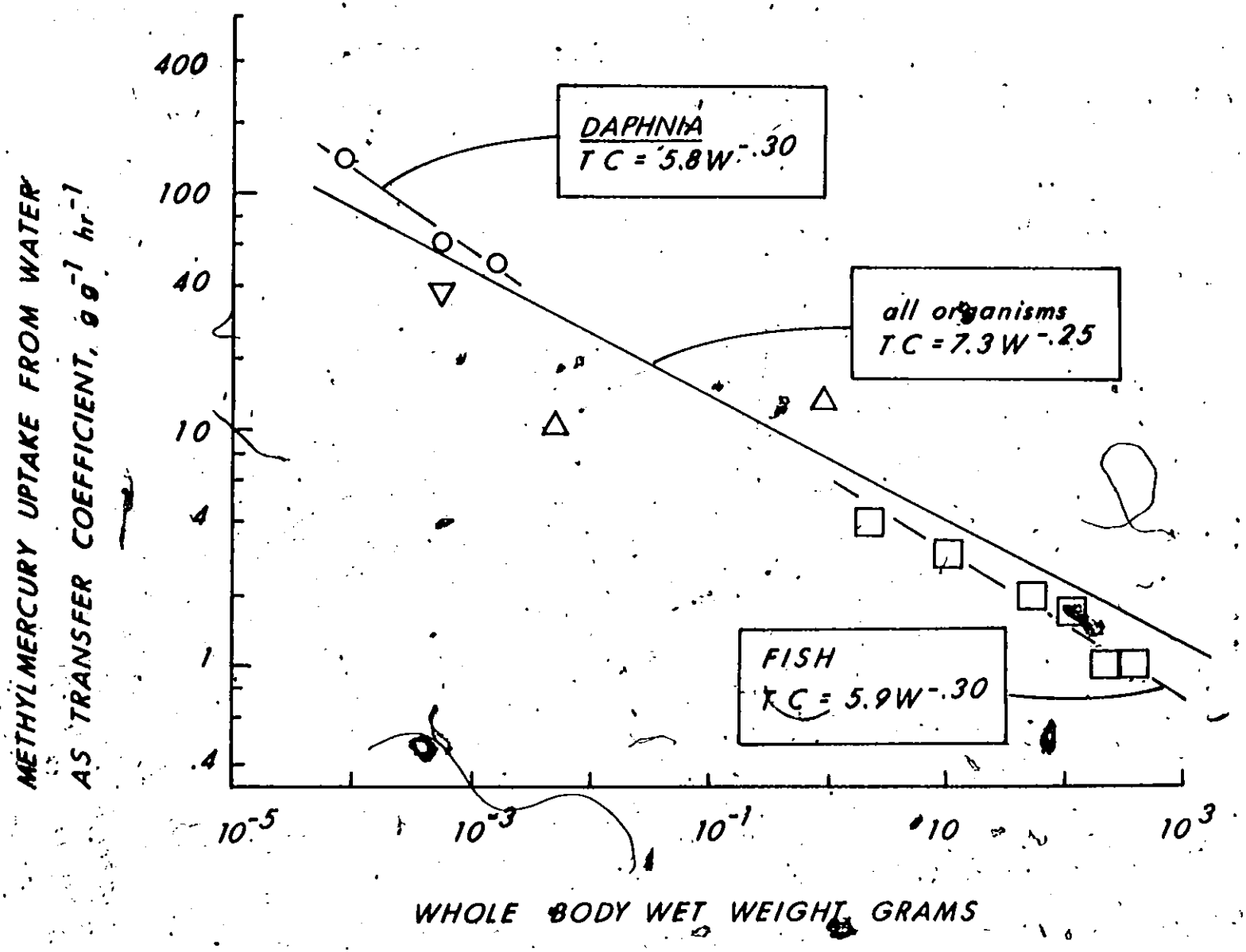

Hyalella azecta (Lloyd, 1979)


Lysmata seticaudata (Fowler et al, 1978)


Several species of fresh water fish (DeFreitas and Hart, 1975)

Solid line in upper left fits data for body size experiment in the present study. Solid line in lower right fits data from DeFreitas and Hart (1975). Regression lines fitted by method of least squares.

FIG 21


400
100
40
10
4
1
.4
DAPHNIA
TC = 5.8W^-.30
all organisms
TC = 7.3W^-.25
FISH
TC = 5.9W^-.30
10^-5
10^-3
10^-1
10
10^3
METHYLMERCURY UPTAKE FROM WATER
AS TRANSFER COEFFICIENT, g g^-1 hr^-1
WHOLE BODY WET WEIGHT, GRAMS

as they appear to be, it could be a very useful tool in making valid predictions of parameter values for mercury uptake from water. As shown by Norstrom et al (1976), the ability to use body size as a control factor in bioaccumulation processes greatly simplifies the mathematical simulation of a process that is actually under the control of a number of biological processes all of which are much more difficult to define and measure than body weight. In this connection it has been necessary to reduce the complexity of the two compartment model for methylmercury accumulation by Daphnia developed from our results, since simplicity of end use is particularly important in this type of applied research. In the case of Daphnia, as in all other methylmercury accumulation studies cited, the fast-clearing compartment can account for only a small (< 10%) fraction of the total mercury body burden due to uptake via the water vector under prolonged exposure. Thus bioaccumulation kinetics of a wide variety of animals can be approximated by using a one compartment two-parameter model describing bioaccumulation kinetics of the slow-clearing compartment.

Clearance from the slow-clearing compartment shows an inverse relationship to body weight which appears to have an important element of generality in pollutant bioaccumulation. In Fig. 22 the values for methylmercury fractional clearance from the slow compartment in Daphnia are plotted against body weight in

Fig. 22. Relationship between body weight and fractional clearance rate of methylmercury in several species of Crustacea and fish.

▲ Daphnia magna, water vector (present study)

△ Daphnia magna, food vector (present study)

● Daphnia pulex, combined water and food (Huckabee et al, 1975)

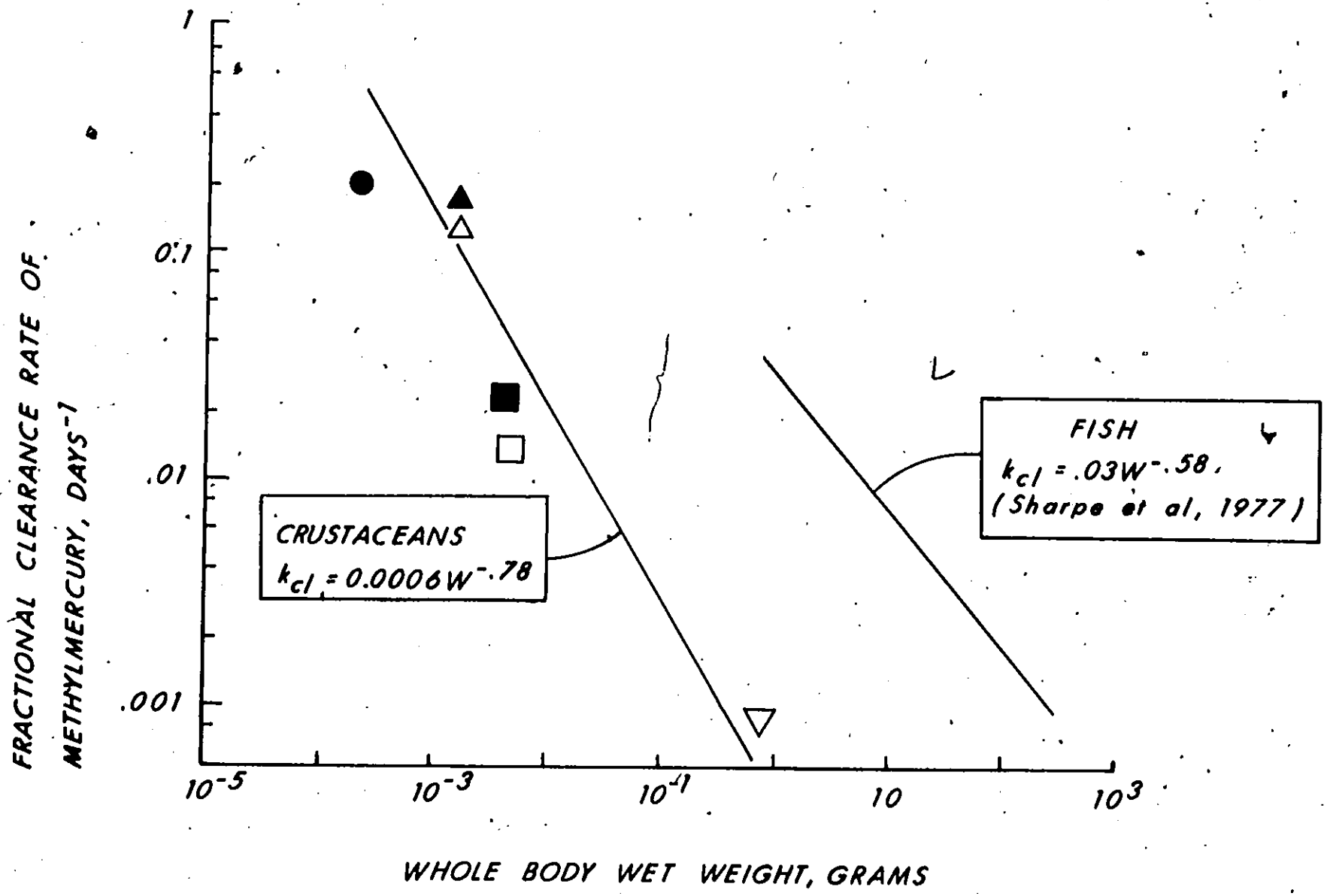
■ Hyalella azteca, water vector (Lloyd, 1979)

□ Hyalella azteca, food vector (Lloyd, 1979)

▽ Lysmata seticaudata, water vector (Fowler et al, 1978)

The best fitting regression line was determined by method of least squares. The line in the lower right is the body size fractional clearance rate for the slow compartment in fish from Sharpe (1975).

FIG 22


FRACTIONAL CLEARANCE RATE OF METHYLMERCURY, DAYS⁻¹
1
0.1
.01
.001
10⁻⁵
10⁻³
10⁻¹
10
10³
WHOLE BODY WET WEIGHT, GRAMS
CRUSTACEANS
$k_{cl} = 0.0006W^{-.78}$
FISH
$k_{cl} = .03W^{-.58}$,
(Sharpe et al, 1977)

grams, along with the equivalent values for D. pulex (Huckabee et al, 1975); the amphipod Hyalella azteca (Lloyd, 1979); and the shrimp Lysmata seticaudata (Fowler et al, 1978). Fractional clearance rates vary considerably but there is a clear trend toward a negative correlation with body size, which is defined by

$$K_{cl} = .0006W^{-.78}$$

where K_{cl} is the fractional clearance rate in hr^{-1} and W is the wet weight in grams.

This body weight-clearance rate relationship for Crustacea has a similar slope to that for fish, -.58 (Sharpe, 1975), but mercury clearance for a crustacean appears to be much slower, by at least a factor of 10, than that of fish. If the trend suggested by these data accurately reflects a significant difference in clearance between crustaceans and fish and the toxic response to tissue levels of mercury is similar in both, then crustaceans appear to be at a clear disadvantage in dealing with exposures to methylmercury, since uptake of mercury is similar in the two groups, but clearance in Crustacea is much slower than in fish. It is important to gather additional data to confirm this trend, and to establish whether other chemicals are handled in a similar fashion by crustaceans and fish.

One important aspect of this relationship of uptake and clearance rate constants to body weight is that the fractional

clearance rate relationship declines more steeply with increasing body weight than does the transfer coefficient for methylmercury uptake. This means that larger, older animals can generally be expected to accumulate higher concentrations of methylmercury from water than smaller, younger animals. The same relationship is true for food.

A Model of Methylmercury Bioaccumulation in Daphnia magna.

In this final section of the discussion a pollutant accumulation model is developed on the basis of information on methylmercury dynamics from the present work coupled to existing information on Daphnia bioenergetics. The goal of this exercise in simulation is to predict the age-specific methylmercury concentration in Daphnia tissue over its lifespan.

Methylmercury intake via food and water vectors, as well as the ingestion rate for food in Daphnia, are all driven by the filtration rate associated with the feeding process. Growth is simulated using a modified version of the Sinko and Streifer (1969) model in which energy flow is calculated using body size-specific and age-specific parameter values. The ingestion rate of food is determined by filtration rate and ambient food density according to the following relationship

$$I = C_F \cdot f \cdot W^{\alpha}$$

where I is the ingestión rate in grams of food per animal per hour, C_F is ambient food density in grams of food per litre, f is the filtration rate coefficient in ml per animal of one gram body weight per hour, W is the dry weight in grams, and α is the body weight exponent for ingestion rate which was assigned a value of 0.8. In the present case $C_F = .002$ g l^{-1} and $f = 3800$ ml animal^{-1} hr^{-1}.

Immature Daphnia are assumed to grow at a rate that is determined by the ingestion rate and the food conversion efficiency for growth, g_i, as in

$$G_i = g_i \cdot e_p \cdot I$$

where G_i is the growth rate in grams per animal per hour and e_p is the assimilation efficiency for food. In this case $e_p = .5$ and $g_i = .8$. At maturity the fraction of assimilated food devoted to growth declines to .20 in the first adult instar and declines further with increasing age. An empirical relationship between food conversion efficiency and age was determined from the data of Crabtree (1975) in which

$$g_a = c \cdot e^{-a(A-A_{mat})}$$

where A and A_{mat} are attained age and age at maturity in days, and c and a are empirical constants with values of 0.2 and 0.1, respectively. Age at maturity, A_{mat}, is assumed to be 10 days after Anderson (1932).

The uptake of methylmercury from food and water were calculated separately. The methylmercury content of Daphnia at the end of the n^{th} day, Yn, due to methylmercury uptake from food, is the amount of mercury present at the end of the previous day, Yn - 1, plus the amount accumulated during the n^{th} day, less the amount lost during the n^{th} day,

$$Y_n = Y_{n-1} \cdot e^{-k^F_{cl} \cdot 24} + \frac{I \cdot P_F \cdot e_{Hg}}{k^F_{cl}} \cdot (1 - e^{-k^F_{cl} \cdot 24})$$

where k^F_{cl} is the fractional clearance rate for ingested methylmercury, Y_n and Y_{n-1} represent the mercury content of Daphnia in ug Hg Daphnia^{-1}, P_F is the methylmercury concentration in food in ug Hg g^{-1} and e_{Hg} is the assimilation efficiency of methylmercury from the diet. Daphnia are assumed to feed constantly. From the present work k^F_{cl} = .005 hr^{-1}, and e_{Hg} = .8. The concentration of methylmercury in the diet, P_F, is related to methylmercury concentration in the water by

$$P_F = CF_{F/W} \cdot P_W$$

where W is the methylmercury concentration in water in ug ml^{-1} and $CF_{F/W}$ is the concentration factor for methylmercury between food and water. This constant was assigned a value of 50,000 after Glooschenko (1969).

The methylmercury concentration in Daphnia at the end of the n^{th} day, Y_n, due to uptake from water can be calculated in a manner similar to that for mercury taken up from food using the following expression

$$Y_n = A_n e^{-k^a_{cl} \cdot 24} + (U_a / k^a_{cl}) \cdot P_W \cdot (1 - e^{-k^a_{cl} \cdot 24}) +$$

$$B_n e^{-k^b_{cl} \cdot 24} + (U_b / k^b_{cl}) \cdot P_W \cdot (1 - e^{-k^b_{cl} \cdot 24})$$

where A_n and B_n are the mercury content in fast-and slow-clearing compartments, respectively, in ug Hg Daphnia^{-1}, k^a_{cl} and k^b_{cl} are the fractional clearance rates for fast- and slow-clearing compartments in hr^{-1}, U_a and U_b are the uptake rates into fast- and slow-clearing compartments in ml Daphnia^{-1} hr^{-1}, and P_w is the methylmercury concentration in the water in ug Hg ml^{-1}. The fractional clearance rate of the slow-clearing compartment, k^b_{cl}, is a constant with a value of .007 hr^{-1}. The fractional clearance rate for the fast compartment is related to body size by the following

$$k^a_{cl} = 1.89W^{.22}$$

where W is the dry weight in grams.

The whole body methylmercury uptake rate from water, U, is the sum of rates of uptake of the fast, U_a, and slow, U_b, compartments

$$U = U_a + U_b .$$

The whole body uptake rate from water was shown above to be related to body size with a body weight exponent that is similar to that of the filtration rate,

$$U = (I/CF) \cdot e_{Hg \cdot w} \cdot P_w$$

where $e_{Hg \cdot w}$ is the assimilation efficiency of methylmercury from the filtered water. The value of this constant is set at .3 based on empirical measurements of filtration rate and whole body methylmercury uptake rates from the present study. The

proportion of the whole body mercury uptake that reaches the fast-clearing compartment is defined by

$$U_a = s_a \cdot U$$

where s_a is an empirical coefficient based on data from the present work.

The methylmercury content of Daphnia due to uptake via food and water, as well as growth, were calculated daily beginning at birth. The methylmercury concentration in Daphnia was calculated daily from the estimates of methylmercury content of Daphnia and body weight.

For the sake of simplicity the environmental conditions of food density (.002 g l^{-1}) and methylmercury concentration in water (.001 x 10^{-3} ug Hg ml^{-1}) were held constant throughout the lifespan of Daphnia. Since the present study failed to show any detectable transfer of methylmercury from methylmercury laden adults to offspring, it was assumed that neonates contained no methylmercury at birth.

The simulated growth of Daphnia was in close agreement with published information on growth in Daphnia magna. The validity of model predictions of methylmercury concentrations in Daphnia tissue was tested by comparing estimated Daphnia/water concentration factors for 21 day old animals against concentration factor values estimated from the data of Beisinger et al (1980).

The latter exposed Daphnia magna to methylmercury under feeding conditions from birth to age 21 days at concentrations of methylmercury in the medium of .04 to .26 x 10^{-3} ug Hg ml^{-1} and observed concentration factors between Daphnia and water of 400,000 to 700,000 after 21 days of exposure. The model-generated concentration factor value, 340,000, agreed well with these values (Fig. 23C).

By age 40 days (= 40 days of exposure) the simulated methylmercury concentration in Daphnia is within 10% of the maximum theoretical concentration. The concentration factor at this age is slightly greater than 400,000. Due to the very rapid rate of methylmercury uptake in immatures, young Daphnia achieve approximately 70% of the theoretical maximum methylmercury concentration by the age of 5 days. If the critical tissue concentrations for toxic responses are the same for all ages of Daphnia, then animals can be expected to succumb as adults to ambient methylmercury concentrations which were innocuous to them as immatures.

To assess the relative importance of food and water vectors in methylmercury uptake, methylmercury tissue concentrations due to uptake by food and water were estimated separately (Fig. 23B). This result suggests that both food and water vectors contribute appreciably to methylmercury uptake in Daphnia. DeFreitas et al (1977) reached a similar conclusion for methylmercury dynamics in fish. Since the food vector appears to play

Fig. 23. Simulated tissue concentrations of methylmercury in Daphnia. A. Methylmercury is accumulated from only water when exposure is begun at birth and at age 10 days. B. Methylmercury is accumulated via food and water vectors considered separately. C. Methylmercury is accumulated via food and water vectors combined. For equations and parameter values used in this simulation see text on pages 147 - 151.

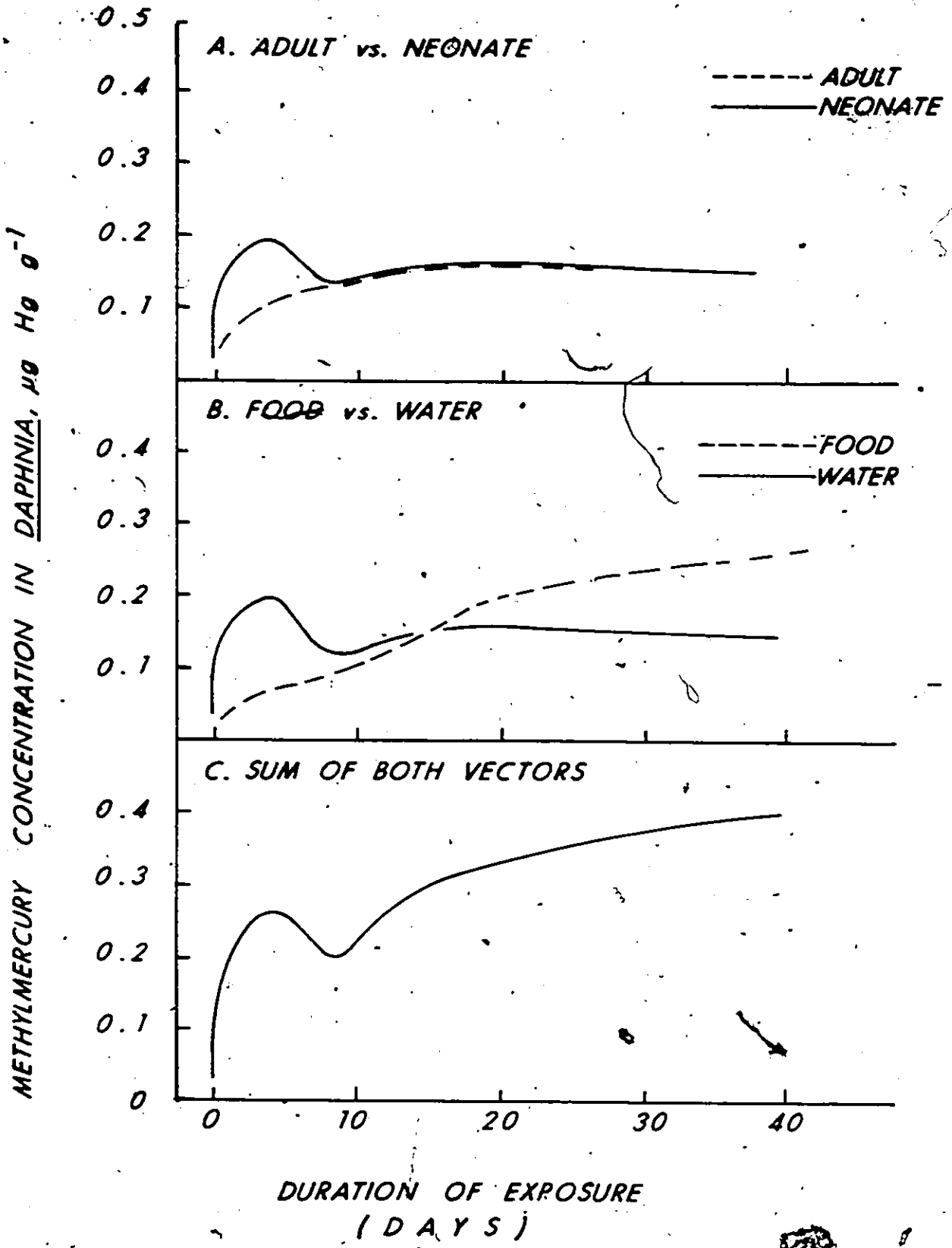

A. ADULT vs. NEONATE
ADULT
NEONATE
B. FOOD vs. WATER
FOOD
WATER
C. SUM OF BOTH VECTORS
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
METHYLMERCURY CONCENTRATION IN DAPHNIA, µg Hg g⁻¹
DURATION OF EXPOSURE
(DAYS)

FIG 23

an important role in methylmercury uptake, it is important that the toxicity of methylmercury in the diet be considered in the hazard assessment context as well as the toxicity of water-borne mercury.

Fig. 23C shows the accumulation of methylmercury in Daphnia when exposure is started at different ages. When exposure is begun at birth animals experience far greater concentrations of methylmercury in the tissues during the first five days of exposure than when exposure is commenced at age 10 days, although the simulated tissue concentrations are roughly equal by the tenth day of exposure. It is not surprising, therefore, that immatures are more sensitive to poisons than adults in toxicity tests involving acute exposures of less than five days duration (Breukleman, 1932).

SUMMARY

The results on mercury dynamics in Daphnia magna presented in this thesis define methylmercury bioaccumulation potential in terms of uptake and retention of mercury from water and food. Changes in these processes with temperature and with age of Daphnia have also been considered. These results on mercury dynamics have been used in conjunction with published information on Daphnia bioenergetics in the development of a pollutant accumulation model for Daphnia designed to be applicable in natural ecosystems as well as laboratory test situations. The results are summarized below.

a) Accumulation of methylmercury via the water vector could be described mathematically as a two-compartment system with no apparent exchange between compartments. Biological half-times for methylmercury clearance from the two compartments were 2.5 hours and four days, respectively. Accumulation of inorganic mercury from water was a multi-compartment process. Clearance of inorganic mercury was faster than that of methylmercury.

b) Methylmercury uptake followed first order mass action kinetics. The observed values for whole body transfer coefficients, i.e., the sum of fast and slow compartment transfer coefficients, were variable, ranging from 1000 to

about 8000 grams of water per gram of Daphnia per hour. Slow compartment transfer coefficient values ranged from 500 to 1600 grams of water per gram of Daphnia per hour. The uptake rate of methylmercury increased proportionately with the .63 power of body weight. This relationship of uptake rate to body weight would have been expected if the methylmercury uptake rate had increased in proportion to metabolic rate. The increase in methylmercury uptake rate with increasing temperature over the temperature range 5 - 20°C supports the relationship between respiration rate and methylmercury uptake rate. The uptake rate of inorganic mercury from water was similar to that of methylmercury.

c) Assimilation efficiency from food strongly favours the preferential uptake of methylmercury over inorganic mercury. Assimilation ranged from 60 - 90% for methylmercury and 5 - 20% for inorganic mercury. The rate of uptake of methylmercury into the tissues was positively related to ambient food density due to a strong positive effect of food density on ingestion rate, despite a small reduction in assimilation efficiency with increased ingestion rate. The uptake rate of methylmercury into the tissues varied proportionately with the .82 power of body weight. This reflects the relationship of ingestion rate with body weight since assimilation efficiency is

independent of body weight. Temperature has a pronounced effect on the rate of methylmercury uptake into the tissues through a small effect on ingestion rate and a much larger effect on assimilation efficiency with an optimum temperature of about 20°C.

d) Values of the fractional clearance rate of methylmercury assimilated from food were similar to those of slow compartment of methylmercury taken up from water. Fractional clearance rates of methylmercury taken up from both food and water were independent of body size and temperature. Clearance of methylmercury was unaffected by moulting. Inorganic mercury assimilated from food was cleared about 10 times faster than methylmercury.

e) Over a wide range of aquatic species ranging in size from 1×10^{-4} to 100 grams wet weight, transfer coefficients for methylmercury uptake from water decline with an increase in body weight. The transfer coefficient of methylmercury uptake (TC) and wet weight in grams (W) can be related by the power function $TC = 7.3W^{-.25}$. Clearance of methylmercury from several crustacean species also declines with body weight. The fractional clearance rate (K_{cl}) was related to body weight by the power function $K_{cl} = .0006W^{-.78}$. The decline in clearance rate with body size in Crustacea is similar in slope to that

for fish, but the fractional clearance rate of fish appears to be approximately 50 times faster than for a crustacean of the same weight.

f) Animals exposed to inorganic mercury via the water vector displayed a very small compartment with a fractional clearance rate similar to that of the slow-clearing compartment of methylmercury. This small compartment in the inorganic mercury-exposed Daphnia may be methylmercury, raising the possibility that Daphnia may be capable of in vivo conversion of inorganic mercury to methylmercury.

g) The information on methylmercury uptake and clearance developed here has been coupled to published information on Daphnia energetics to produce a bioenergetic based methylmercury bioaccumulation model for Daphnia. This model was used to estimate the concentration of methylmercury in an individual Daphnia exposed continuously from birth to constant conditions of methylmercury contaminated food and water. The results of this simulation suggest that food and water mercury uptake vectors may be important in accounting for the mercury body burden in Daphnia. Also, Daphnia may achieve almost 70% of the maximum body burden by age five days, so that the concentration of methylmercury in the tissues will change little in the remaining 40 days of life.

Many gaps still exist in our understanding of methylmercury bioaccumulation in microcrustaceans. Several obvious areas requiring further work are the possibility of in vivo methylation of inorganic mercury in Daphnia; the effect of water quality on mercury uptake through influence on the chemical speciation of mercury and on the physiological function of Daphnia; and the possibility of transfer of mercury from the adult female to the egg.

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