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Ecological Implications of Dimethyl Mercury in an Aquatic Food Chain

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ABSTRACT

Laboratory studies indicate dimethyl mercury may be a major product of microbial methylation of inorganic mercury Although another methylation product, monomethyl mercury, has been extensively studied the physical, chemical, and biological factors affecting the transport and food chain distribution of dimethyl mercury hav remained unclear. This report represents results of laboratory studies of volatilization rates from water as a function of temperature and mixing conditions, uptake kinetics and equilibrium concentrations in algae, *Daphnia*, and fish, toxicity to fish, and studies of metabolism of dimethyl mercury by microbes. Mercury-203 labeled dimethyl mercury was used in the study, and in all tests the organisms used were live counted A unique method was developed for measuring dimethyl mercury uptake in algae. In water, dimethyl mercury was found to behave similarly to non-reactive gases such as oxygen Volatilization occurred rapidly

ACKNOWLEDGMENTS

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INTRODUCTION

The Problem of Mercury Pollution

Current concern

Although the toxic effects of mercury have been recognized since ancient times (Goldwater, 1971) the recent flurry of interest in mercury as an environmental pollutant has arisen largely from the belated realization that inorganic mercury, the most common form discharged into the environment by man, may be microbially converted to organomercury compounds presenting far more hazards to humans than their precursors (Grant, 1971). This discovery has been accompanied by an intensive effort to measure mercury concentrations in human food supplies, especially fishes (Grant, 1971).

Mercury toxicity

The extraordinary toxicity of mercury compounds has long been recognized. In medieval England metallic mercury was used to induce abortions and to commit suicide (Wood, 1971). More recent testimony as to the toxicity of mercury was given by the Minimata disaster in Japan, where 111 cases of mercury poisoning were reported, with 46 deaths. Poisoning was attributed to consumption of contaminated seafood (Gavis and Ferguson, 1972).

This toxicity is attributable to the tendency of mercury to form covalent linkages with sulfur. In animal tissue, mercurials even in low concentrations are capable of inactivating sulfhydryl enzymes and thus interfering with cellular metabolism and function (Goodman and Gilman, 1970).

There is no evidence that ionized organomercurials possess a different mode of toxic action from mercuric ions (Hassal, 1969). The greater apparent toxicity of organomercurials, and especially methyl mercury compounds, may well be entirely due to differences in retention and distribution. For example, humans were found to absorb only one percent of a mercuric chloride dose ingested, but absorbed 98 percent of a comparable dose of monomethyl mercury (Environmental Science and Technology, 1970). Elimination of monomethyl mercury from the body is a slow process; the half-life in humans is about 70 days (Hammond, 1971).

Dimethyl mercury toxicity will be discussed in a later section, but it is worth pointing out here that its mode of toxic action does not involve the complexing of sulfhydryl groups as for other mercurials (Gavis and Ferguson, 1972).

Sources of mercury

Mercury has hundreds of uses, and except for the estimated 18 percent of the annual consumption which is recycled (Wallace et al., 1971) all mercury used may be assumed to ultimately end up "in the environment." A summary of sources of environmental mercury based on data for 1968 is shown in Table 1.

The category of most interest in this discussion is discharges to water. The two major contributors in this category, chlor-alkali plants and the pulp and paper industry, have drastically reduced emissions since the onset of concern about mercury pollution. The latter have completely discontinued use of mercurials, and U.S. industry as a whole has reduced mercury emissions by 86 percent since 1970 (Environmental Science and Technology, 1970). This means that the problem of mercury in water has been reduced to one of describing and dealing with the consequences of mercury deposited to waterways in times past.

Historically most mercury discharged to water bodies has been in the ionic form. Such discharges have rarely resulted in hazardous mercury concentrations in water because of the tendency of ionic mercury to be adsorbed onto sediments (Gavis and Ferguson, 1972).

Mercury Methylation

Significance

Methylation is the process by which inorganic mercury is converted to either dimethyl or monomethyl mercury. Based on the nature of man-made and natural sources, it appears that most discharges of mercury to the environment have occurred as inorganic mercury, and practically none as methyl mercury (Hanson, 1971; Wallace et al., 1971). There is no record of dimethyl mercury being discharged as such to the environment. For these reasons, it may be safely asserted that virtually all methyl mercury compounds in the environment are the

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Table 1. Summary of man-made discharges of mercury to the environment.

Source of mercury	Primary chemical form	Amount added to environment, millions of lbs/yr
Primarily discharged to water		
Chlor-alkali process	Hg^{++}	1.33
Pulp and paper	Phenyl mercuric acetate	0.06
Primarily discharged to air		
Coal combustion	Inorganic	2.08
Mercury mining and processing	Inorganic	0.17
Primarily discharged to land		
Electrical and lab equipment	Hg^0	1.30
Agricultural	Organic	0.28
Miscellaneous		
Paints	Organic	0.68
Dental	Hg^0	0.23
Catalysts	Organic	0.11
Pharmaceutical	Organic	0.06
Total		6.30

Reference: Calculated from Wallace et al. (1971) for 1968.

result of biological synthesis, and that all dimethyl mercury found in nature is of this origin.

Agents and mechanisms

The ability to methylate inorganic mercury appears to be widespread in nature. Methylation has been reported to occur in biologically active sludges (Jensen and Jernelov, 1969), fish homogenates (Jensen and Jernelov, 1969), surface mucous from fish (Jernelov, 1970), calf's livers (Jernelov, 1970), and methanogenic bacterial cell extracts (Wood et al., 1968). Methylation has also been shown to occur non-biologically when inorganic mercury was reacted with methylcobalamin, a vitamin B_{12} derivative and a known methyl donor in biological systems (Imura et al., 1971; Bertilsson and Neujahr, 1971; Wood, 1971).

It has been suggested that any organism capable of synthesizing methylcobalamin has the potential for synthesis of methyl mercury (Wood, 1971). This would include humans and other animals (Conn and Stumpf, 1966; Imura et al., 1971) as well as many microorganisms (Wood, 1971). Studies completed thus far suggest that methylation occurs primarily in sediments (Wallace et al., 1971; Jensen and Jernelov, 1969).

The ubiquity of biological methylation of mercury in nature may be attributed in part to the variety of biochemical pathways available. Wood (1971) has identified five such pathways, both enzymatic and non-enzymatic and aerobic and anaerobic.

The state of research in this area does not allow prediction of the dominant methylation pathway in a given sediment system. It seems probable that in biologically active sediment systems more than one pathway functions simultaneously, and that this flexibility allows methylation to continue in mercury-contaminated sediments despite changes in temperature, oxygen concentration, pH, or biological makeup of the sediment system.

Dimethyl mercury synthesis

Dimethyl mercury synthesis has been shown experimentally to occur in sediments (Jensen and Jernelov, 1969), and the biochemical methylation pathways identified by Wood (1971) permit dimethyl mercury synthesis enzymatically and non-enzymatically and under aerobic and anaerobic conditions.

Both mono and dimethyl mercury were found to be produced in all reported methylation studies which were designed to measure both forms (Jensen and Jernelov, 1969; Wood et al., 1968; Imura et al., 1971). Wood et al. (1968) have concluded that dimethyl mercury is the ultimate product of the methylation process.

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The conditions favoring the formation of dimethyl mercury over the monomethyl form have not been explicitly studied. In studies of non-biological methylation of inorganic mercury using methylcobalamin, Imura et al. (1971) found the initial product to be dimethyl mercury: this immediately reacted with mercuric ions in solution to form monomethyl mercury. This suggests that dimethyl mercury formation would be favored in situations in which the concentrations of methyl donors in the sediments were large relative to the inorganic mercury concentration. However, this hypothesis remains untested.

Ecological effects of methylation

From an ecological viewpoint, methylation may be either beneficial or harmful, depending on the compound synthesized. Monomethyl mercury synthesis is unquestionably a harmful process because the product is far more toxic than its inorganic precursor. Moreover, mercury in this form is no longer immobilized in sediments but is rather released into water where it may be rapidly taken up by the biota (Wallace et al., 1971).

Dimethyl mercury synthesis, on the other hand, may be ecologically beneficial because of the well recognized tendency of the compound to volatilize from water (Wolfe et al., 1972).

Dimethyl Mercury

Dimethyl mercury apparently has no industrial or other uses, but its significance in organometallic chemistry and in environmental studies has resulted in a substantial body of information on the compound.

Chemical properties

Although the environmental significance of dimethyl mercury has only lately been recognized, the compound has been known to exist at least since the middle of the 19th Century (Ostlund, 1969). A variety of physical and chemical properties have been determined and are summarized in Table 2.

From an environmental point of view, the properties in Table 2 which are of most interest are the high volatility and the relatively high solubility in water. A solubility limit at room temperature of 10^{-2} M corresponds to about 2000 mg/l. The slowness of the methylation process, combined with the tendency of the com-

Table 2. Summary of properties of dimethyl mercury.

Property	Value	Reference
General:		
Chemical formula	$Hg(CH_3)_2$	Weast, 1971
Atomic weight	230.66	Weast, 1971
Toxicity	No data in literature	
Physical.		
Form at room temperature	Colorless liquid, sweet odor	Weast, 1971
Boiling point	96°C	Weast, 1971
Vapor pressure equation	$\log_{10} P_{mm} = 7.017$ $- \dfrac{1342}{232 + T°C}$	Long and Cattanach, 1961
Solubility:		
ether, alcohol	Soluble	Weast, 1971
water	10^{-2}M	Wolfe et al., 1972
Thermodynamic, at 298.15°K:		
Enthalphy of formation	14.3 kcal/mol (liquid) 22.56 kcal/mol (gas)	U.S. National Bureau of
Gibbs energy of formation	33.5 kcal/mol (liquid) 34.9 kcal/mol (gas)	Standards
Enthalphy	4.29 kcal/mol (gas)	
Enthropy	50 cal/deg-mol (liquid) 73 cal/deg-mol (gas)	
Heat capacity	19.9 cal/deg-mol (gas)	

pound to volatilize from water suggests that this solubility limit is unlikely to be approached in nature.

Chemistry in aqueous solution

The studies reported by Wolfe et al. (1972) were exclusively concerned with the chemistry of dimethyl mercury in water. It was found that dimethyl mercury was stable in aqueous solution under conditions prevailing in most natural environments. No photodegradation of aqueous dimethyl mercury was observed in sunlight, and decomposition due to low pH was found to occur very slowly. At pH 5, the half-life of dimethyl mercury was calculated to be about 33 years at 25°C. Under basic conditions, dimethyl mercury was found to be stable for 20 hours in 1 M KOH, and for at least 24 hours in O^2 saturated water. Both of these experiments were conducted at 85°C, indicating the thermal stability of the compound.

One reaction that did occur rapidly was the desymmetrization reaction:

$$CH_3Hg\ CH_3 + 2Hg^{++} \rightarrow 2CH_3Hg^{+}$$
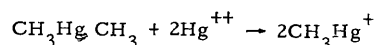
This reaction occurs virtually instantaneously at high Hg^{++} concentrations. Ostlund (1969), in fact, used mercuric solutions as vapor traps for dimethyl mercury volatilized from mice.

Biological interactions

A review of the literature revealed only one study involving interactions between dimethyl mercury and living organisms. Ostlund (1969) injected sublethal doses of Hg-203 labeled dimethyl mercury into mice, and determined partitioning within the animals and the kinetics and modes of loss.

He found the dimethyl mercury was rapidly partitioned into fat deposits within the mice, and that excretion was very rapid. Half the dimethyl mercury loss occurred in the first 30 minutes, and virtually all loss occurred within 3 hours.

The mice were dosed with dimethyl mercury in two ways; by inhalation and intravenously. Excretion kinetics were virtually the same in both cases, and in both cases the primary mode of excretion was exhalation. To a much lesser extent losses occurred through the skin. These results suggest that dimethyl mercury is readily transported through biological tissue.

Ostlund further reported that metabolic conversion of about 10 to 20 percent of the added dimethyl mercury occurred, and that the product thus formed was apparently monomethyl mercury As will be shown, results from this study suggest that the apparent metabolic conversion to monomethyl mercury may in fact have resulted from radiolysis of the stock solution which occurred prior to injection.

No data were found in the literature regarding the toxicity of dimethyl mercury, although it has been suggested that the compound is biologically inert (Jacobson, 1972). This supposition may be supported on two bases. First, the classical mode of mercury toxicity by complexing of sulfhydryl groups does not apply to a nonionized form such as dimethyl mercury (Gavis and Ferguson, 1972). Secondly, Ostlund's study suggests animals excrete dimethyl mercury rapidly with little conversion to other forms.

Strictly speaking, of course, it is obvious that high concentrations of dimethyl mercury will produce toxic effects via mechanisms other than sulfhydryl group complexing. The question is whether concentrations of dimethyl mercury in nature are high enough for such effects to be manifested.

There are no reports in the literature of studies aimed at demonstrating dimethyl mercury synthesis in natural environments, or at detecting dimethyl mercury in natural waters (Gavis and Ferguson, 1972). Thus it must be admitted that a concern for dimethyl mercury in the environment is not yet based on hard in situ data confirming its presence. On the other hand, the work of Jensen and Jernelov (1969) and Wood et al. (1968) strongly suggest that dimethyl mercury is in fact present in natural environments.

Study Rationale and Objectives

General

Although previous studies (Ostlund, 1965; Wolfe et al., 1972) have shown some ecologically significant properties of dimethyl mercury, a great many questions remain unanswered. Three main areas of interest may be identified as transport processes within aqueous ecosystems, processes of synthesis and degradation, and toxic effects on biota. The primary objective of this study was to identify and answer the most important questions arising within the first area of transport processes. Additionally, limited forays were made into the other areas.

The processes available in nature for the transport and distribution of dimethyl mercury are shown in Figure 1. Each of the processes shown has associated with it a specific chemical or biological mechanism, which includes kinetics and equilibria. None of these properties have been previously identified for any of the processes shown in Figure 1.

Volatilization

The tendency of dimethyl mercury to volatilize from water is well recognized (Wolfe et al., 1972; Jensen and Jernelov, 1969) but the kinetic model involved and the factors affecting kinetics have not been elucidated. Because of its non-polarity, dimethyl mercury behaves similarly to a gas such as oxygen in water (Wolfe et al.,
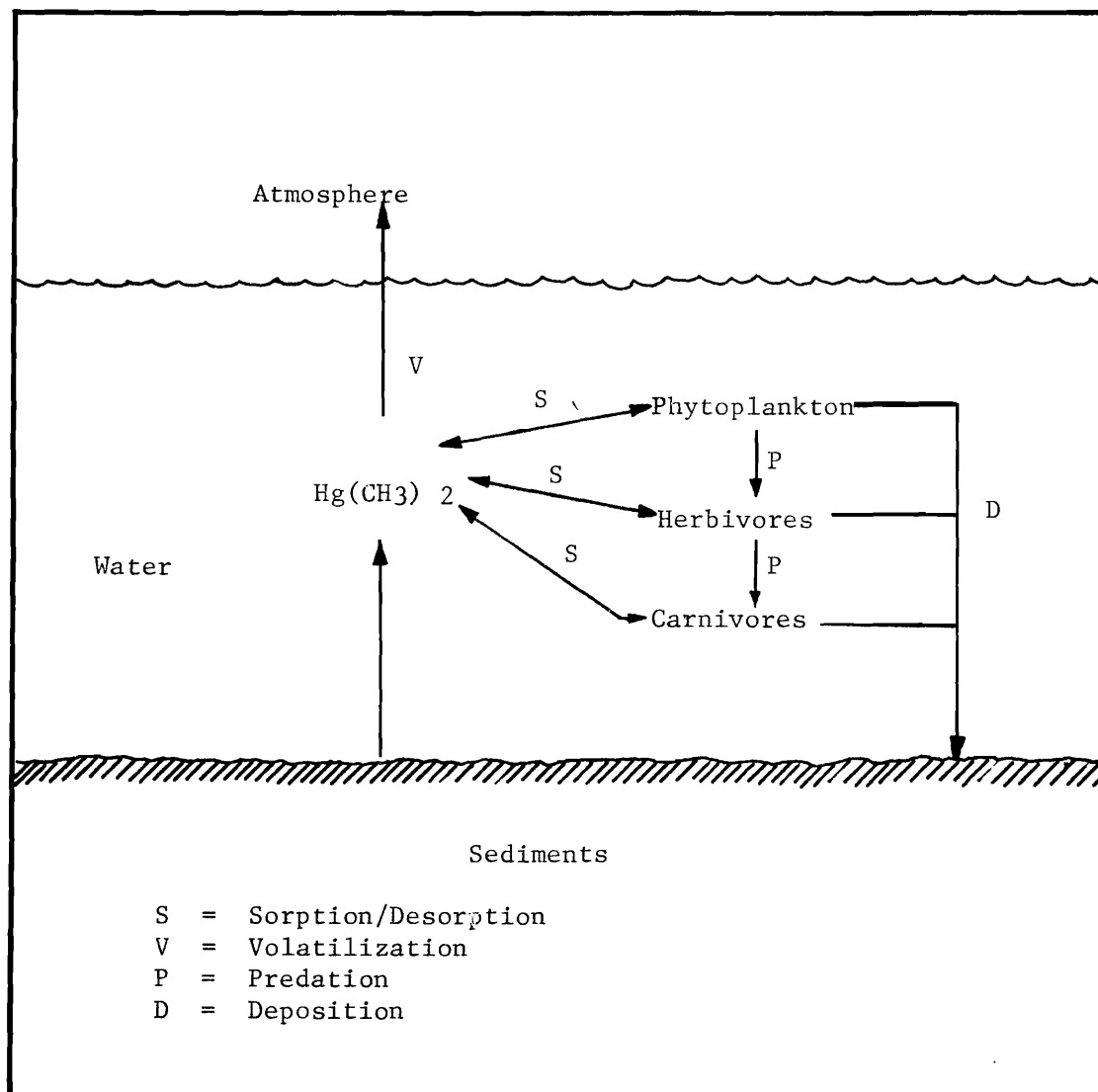
Figure 1. Transport processes for dimethyl mercury.

1972) and this suggests that the variables most important in oxygen transfer should be investigated. These are temperature and mixing.

Biological uptake and loss

A description of the dimethyl mercury uptake and loss processes by aquatic organisms has obvious value in understanding the ecological significance of the compound. In order to study the problem on a broad basis, species from three trophic levels were selected for use: the green alga *Selenastrum capricornutum*, and herbivore *Daphnia magna*, and at the carnivore level, the fathead minnow, *Pimephales promelas*.

For each species the goal was to define the equilibrium uptake, and the kinetic processes involved in reaching it.

Miscellaneous studies

During the conduct of studies of transport processes involving dimethyl mercury, it was found useful to know certain other properties as well. It was decided to investigate the following: a) the lipid-water partition coefficient for dimethyl mercury, b) the acute toxicity of dimethyl mercury to fish, and c) whether dimethyl mercury is readily metabolized in bacteria-containing waters used in culturing fish, or in bacterized algal cultures.

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Summary of study objectives

The objectives of these studies were:

1. To define the kinetics of volatilization of dimethyl mercury from aqueous solution as a function of temperature and mixing conditions.
2. To describe the processes of uptake and loss of dimethyl mercury by aquatic organisms at three trophic levels.
3. To conduct a preliminary investigation of the following phenomena involving dimethyl mercury:
   a) The metabolism of dimethyl mercury by microbial populations.
   b) The toxicity of dimethyl mercury to fish.

This study was conducted using mercury-203 labeled dimethyl mercury. This permitted rapid nondestructive determinations of dimethyl mercury concentrations. The use of radiolabeled dimethyl mercury immensely simplified the conduct of this study, and without it certain experiments, such as measurement of uptake in algae, could not have been performed at all.

Source and Properties

Radiolabeled dimethyl mercury was purchased from ICN International Chemical and Nuclear Corp, of Irvine, California, as a special synthesis. The standard method using the Grignard reagent was used in synthesis, with Hg^{++}-203 in place of stable Hg^{++}-200 (Jimruska, 1972). The properties of the labeled compound and the isotope on which it is based are summarized in Table 3.

Table 3. Characteristics of the radiolabeled dimethyl mercury used.

Characteristic	Value
Labeled molecule	Hg-203
Specific activity	5 m C/g Hg
Chemical form	$(CH_3)_2$ Hg in either solution
Radiochemical purity	99%
Emission characteristics:[a]	
Half-life	46.57 days
Beta energy	210 KeV
Gamma energy	279.2 KeV

[a]Source: Weast (1971).

Methods of Detection

Beta emissions

Radioactive mercury was detected on the basis of both beta and gamma emissions. Beta emissions were detected using liquid scintillation counting. This technique was used only for liquid samples. These were added to counting vials containing scintillation cocktail, a teflon-lined screw cap was added, and the mixture shaken to result in a single-phase clear liquid. In general, 2 ml of sample was added to 15 ml of counting cocktail.

The counting cocktail used was Aquasol, a xylene-based solution purchased from New England Nuclear, Inc., Boston, Massachusetts. The vials were of the standard borosilicate glass variety with a neck diameter of 24 mm. The plastic caps were modified by the replacement of the polyethylene liners with cap liners prepared from 0.8 mm sheet teflon. This was done to preclude volatile dimethyl mercury vapors from being absorbed by the cap or its liner, and thus being lost for counting purposes.

The efficacy of this system of cocktail, vial, and cap in retaining dimethyl mercury activity was verified in two ways. First, it was found repeatedly that a sample could be recounted days later with no loss of activity except that due to decay. Secondly, it was found that once a sample was added to a vial and mixed with the counting cocktail, the cap could be removed for at least 12 hours without loss of activity. It was not determined whether this was due to some reaction with the cocktail producing a non-volatile product, or to a low diffusion rate of the dimethyl mercury from the cocktail.

The samples thus prepared were counted using a Beckman CPM 100 Liquid Scintillation system at ambient temperature. The tritium-carbon 14 channel was used, which resulted in a counting efficiency of 58.4 percent with a background of 25 - 35 counts per minute (cpm).

Gamma emissions

Gamma emissions of Hg-203 labeled dimethyl mercury were detected using a system based on a well-type thallium-activated sodium iodide scintillation crystal. The equipment used was manufactured by Baird Atomic, Inc. of Cambridge, Massachusetts, and consisted of the following: Scintillation detector, Model 810; Multiscaler II, Model 132, operated at 1500 V; and Precision Timer, Model 630.

This apparatus had no provision for counting discrete energy spectra, and hence the background count was high relative to system geometry. In most cases background was around 400 cpm. Counting efficiency for samples in the well was found to be 56.8 percent based on a $Hg^{203}(NO_3)_2$ standard.

Gamma counting was used for most of the dimethyl mercury determinations made in this study. The specific techniques used depended on the sample being counted, and will be detailed in chapters on specific studies.

Radiolysis and Chemical Purification

Autoradiolysis is the chemical decomposition of a labeled molecule due to its own radioactive emissions. This phenomenon was found to occur in the stock Hg-203 labeled dimethyl mercury used in these studies and was perhaps the single greatest source of experimental problems.

Mechanism and products

The exact mechanism involved in the radiolysis of dimethyl mercury was not elucidated, but the following inferences may be made. First, the process is apparently due to beta emissions rather than gamma rays (Jimruska, 1972), as has been repeatedly found for C^{14} labeled compounds. Secondly, there is reason to believe that the end product of this process is monomethyl mercury. This is based on the fact that the autoradiolysis of Hg-203 labeled monomethyl mercury results in the splitting off of the methyl group (Jimruska, 1972), suggesting that cleavage of one or both methyl groups may occur in radiolysis of dimethyl mercury. Any mercuric ions thus formed would immediately react with dimethyl mercury to form monomethyl mercury (Wolfe et al., 1972). This identification was not experimentally verified, and therefore the radiolysis product will be referred to in this report as "non-volatile mercury" or "non-volatile residue."

The problem of autoradiolysis was attacked on two fronts: first, steps were taken to slow the rate of this process in the ether stock solutions; and second, ways were developed to remove the non-volatile contaminant before adding stock to test solutions.

Methods of slowing radiolysis

The available means of minimizing autoradiolysis during storage consist of proper selections of solvent, storage temperature, and specific activity. In addition, free radical scavengers may be employed (Nuclear-Chicago Corporation, 1965). Of the solvents available aromatic compounds such as benzene are the most desirable because of their ability to dissipate absorbed radiation energy without transferring it to solute molecules (Nuclear-Chicago Corporation, 1965). However the toxicity of benzene and its derivatives to aquatic biota (McKee and Wolf, 1963) precluded its use, and therefore the labeled dimethyl mercury remained in its original ethyl ether solution during storage.

In general, low temperatures have been found to inhibit autoradiolysis (Nuclear-Chicago Corporation,

1965), and stock solutions were therefore stored at the lowest feasible temperature, which was -20°C. A lower specific activity may also help minimize radiolysis (Nuclear-Chicago Corporation, 1965) and to this end stock solutions were diluted with unlabeled dimethyl mercury to reduce specific activity by at least a factor of 10. The diluent used for the unlabeled mercury was ethyl alcohol, so adding unlabeled dimethyl mercury involved adding a small amount of ethyl alcohol to the stock solutions. This ethyl alcohol addition may have been useful in itself in minimizing radiolysis, because there is some evidence that this substance is an effective free radical scavenger at low concentrations (Bayly and Evans, 1966).

Chemical purification

Removal of all possible non-volatile mercury contaminant was found to be an absolute necessity for all experiments in which labeled dimethyl mercury was used in conjunction with living organisms. This was because the non-volatile product, which was very likely monomethyl mercury, tended to be almost completely absorbed into biological tissue, while dimethyl mercury was absorbed to a much lesser extent.

The result was that if even one or two percent of the added radiomercury was in the non-volatile form, a majority of the radioactive mercury uptake would be in a form other than dimethyl mercury. While this could be corrected, it was still of vital importance that all possible contaminant be removed from stock solutions.

After struggling in algal uptake experiments with problems engendered by the tendency of the non-volatile mercury contaminant to be adsorbed onto algal cells, the idea was conceived of using the algal cells themselves to remove the contaminant. This approach proved to be an effective solution to the problem.

The method worked as follows: about 15 ml of thick algal suspension was prepared by centrifuging. This was used to fill a 12 ml glass centrifuge tube. Labeled dimethyl mercury in ether solution was injected into the algal suspension in the tube using a 50 µl Hamilton syringe. It was found necessary to chill the syringe to -20°C in order to accurately measure volumes of the volatile ether solution.

Immediately after adding the isotope to the centrifuge tube, a teflon-lined screw cap was added and the sealed tube was allowed to stand about 30 minutes to allow the monomethyl mercury to be absorbed. Then the tube was placed in the centrifuge and the algae concentrated into a pellet. The supernatant was used as the stock dimethyl mercury solution, and the algal pellet discarded. This method was capable of removing over 90 percent of the original non-volatile mercury contaminant.

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VOLATILIZATION STUDIES

Experimental Procedures

The basic objective of volatilization studies was to define the kinetic process involved in dimethyl mercury volatilization from aqueous solution and to describe the effects of two variables thought to be most important; mixing and temperature.

Three temperatures and four mixing rates were selected. The temperatures were $10°$, $20°$, and $30°C$, intended as a fair approximation of the range of temperatures found in natural waters. Mixing conditions found in nature are not readily duplicated in laboratory environments, so it was arbitrarily decided to use four mixing speeds of 0, 20, 40, and 60 rpm on the paddle-type stirrers used.

NAAM algal culture medium was used in order to provide a medium comparable to natural waters but having a defined chemical composition. This medium is completely inorganic, buffered to maintain pH near neutrality, and has a total dissolved solids concentration of less than 70 mg/l (Weiss and Helms, 1971). The composition of NAAM medium is given in Appendix A.

Volatilization tests were conducted in 600 ml pyrex beakers containing 500 ml of NAAM medium. Temperature was controlled using water baths (Neslab Model RTZ), and stirring was done with paddle-type stirrer units equipped with a gage to indicate speed in revolutions per minute (Phipps and Bird, Inc., Richmond, Virginia).

In order to control the air-water interface area and to allow calculation of power inputs, it was necessary to suppress vortex formation (Fair and Geyer, 1954). This was done with vinyl baffles, which were positioned across the beaker to bisect the surface of the water. The apparatus used is shown in Figure 2.

Experiments were begun by adding Hg^{203} dimethyl mercury to each beaker with a pipet. Initial concentrations were about 0.4 mg/l. Samples were taken after about 30 seconds of stirring and at regular intervals of one or 1.5 hours thereafter. In all cases 2 ml samples were added to numbered vials containing 15 ml Aquasol for subsequent counting of beta emissions. Duplicate beakers were run for each set of experimental conditions. A high degree of precision was found in sampling and analysis of aqueous labeled dimethyl mercury solutions. In trial runs, the

Top view

Side view
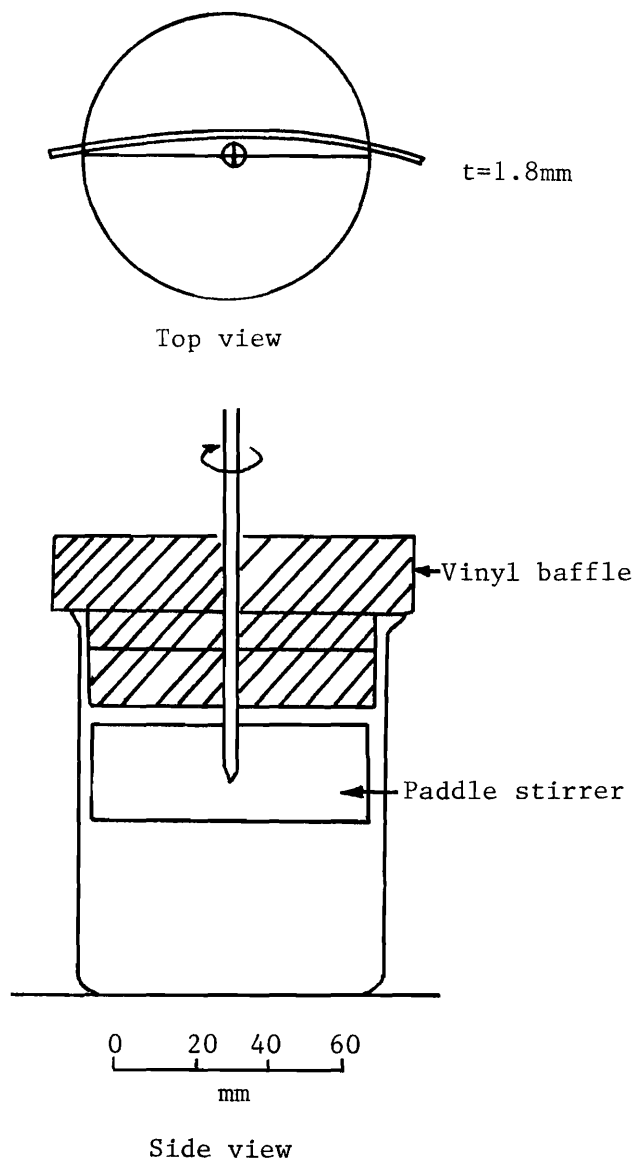
Figure 2. Schematic view of apparatus used in volatilization tests.

coefficient of variation was less than 4 percent. This permitted single samples to be taken at each sampling period.

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Results and Discussion

Data analysis

Raw data for volatilization experiments consisted of count per minute data for the 2 ml samples taken periodically throughout the runs. These data could not be taken as directly proportional to dimethyl mercury concentrations because it was found that about 30 percent of the Hg^{203} activity originally added to each beaker was in a form other than dimethyl mercury. This non-volatile component was the result of radiolysis which had previously occurred in the dimethyl mercury stock solution.

To correct for this it was necessary to subtract from each data point the counts per minute attributable to non-volatile mercury. The magnitude of this correction was difficult to determine exactly because the residue left after the dimethyl mercury had evaporated was not solely in solution; a small portion had been absorbed onto the vinyl baffles and onto the stirrers.

For each beaker an estimate was made of the residue correction based on the radioactive residue remaining in solution plus estimates of the absorption to baffles and paddles. This estimate was then refined by a slight adjustment so that corrected data points plotted as an approximate straight line on a semilogarithmic plot of counts per minute versus time, in conformance with the kinetic model to be described below. Practically speaking, this refinement affected only data points for low dimethyl mercury concentrations. Rate constants were found to be insensitive to the size of this final correction. Raw data, together with the correction described, is shown in Appendix B.

Rate constants were determined using a least squares computer program which minimized deviations of the fitted curve from the data points. The curve used in this context was based on the kinetic model described in the following section. The computer program was prepared especially for this application and is presented in Appendix C.

Kinetic model

The kinetics of dimethyl mercury volatilization from aqueous solution in stirred systems were found to conform to a simple first-order model in which the rate of loss was proportional to the concentration of dimethyl mercury in solution. Mathematically this may be expressed as follows:

$$\frac{dM}{dt} = - kt \quad \ldots \ldots \ldots \ldots \ldots \ldots \quad (1)$$

where M is the mass of dimethyl mercury, k is the rate constant and t is time.

This model is identical to that developed for sparingly soluble gases such as oxygen or CO_2 whose mass transfer is controlled by liquid film resistance (Eckenfelder, 1966). Equation 1 can be rewritten in this context as

$$\frac{dC}{dt} = - K_L aC \quad \ldots \ldots \ldots \ldots \ldots \quad (2)$$

where K_L is liquid film transfer coefficient and a is the ratio of air-water interface area to volume.

Equation 2 can be integrated to yield the following expression:

$$C/C_o = e^{-K_L at} \quad \ldots \ldots \ldots \ldots \ldots \quad (3)$$

where C_o is the concentration at t = 0.

For tests conducted using a given test vessel it is clear that a, the surface area to volume ratio, is a constant. The only remaining parameter is K_L, and it is this coefficient which varies with temperature and mixing conditions. For these experiments the surface area of the beakers was carefully measured at 46.40 cm^2. With a volume of 500 ml, the surface area to volume ratio is 0.0928 cm^{-1}.

Effect of temperature

Volatilization runs were conducted at a paddle speed of 40 rpm at three temperatures: $10°$, $20°$, and $30°C$. Results from all three runs are plotted in Figure 3 as a percentage of original concentration. As noted earlier, lines of best fit were determined by a computer program using the least squares criterion.

It is clear from Figure 3 that volatilization proceeds faster at higher temperatures. A summary of the values of K_L determined from the above data is presented in Table 4.

Table 4. Summary of values of K_L as a function of temperature at 40 rpm paddle speed.

Temperature °C	K_L, cm/hr
10	5.2
20	6.9
30	9.0

It has been shown by Arrhenius (Moore, 1962) that the activation energy, E_a, of a process is related to temperature and rate constant by the following expression
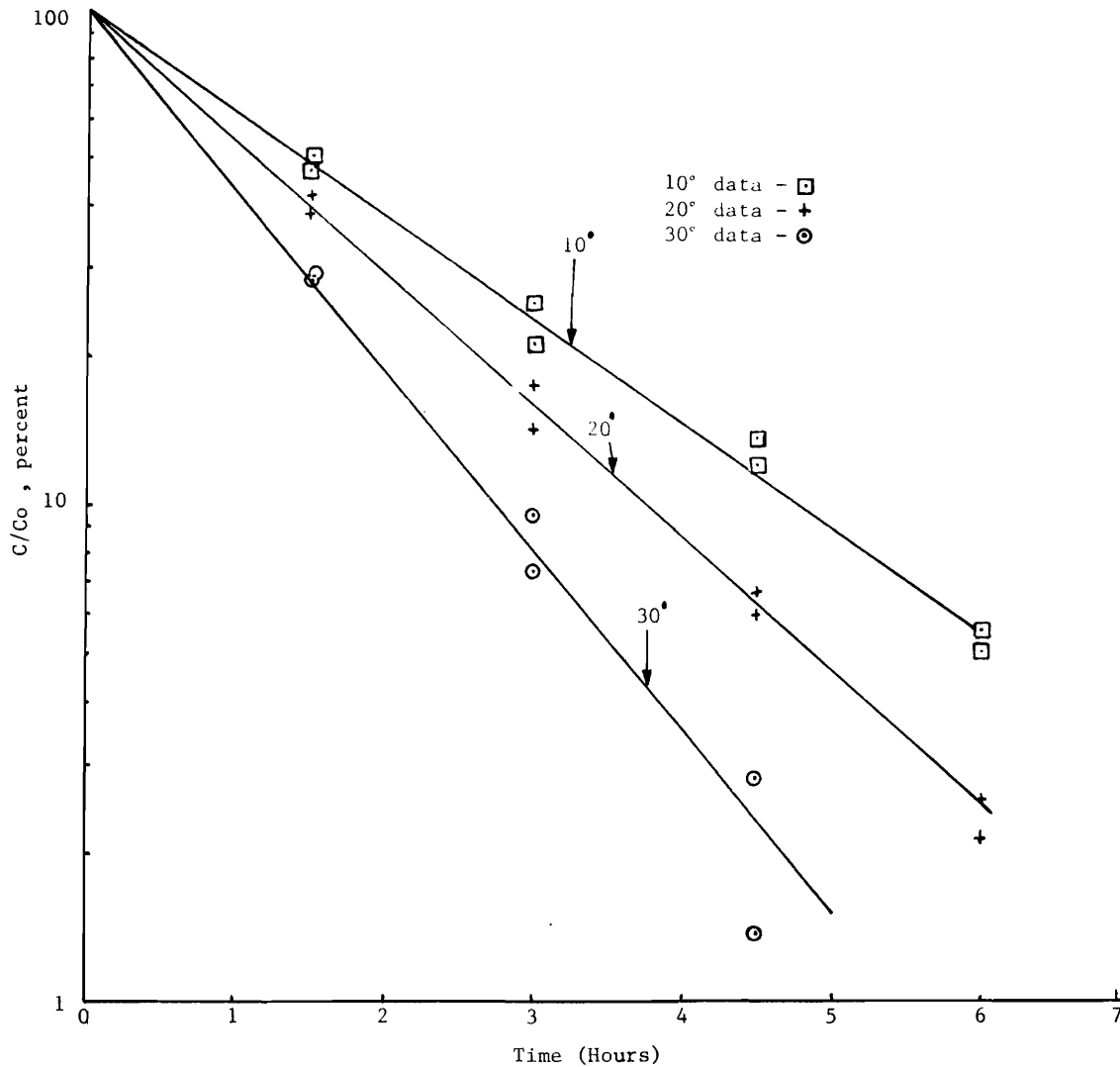
Figure 3. Dimethyl mercury concentration as a function of time for three temperatures at 40 rpm paddle speed.

$$\ln K = -\frac{E_a}{RT} + \ln A \dots \dots \dots \dots (4)$$

where A is a constant of integration and R is the ideal gas constant. Stated graphically, the activation energy of the volatilization process may be found based on the slope of a plot of $\ln K_L$ versus the reciprocal of the absolute temperature. This plot is shown as Figure 4.

The linear relationship predicted by Arrhenius was confirmed and is shown in Figure 4. Based on this plot the activation energy of the volatilization process may be calculated to be 4735 cal/mole.

A relationship between rate constant and temperature of the form

$$\frac{(K_L)_T}{(K_L)_{20}} = A^{(T-20)^\circ C} \dots \dots \dots \dots (5)$$

has been empirically defined for oxygen transfer. In this equation $(K_L)_T$ and $(K_L)_{20}$ are values of K_L at some given temperature T° and $20^\circ C$ respectively, and A is a constant. For oxygen transfer in water A has been found to be 1.028 (Eckenfelder, 1966).

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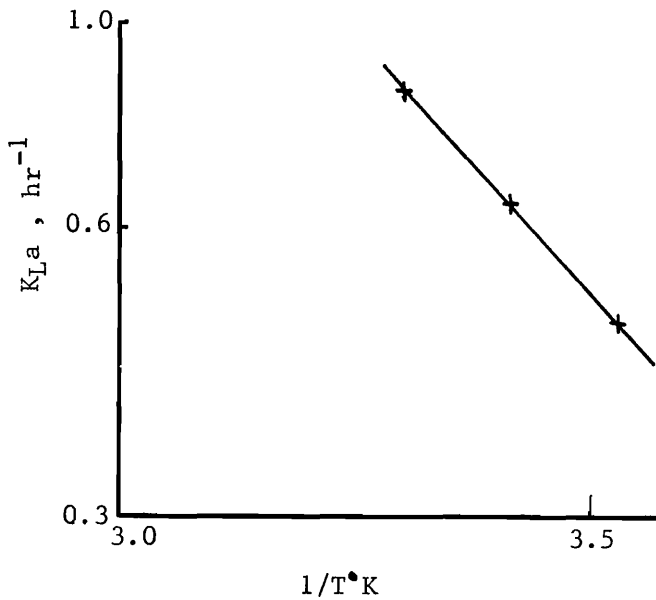
Figure 4. Arrhenius plot for dimethyl mercury volatilization from water.
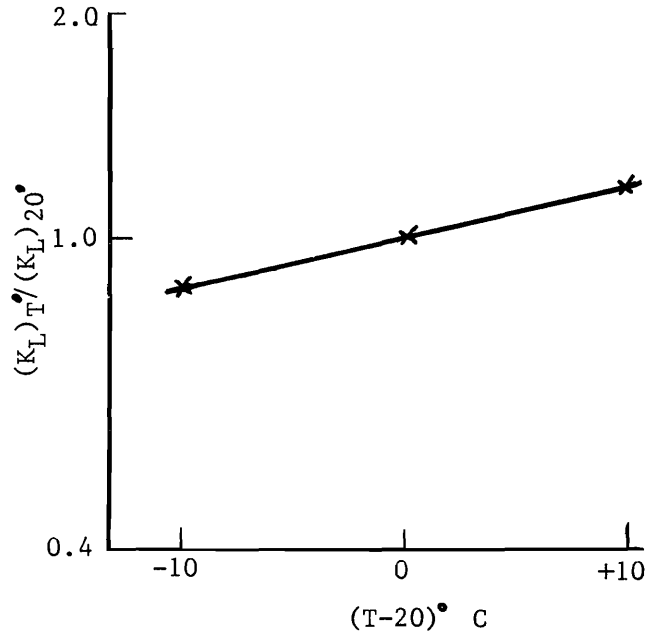
Figure 5. $(K_L)_T/(K_L)_{20°}$ versus $(T - 20)°C$ for dimethyl mercury volatilization from water.

A similar relationship for dimethyl mercury is shown in Figure 5. As was true for the Arrhenius plot, the predicted linearity of the relationship between temperature and rate constant is confirmed. The value of the constant A in Equation 5 may be determined from the slope of the line. This value was calculated to be 1.027, which is virtually identical to the corresponding constant for oxygen transfer.

Effect of stirring

The effect of stirring was assessed by volatilization experiments conducted at 20°C at paddle speeds of 20, 40, and 60 rpm as well as an unstirred beaker. Results of these evaluations are plotted in Figure 6.

As expected, more rapid stirring resulted in an increase in the volatilization rate. Values of the rate constants are summarized in Table 5, together with

Table 5. Summary of values of K_L as a function of stirring speed at 20°C.

Stirring speed, rpm	Specific power, watts/l	K_L, cm/hr
0	10	0.811
20	$1.73 \cdot 10^{-9}$	4.504
40	$1.39 \cdot 10^{-8}$	6.918
60	$4.68 \cdot 10^{-8}$	7.274

specific power levels calculated according to Fair and Geyer (1954).

Once again using gas transfer theory, the relationship between the rate constant K_L and the mixing velocity has been found to be of the form:

$$K_L = cV^n$$

where c and n are constants, and V is the velocity of the water (Tsivoglou and Wallace, 1972). A variety of values have been proposed for n, but the value of 0.5 proposed by O'Connor and Dobbins (1956) appears to be based on the best laboratory data.

From the data for dimethyl mercury developed in this study, the value of n was found by plotting values of K_L versus paddle speed on log-log paper, developing a line of best fit by the least squares method, and calculating n as the slope of this line. This plot is shown in Figure 7. The value for n calculated from the slope of the line was 0.429. This is similar to the value of 0.5 found by O'Connor and Dobbins. A plot of K_L versus the square root of paddle velocity, shown as Figure 8, confirms that this model provides a good approximation of the experimental results.

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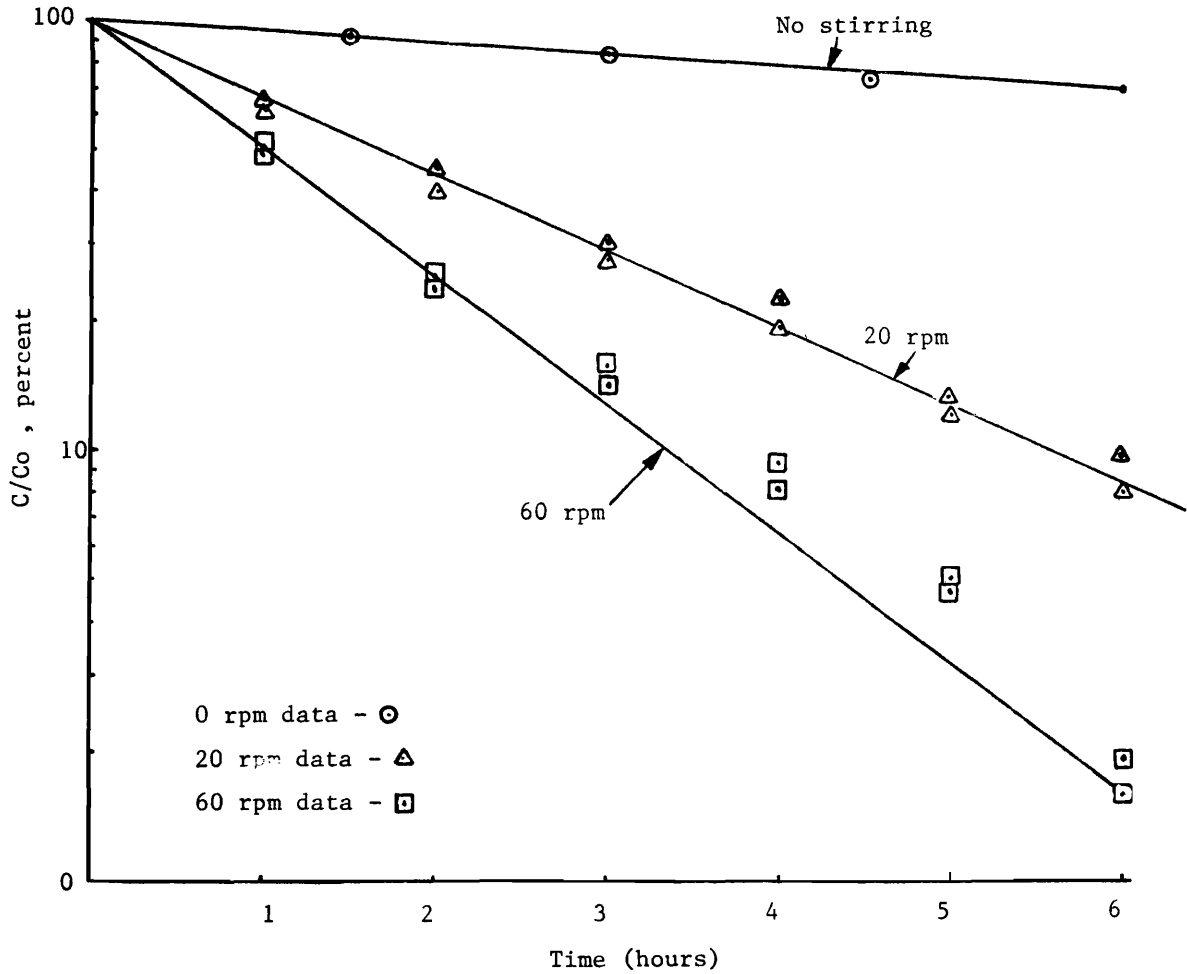
Figure 6. Dimethyl mercury concentration as a function of time for three paddle speeds at 20°C.

Comparison with oxygen data

It has been shown on theoretical grounds that the ratio of K_L values for any pair of gases A and B will be equal to the inverse of the ratio of their molecular diameters (Tsivoglou and Wallace, 1972). If d is the molecular diameter, this may be expressed as:

$$\frac{(K_L)_A}{(K_L)_B} = \frac{d_B}{d_A}$$

Dimethyl mercury has been shown to behave like a sparingly soluble gas in aqueous solution, and this led Wolfe et al. (1972) to suggest the following relation holds:

$$\frac{(K_L)_{O_2}}{(K_L)_{me_2Hg}} = \frac{d_{me_2Hg}}{d_{O_2}} = 2.4$$

This relationship may be tested using K_L values for dimethyl mercury determined in this study in conjunction with K_L values for oxygen found in the literature. An oxygen transfer experiment summarized in Eckenfelder (1966) gave a transfer constant of 25.2 cm/hr at 20°C for a velocity equivalent to a paddle speed of 120 rpm. If the line relating K_L to paddle speed for dimethyl mercury (Figure 8) were extrapolated to a point corresponding to 120 rpm, K_L may be estimated at 10.6 cm/hr. The ratio of transfer constants is 25.2/10.6, or 2.38.

This is in excellent agreement with the relationship proposed by Wolfe. This model provides a simple means of estimating dimethyl mercury transfer coefficients for streams for which reaeration constants have been previously determined.
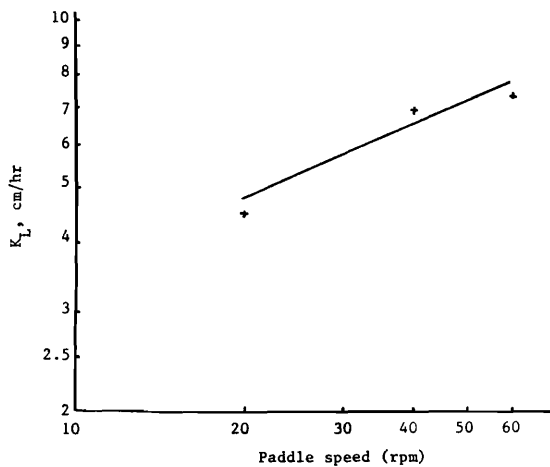
13

Figure 7. Plot of log K_L against log paddle speed for dimethyl mercury volatilization from water.

Summary

In aqueous solution dimethyl mercury was found to behave like a sparingly soluble gas such as oxygen or carbon dioxide. The volatilization of dimethyl mercury from water followed the relation

$$\frac{dc}{dt} = - K_L a$$

K_L was found to vary with temperature according to the relation

$$(K_L)_T / (K_L)_{20} = A^{(T-20)}$$

where c is the concentration in water, t is time, K_L is the first-order transfer constant and a is the surface area to volume ratio.
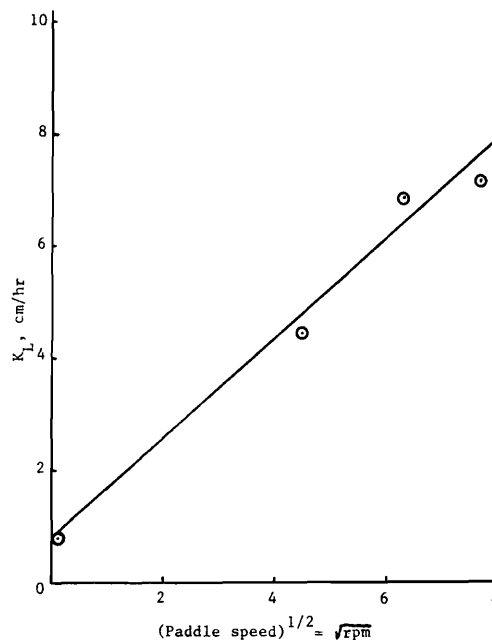
Figure 8. K_L as a function of the square root of paddle speed for dimethyl mercury volatilization from water.

where T is the temperature in degrees centigrade and A is a constant. The value of A was 1.027, almost identical to the corresponding value for oxygen.

Increasing the stirring rates gave higher transfer constants. K_L values were found increased with the square root of the water velocity, the same relationship as has been shown for other gases.

The absolute value of K_L for dimethyl mercury has been estimated to be less than that of oxygen by a factor of 2.4 (Wolfe et al., 1972). Results of these experiments confirm this estimate. This provides a basis for estimating dimethyl mercury transfer constants for streams for which reaeration constants have been previously determined.

STUDIES OF FISH AND *DAPHNIA*

Experimental Procedures

The purposes of the fish and *Daphnia* studies were to determine the equilibrium uptake of dimethyl mercury and to define the kinetics of the process.

Culturing and characterization of test organisms

Fish. At the carnivore trophic level, the organism selected for test was the fathead minnow, *Pimephales promelas.* This fish is widely used as a test organism for toxicity tests (McKee and Wolf, 1963) and for this reason, techniques of culturing are well understood. The fish used were purchased as adults from a commercial supplier (Ozark Fisheries, Inc., Stoutland, Missouri) and cultured in a 490 l tank maintained at 24°C. Water used was dechlorinated Logan City tap water, a moderately hard groundwater supply.

The fathead minnows used were 4 to 5 cm long and weights ranged from 0.8 g to 1.8 g. The relatively small size of the minnows was an important advantage, because it permitted the entire fish to be placed in the gamma counting well when assaying radioactive content.

Fish were selected at random for use in experiments and no characterization was attempted until completion of the experiments. At this time the fish were killed by severing the spinal cord just behind the brain. They were then blotted briefly and weighed. Length was determined as "Standard Length" from snout to base of caudal fin (Lowe-McConnell, 1968). The fish were then placed in numbered envelopes and placed in a freezer at -20°C.

Lipid content was run on these fish according to the method of Bragdon (1951). This method employs an exhaustive extraction with hexane, followed by a chromic acid oxidation step. Lipid content is related to the amount of reduced chromate ion produced, as determined colorimetrically at 50 mµ. Results are based on a steric acid calibration curve.

When used for toxicity experiments, fish were placed in 2.4 l bottles sealed with screw caps. Results of toxicity tests did not warrant any characterization of fish.

Daphnia. At the herbivore level, the water flea, *Daphnia magna,* was selected for test. Because of their wide distribution in aquatic environments around the world, and because they are an important food source for a variety of fishes, *Daphnia* make an excellent representative of the second trophic level (Lammering, 1964).

The *D. magna* population used in these tests was cultured from a sample obtained from the Environmental Protection Agency Laboratory in Duluth, Minnesota. A 20 l glass-walled aquarium was used. The aqueous medium was dechlorinated tap water enriched with about 100 g of horse manure. The *Daphnia* were fed with about 1 l of a 40 mg/l *Selenastrum capricornutum* suspension added every other day.

Under these conditions, the *D. magna* culture was maintained without difficulty for about 6 months. The only maintenance required was an occasional removal of bacterial and algal slime growths from the side walls of the aquarium.

Only larger individuals were selected for testing, on the theory that their lower surface area to volume ratios would tend to minimize volatilization losses of dimethyl mercury during preparation for counting. Another advantage of using larger *Daphnia* was that coarser netting could be used to contain the animals during testing, thus allowing better water circulation.

Daphnia selected for use were those passing through netting with a 2.5 mm opening, and retained on netting with a 1.81 mm opening. Netting used to contain the *Daphnia* during testing had an opening of 1.23 mm.

At the end of experiments, test organisms were placed in a 100°C oven overnight, and dry weight determined the following day.

Measurement of dimethyl mercury content

Fish. For all experiments involving dimethyl mercury uptake or loss, fish were placed in specially-designed glass tubes. These tubes, shown in Figure 9, permitted removal of the fish from the test solution for live counting without handling. The tubes had an outside diameter of 16 mm, which was just small enough to permit them to be placed in the 17 mm diameter gamma counting well.
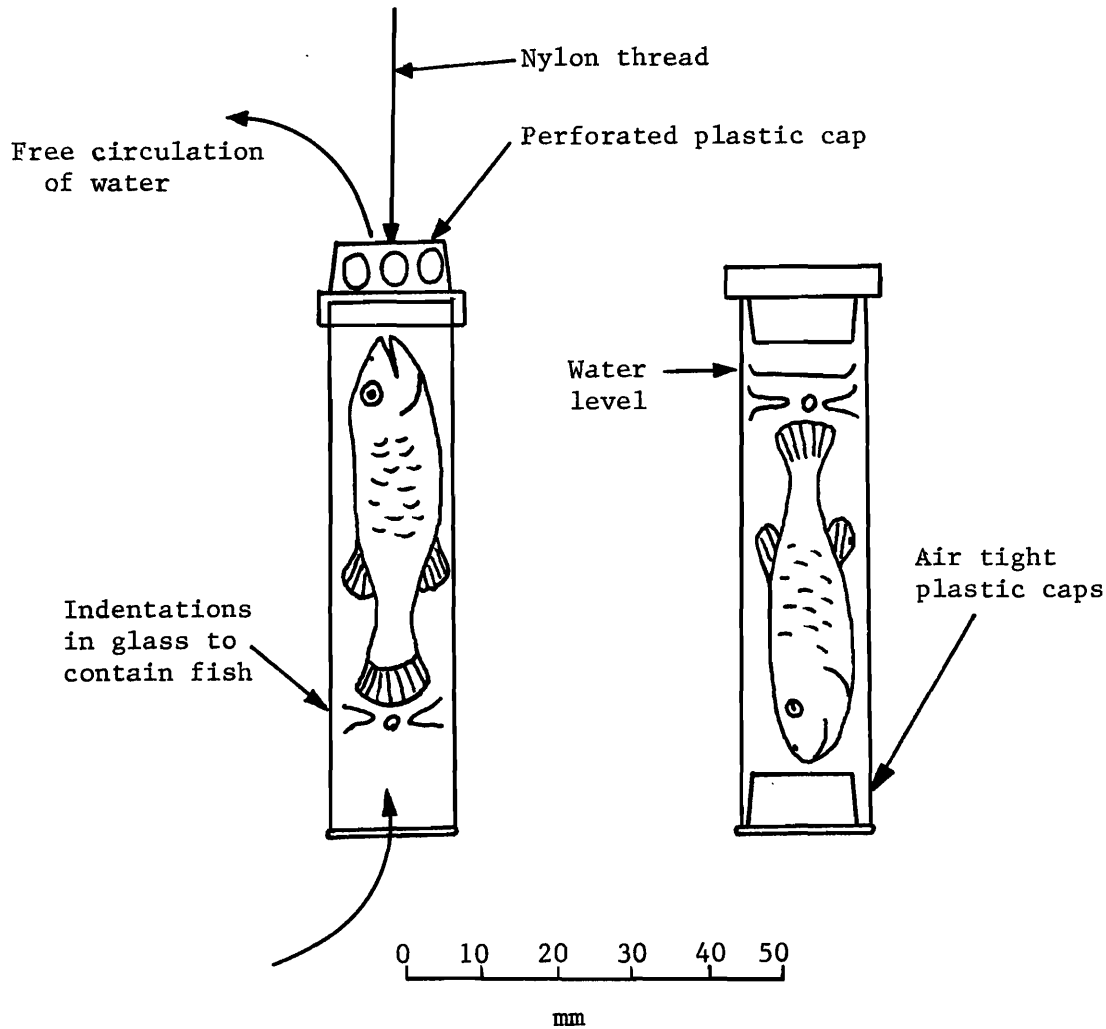
15

Figure 9. Apparatus used for dimethyl mercury uptake and loss studies involving fish.

When the fish were in dimethyl mercury solutions, special precautions were taken to minimize volatilization of dimethyl mercury. A stoppered vessel was used and filled to the point of smallest air-water interface. This apparatus is shown in Figure 10.

The procedure used to determine dimethyl mercury content was as follows: the rubber stopper was removed and the selected fish was extracted from the dimethyl mercury solution and dipped briefly in mercury free (tap) water. Then the perforated plastic cap was quickly removed and replaced with a watertight stopper. The whole assembly was then inverted and filled with mercury-free water, and another stopper was added to the "tail" end. The fish was then ready for radiation counting without fear of dimethyl mercury loss.

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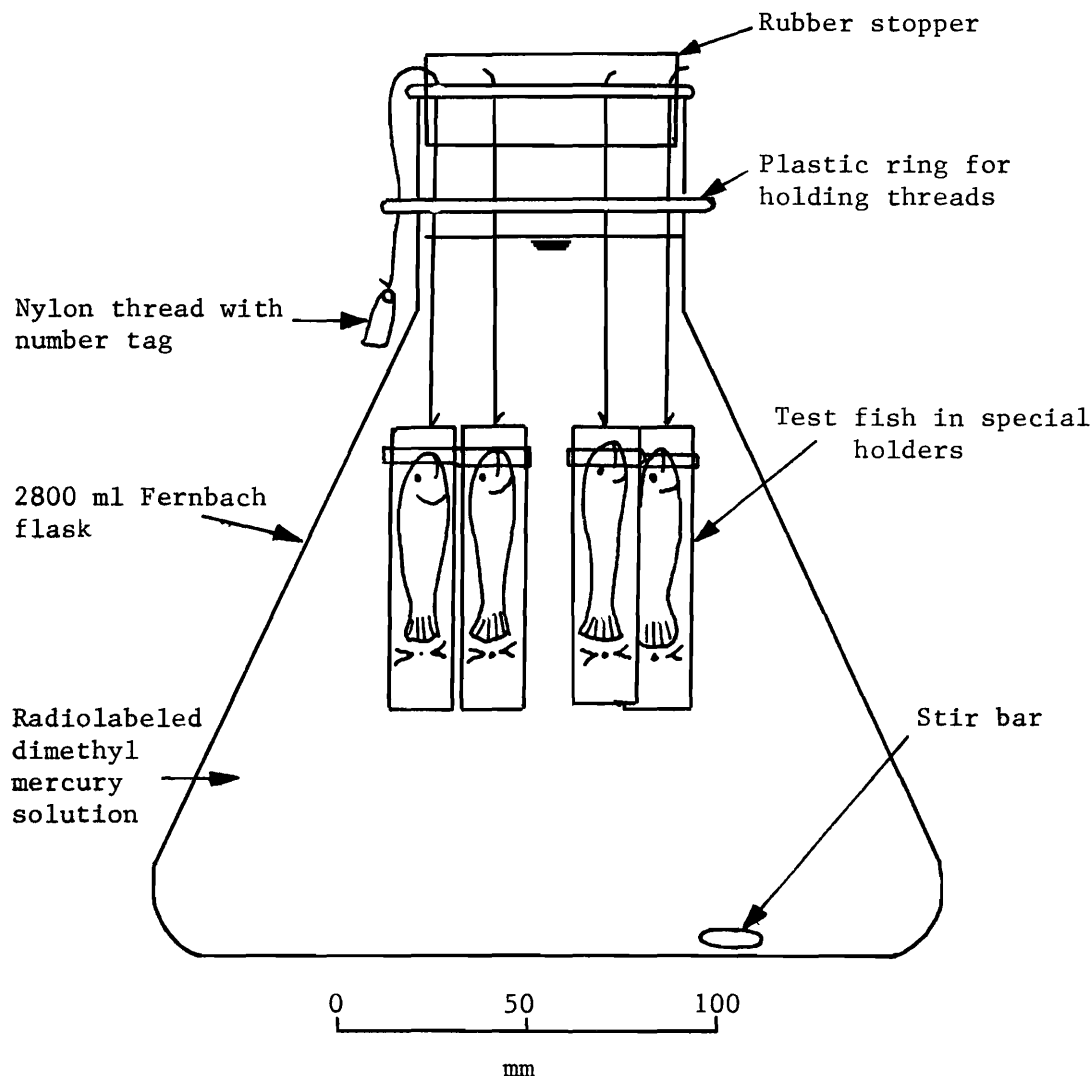
Figure 10. Apparatus used for dimethyl mercury uptake experiments.

With reference to Figure 9, the procedure outlined above consisted of going from configuration a) to configuration b). It was consistently possible to complete this sequence in less than 10 seconds. When counting was completed, the procedure was reversed to replace the fish in water. The test fish appeared little affected by this handling. In some experiments, test fish survived ten repetitions of this procedure. No mortality occurred in any of the runs reported.

A non-volatile mercury residue was present in all radiolabeled dimethyl mercury solutions. This radioactive contaminant showed no tendency to adsorb onto the glass tubes which held the fish, but did adsorb onto the plastic caps. This presented no counting problem because the caps which were on the tubes when in the dimethyl mercury solutions were replaced with other caps before counting.

To determine the effect of fish activity levels on dimethyl mercury loss kinetics, the apparatus shown in Figure 11 was used. In this apparatus, water was forced to pass from the left compartment to the right through the tubes past the fish. Thus, the velocity against which the fish were required to swim could be set by varying the pump speed. Fish were removed from this apparatus at defined intervals and prepared for counting as described above.

Daphnia. The apparatus used for *Daphnia* studies was similar to that used for the fish. The animals were placed in containers consisting of 16 mm OD glass tubing 3 cm in length with nylon netting in each end. The netting was held in place with plastic rings made by cutting the ends out of stoppers. This apparatus could accommodate at least 60 free swimming *Daphnia.* The container used is shown in Figure 12. Three such assemblies, each con-

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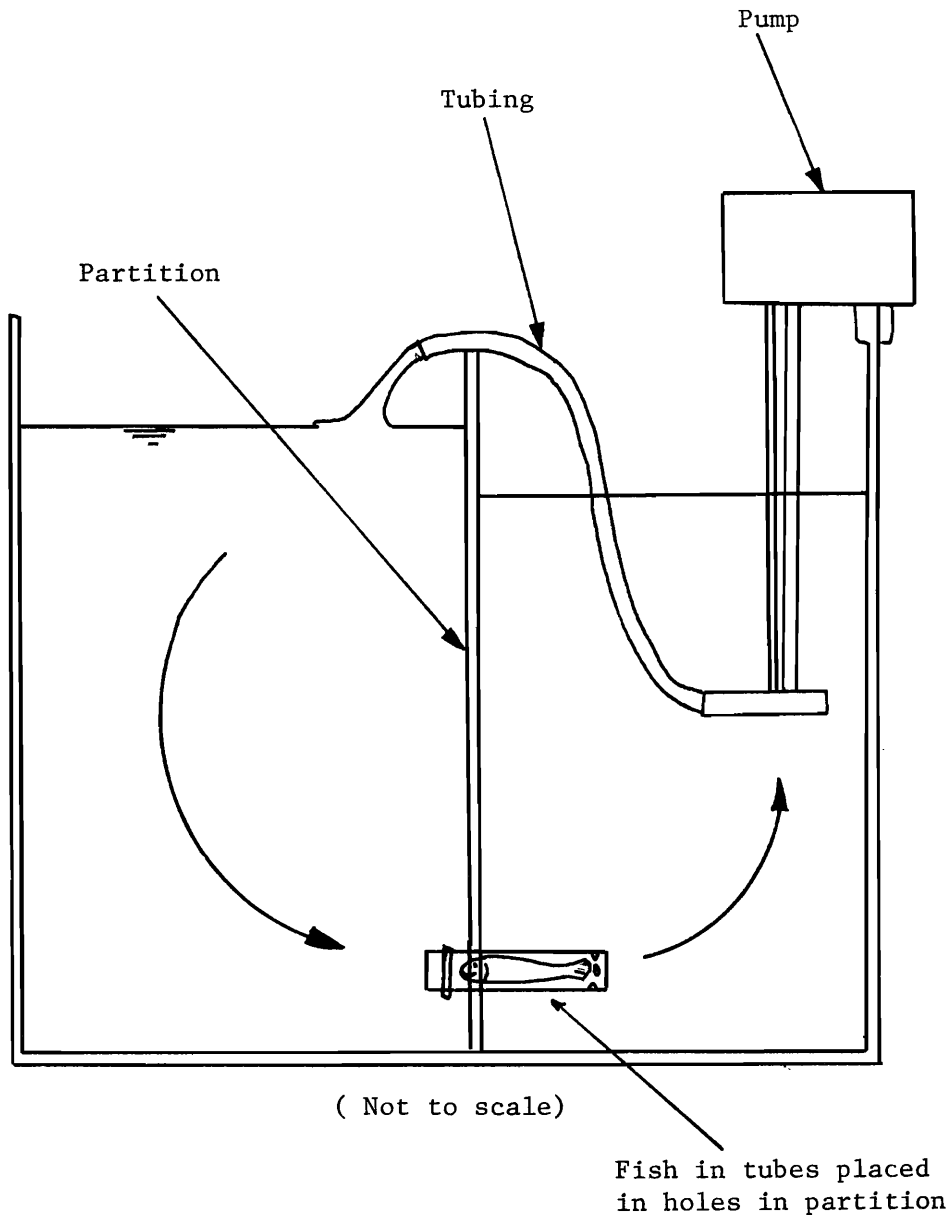
Figure 11. Apparatus used to vary velocity of rinse water past test fish.

taining about 30 to 60 organisms, were used in these experiments.

Daphnia containers were placed in a dimethyl mercury solution contained in a stoppered one liter erlenmeyer flask. The apparatus used was similar to that shown in Figure 10 for fish studies. Despite the stopper, dimethyl mercury volatilization from the system did occur. In nine hours of experiments, about half the initial concentration was lost for the runs reported here. Samples of the water were taken periodically to allow appropriate corrections to be applied when calculating concentration factors.

When removing Daphnia from dimethyl mercury solutions for radiation counting, the procedure followed was similar to that used for fish; the apparatus was removed from the test solution, rinsed briefly, and the netting and rings were replaced with solid stoppers. Water was added to allow the organisms to swim freely during counting. This permitted uniform counting geometry to be maintained and kept the Daphnia healthy. No mortality occurred during the runs reported here.

The presence of non-volatile mercury residues in test solutions made it necessary to measure two values in determining dimethyl mercury uptake: first the tubes

Free circulation of
water
through netting

Nylon thread

Plastic ring

Nylon netting

Daphnia

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0    5    10    15
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Scale in millimeters.
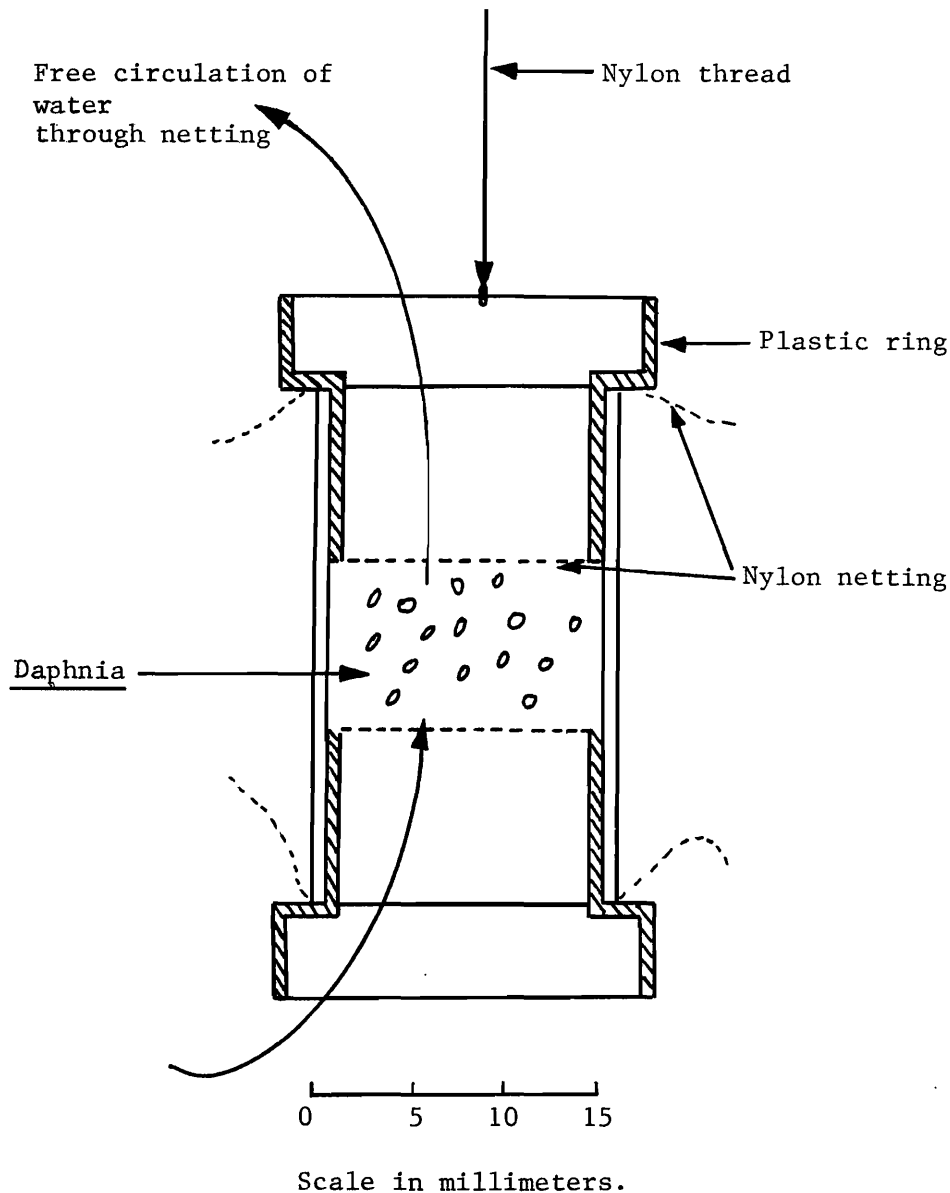
Figure 12. Cross-section of the apparatus used to contain *Daphnia* during dimethyl mercury uptake experiments.

containing *Daphnia* were counted immediately upon removal from the dimethyl mercury test solution; and then they were counted again after the dimethyl mercury was lost during a rinse in plain water. Dimethyl mercury uptake was taken as the difference between the two counts. For runs at $10°$ and $20°$ this procedure was repeated twice; at $30°$ only a single determination was made for each *Daphnia* container.

A water bath was used to control temperature in both the dimethyl mercury test solution and in the rinse bath. At the end of each series of runs, *Daphnia* were remove from the test apparatus and placed in aluminum

cups. After drying overnight in a $100°$ oven, the organisms were weighed and the number of individuals counted.

Concentration factors

Equilibrium uptake data is most meaningfully presented in the form of the concentration factor, defined as:

Concentration factor =

$$\frac{\text{dimethyl mercury concentration in the organism, cpm/g}}{\text{dimethyl mercury concentration in water, cpm/g}}$$

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The concentration factor is a dimensionless parameter which includes both the size of the organism and the concentration of dimethyl mercury in the water (Polikarpov, 1966).

An important underlying assumption in the use of this concept is that the concentration factor is independent of concentration, at least within the relevant range of concentrations. In other words, if this concept is to have any general validity, it must be assumed that doubling the concentration of dimethyl mercury in water will result in a doubling of the concentration in the organism. This concept is expressed graphically in Figure 13 for an idealized case.

As shown in Figure 13, at sufficiently low concentrations the concentration factor, shown as the slope of the uptake line, is in fact a constant. For case 1 the uptake line continues upward at the same slope, but in case 2 the curve flattens out, indicating that the organism has become in effect "saturated." This may be due to literal physical saturation of adsorption sites, or to a biological mechanism in which the elimination rate equals the absorption rate (Rescigno and Segre, 1966).

Given low dimethyl mercury concentrations likely to be present in nature. it is clear that the lower, linear part of the uptake curve is the region of interest. Therefore, in experiments using biota the lowest practical
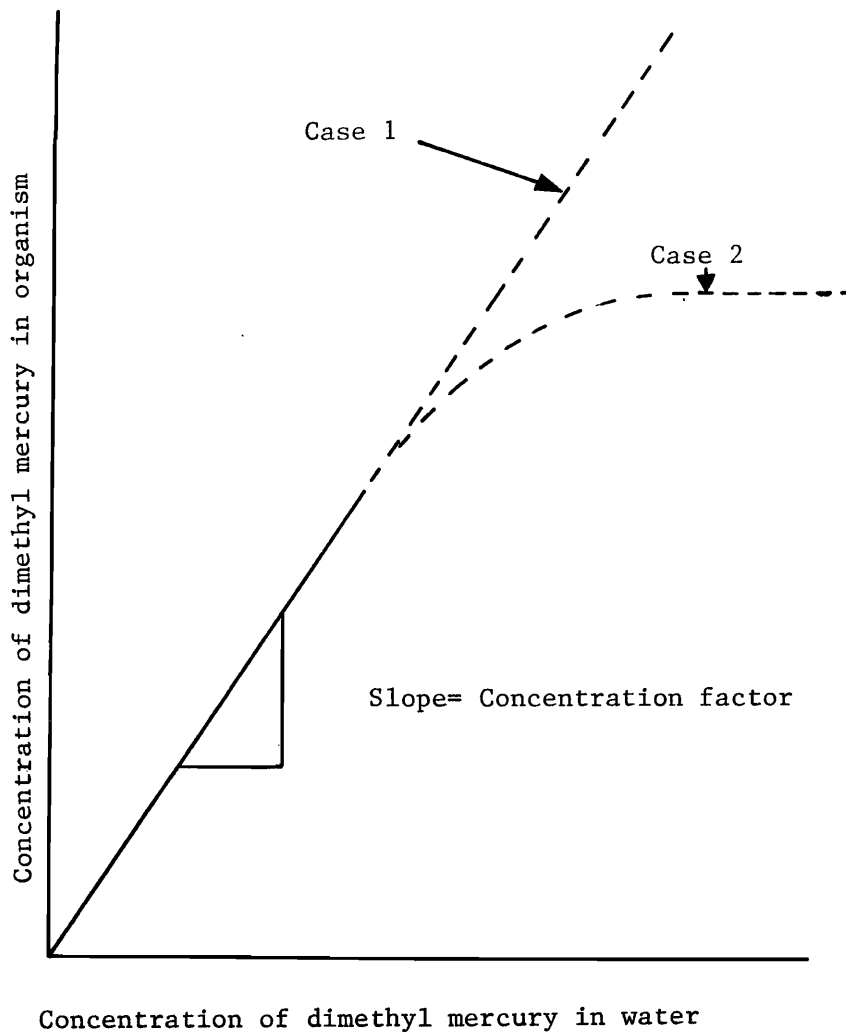
Figure 13. Illustration of the concentration factor concept.

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dimethyl mercury concentrations were employed; this was always less that 0.5 mg/l.

Much lower concentrations were theoretically possible, but the following factors prevented their use. 1) The specific activity of the labeled dimethyl mercury was lowered by adding unlabeled dimethyl mercury. This was done to minimize radiolysis. 2) The high uptake of even small amounts of non-volatile radiomercury in water made it imperative that comparably high dimethyl mercury uptake occur. This required high aqueous concentrations. 3) The live counting techniques used limited counting time to one or two minutes. Therefore, to obtain acceptable counting statistics relatively "hot" labeled dimethyl mercury solutions were necessary.

To test whether a concentration factor obtained at a given aqueous dimethyl mercury concentration falls on the linear part of the uptake curve, it was only necessary to repeat the experiment under the same conditions at another concentration, either higher or lower. If the same answer was obtained, then it may be concluded that both concentrations fall in the linear range. This test was applied to both fish and *Daphnia* runs. As will be shown, results were positive.

Results and Discussion

Fish studies

Of the three organisms used in these studies fish were studied in the greatest detail. This was because only the fish could be studied on an individual basis, and because only the fish had dimethyl mercury uptake and loss rates slow enough to measure. As will be shown, there was considerable variation between individual fish in both equilibrium and kinetic experiments. This made the live counting technique especially advantageous, because the same individual fish could be run under a variety of test conditions. This obviated the need for running a large number of fish in order to get statistically valid averages.

Equilibrium studies. In order to ascertain whether the aqueous dimethyl mercury solution of about 0.4 mg/l was on the linear part of the uptake curve, concentration factors were determined for four fish at 23 °C. Then the concentration of dimethyl mercury was approximately tripled by adding unlabeled dimethyl mercury to the test solution. The same fish were then run again. Results are summarized in Table 6.

Results of this experiment indicate that for concentrations of dimethyl mercury below at least 1.3 mg/l, concentration factors are independent of concentration. Although results for individual fish at the higher concentrations fell slightly above or below earlier results, the mean concentration factors fell within 5 percent of one another.

Table 6. Dimethyl mercury concentration factors for fish at two concentrations of dimethyl mercury in water.

Fish	Approximate me_2Hg concentration, mg/l	
	0.4 Concentration factor	1.3 Concentration factor
1	6.1	6.1
2	4.6	4.0
3	4.6	4.0
4	8.6	8.7
Mean	6.0	5.7

To assess the effect of temperature on equilibrium uptake of dimethyl mercury, four fish were run at three temperatures on the same day. The temperatures used were $18.2°$, $23.2°$, and $27.0°C$. This range was selected as one in which the fish would survive without mortality in the absence of acclimatization. Acclimatization was not possible because the two week delay which it would necessitate between runs could lead to physiological changes in fish, and results would not be strictly comparable. Results of these experiments are summarized in Table 7.

Table 7. Dimethyl mercury concentration factors for fish at different temperatures.

Fish	Temperature, °C			Mean for all temperatures
	18.2	23.2	27.0	
1	5.9	6.1	5.9	6.0
2	3.9	4.0	4.3	4.1
3	4.0	4.1	4.5	4.2
4	7.9	8.7	8.5	8.4
Mean	5.4	5.7	5.8	5.6

As shown in Table 7, equilibrium concentration factors for dimethyl mercury in fish are insensitive to temperature, although a slight upward trend is indicated with higher temperatures. Differences in mean concentration factors at the three temperatures were not statistically significant. These results indicate that in nature, temperature is not a significant factor affecting dimethyl mercury distribution.

The concentration factors presented above show wide variations from one fish to another. This pattern of variation was continued in uptake experiments for a total of 13 test fish. This variation could not be attributed to experimental error, because it was possible to reproduce results from any given fish, as Tables 6 and 7 indicate.

The non-polar nature of dimethyl mercury suggested that concentration factors might correlate with lipid content. This hypothesis was tested by plotting concentration factor against lipid content. This plot is shown as Figure 14, together with the least-squares line of best fit.

The correlation coefficient for this relationship was 0.88, indicating that lipid concentration is indeed the significant variable affecting equilibrium dimethyl mercury uptake. This indicates partitioning of dimethyl mercury into lipid compartments, a finding which is in agreement with the results of Ostlund's (1969) experiments with mice.

From a physiological standpoint, lipids may be considered an inert compartment of fish and dimethyl mercury stored in lipids would be essentially unavailable for metabolic processes.

The intercept of the regression line was 2.52, corresponding to the concentration factor resulting from dimethyl mercury in non-lipid compartments. Of this 2.52 factor, about 36 percent may be explained simply in terms of the water content of the fish.

The slope of the line may be interpreted as a lipid-water partition coefficient for dimethyl mercury of 224. This is not unreasonable in terms of an octyl alcohol-water partition coefficient of 170 determined in the laboratory. Octyl alcohol is commonly used to mimic natural lipids, but partition coefficients would not necessarily be identical (Street, 1973).

Local data indicate that large variations in lipid content from one fish to another within a given species are not uncommon (Street, 1973). In the case of the fathead minnows used, differences may have been due to the presence of eggs, which may contain significant amounts of lipid (Brown, 1957).

In summary, equilibrium concentration factors for dimethyl mercury in fathead minnows were found to correlate with lipid content according to the relation:

Concentration factor = 2.52 + 224 (fraction lipid).

This relation may be a useful means for estimating concentration factors in other species of fish.
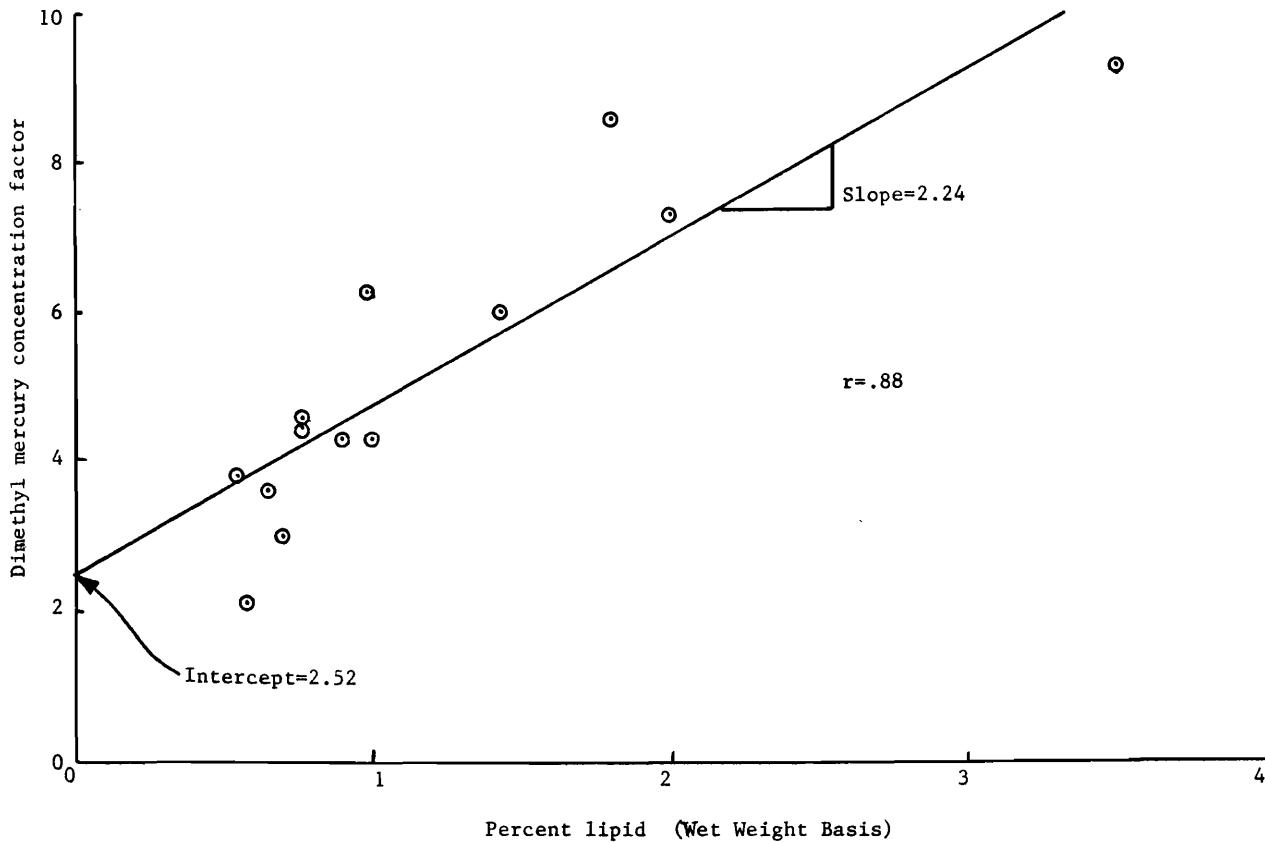
Figure 14. Concentration factors as a function of lipid concentration in fish.

Tests on about ten fish selected at random showed that the dry weights fell in the range of 10 to 15 percent of wet weights. This means that concentration factors determined on a wet weight basis would be about 7 to 10 times higher by dry weight. Therefore dimethyl mercury concentration factors for fathead minnows may be estimated to fall in the range of 30 to 90 on a dry weight basis. This conversion was made to allow comparison with concentration factors found for Daphnia and algae on a dry weight basis.

Kinetic studies. The first series of kinetic studies were run to compare uptake rates with those of elimination. Four fish were placed in a dimethyl mercury solution for 10 minutes and then counted. Then they were replaced in the dimethyl mercury solution and allowed to equilibrate for two hours. At the end of this time the fish were counted, placed in a rinse bath for 10 minutes, and then counted again. Results are summarized in Table 8.

Table 8. Comparison of dimethyl mercury absorption and elimination rates in fathead minnows.

| Fish | Percent of equilibrium uptake transferred to 10 minutes | |
	Absorption	Elimination
1	61.0	60.7
2	71.8	85.6
3	84.6	89.2
4	51.6	45.6
Mean	67.3	70.3

The results summarized in Table 7 show that absorption and elimination rates of dimethyl mercury from fish were essentially identical. Moreover, it was clear that equilibrium was approached in a matter of minutes. These findings suggest that uptake and elimination were governed by the same mechanism, and that this mechanism was extremely rapid. A non-biological physico-chemical mass transfer process was strongly indicated.

From an experimental point of view, the identity of uptake and elimination rates meant that it was necessary to measure only one or the other. Elimination rates were by far the easiest to measure, and all succeeding kinetic studies were conducted on this basis.

To test the hypothesis that the mass transfer of dimethyl mercury occurs by a mechanism independent of the activity level of the fish, the apparatus shown in Figure 11 was used. Four fish were removed from a dimethyl mercury solution, counted for radioactivity, and placed in the rinse apparatus. The velocity against which the fish had to swim was about 15 cm/sec. The fish were

recounted about every 10 minutes to define the elimination curves. The experiment was then repeated using the same fish but the rinse water was merely stirred. Results of both experiments are shown in Figure 15. Fishes 2 and 3 had similar elimination curves, and hence the latter was not plotted.

As shown in Figure 15, elimination rates were similar for both types of rinse systems, and no consistent differences existed between them. This indicates that dimethyl mercury elimination is independent of the activity level of the fish, and supports the thesis that uptake and elimination occur by non-biological mechanisms.

The data shown in Figure 15 suggested a bimodal elimination model. Rapid loss occurred in the first 10 minutes; after this all fish seemed to roughly follow a first-order elimination model with very similar rate constants This pattern held for all eight fish tested at 23°C.

To further investigate this, elimination data for the eight fish were applied to computer program which calculated a first-order rate constant for all data points except the first, and gave the initial amount of rapid loss by extrapolating this line backwards to the t = 0 intercept.

Results are summarized in Table 9.

Table 9. Results of regression analysis for dimethyl mercury elimination from fish.

Fish	Initial loss, c.f. units	First-order rate constant, min[-1]	Correlation Coefficient r
1	1.43	-0.0576	-0.995
2[a]	2.28	-0.0557	-1.00[a]
3[a]	3.00	-0.0546	-1.00[a]
4	1.39	-0.0447	-0.999
5	2.31	-0.0419	-0.992
6	2.72	-0.0283	-0.977
7	3.37	-0.0352	-0.929
8	1.42	-0.0382	-0.995
Mean	2.24	-0.0445	-

Degrees of freedom = 7,6 F = 1,130, not significant at P > 0.75

[a]Final concentrations fell below 5 percent of original value; at this level the counting error was deemed too high to allow use of these data points in this analysis.

The statistical analysis shown in Table 9 indicates that differences in first-order rate constants for the eight fish were not significant, even at the 0.25 level. The initial losses of dimethyl mercury from fish fell within a fairly narrow range of 1.4 to 3.4 concentration units. The mean
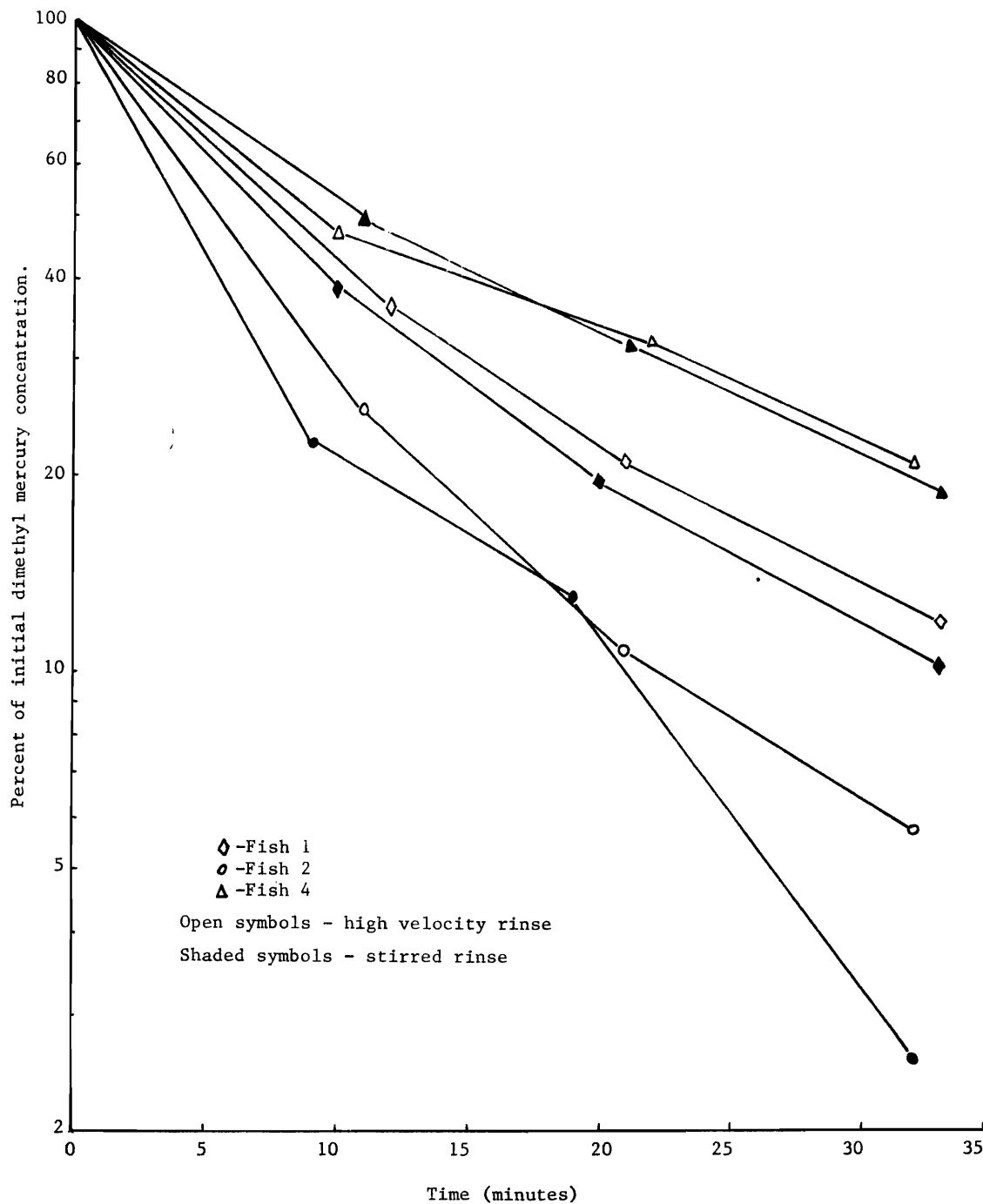
Figure 15. Dimethyl mercury elimination from fish for two types of rinse systems.

value of 2.24 is comparable to the 2.52 concentration factor which was the portion of equilibrium dimethyl mercury uptake found to be in non-lipid compartments. This suggests that the initial rapid dimethyl mercury loss is due to depletion of non-lipid compartments, and that the slower first-order loss which occurs afterwards represents loss from lipid compartments.

The model which this implies may be expressed mathematically as:

$$C = (C_o - 2.52) e^{-kt}$$

where C and C_o represent mercury concentrations in concentration factor units at times t and $t = 0$ respectively.

To test this model, kinetic data for all fish were normalized by subtracting 2.52 concentration factor units from each data point and dividing data for each fish by the adjusted equilibrium uptake. In other words data were normalized to the form:

$$\frac{C - 2.52}{C_o - 2.52}$$

A plot of these values against time is shown in Figure 16, together with a least-squares line of best fit.

If the proposed model for dimethyl mercury loss from fish is valid, two criteria should be met in the normalized plot; the intercept of the line of best fit should be close to 1.0, and the correlation coefficient should approach -1. The first criterion is met fairly well, with an intercept of 0.92. The correlation coefficient was found to be 0.84, reflecting considerable data scatter, but adequate for the purposes of a rough empirical model intended to allow prediction of dimethyl mercury kinetics in fish other than those tested.

The value of the rate constant was calculated to be 0.037 min⁻¹, corresponding to a first-order half-life of about 19 minutes. This is in good agreement with the mean first-order rate constant of 0.045 min⁻¹ shown in Table 9.

In summary, the model developed for dimethyl mercury elimination from fathead minnows may be written as follows:

$$C = (C_o - 2.52)e^{-0.037t}$$

where C and C_o are concentration factors at times t and at equilibrium respectively, and t is measured in minutes.
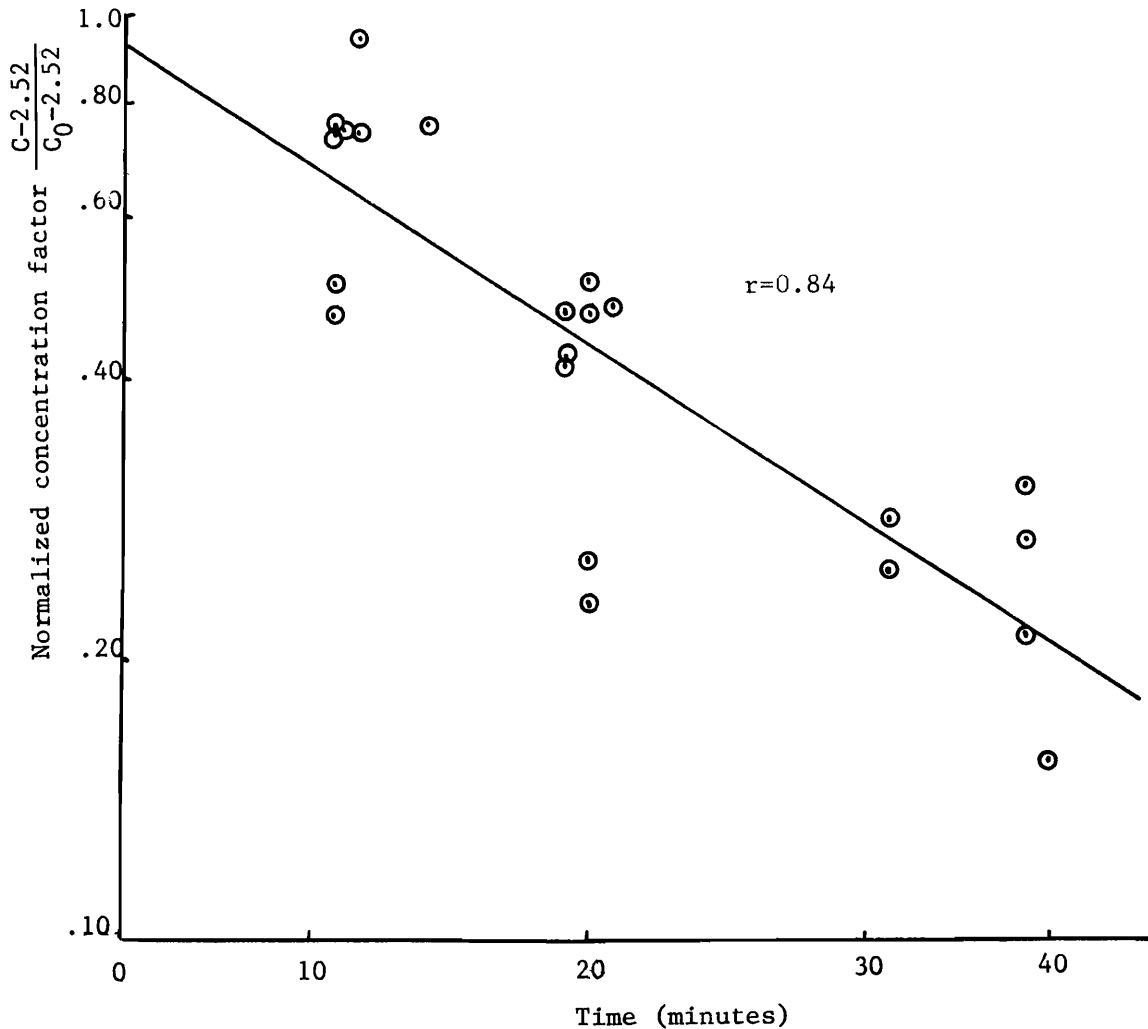
Figure 16. Plot of $\frac{C-2.52}{C_0-2.52}$ versus time.

This model may be useful in predicting dimethyl mercury kinetics in other species of fish, although the rapidity of the process indicates that practically speaking, fish are likely to be continuously in equilibrium with aqueous dimethyl mercury concentrations. This also suggests that food chains are not important in dimethyl mercury uptake.

Toxicity of dimethyl mercury to fish. The measurement of the acute toxicity of dimethyl mercury to fathead minnows was complicated by the need to keep the test fish in sealed containers to prevent dimethyl mercury loss. As a result oxygen depletion-induced mortality began to occur after about 4 hours in both controls and dimethyl mercury solutions, and tests were terminated.

To summarize fragmentary results, no dimethyl mercury toxicity to fish could be demonstrated. At 23°C, five fish were found to survive 100 mg/l dimethyl mercury for four hours; since they had attained equilibrium with the concentration in the water within the first hour, this result suggests that further exposure would not likely result in greater mortality.

Although the above results are inadequate to define a TL_m value for dimethyl mercury, they do at least indicate that it is not likely that dimethyl mercury exerts any significant toxicity to biota in natural waters.

Daphnia studies

Daphnia studies consisted of experiments to determine equilibrium dimethyl mercury concentration factors at three temperatures: 10°, 20°, and 30°C. Results of these runs are presented in Table 10.

Table 10. Summary of dimethyl mercury uptake in *Daphnia magna* **at three temperatures.**

Variable	Temperature, °C		
	10	20	30
Concentration factor, dry wt. basis	69	75	69
Standard error for concentration factor	19	4.7	13
Number of replications	6	6	3

As was the case for fish, concentration factors for *Daphnia* were similar at all three temperatures, and differences were not found to be statistically significant. *Daphnia* concentration factors fell well within the range of estimated dry weight concentration factors for fish, and a similarity of concentration factors for all aquatic animals is suggested. As will be shown in the following chapter, algal concentration factors also fall in this range.

Uptake and loss rates were not determined for *Daphnia.* Surface area to volume ratios for *Daphnia* were estimated to be at least an order of magnitude higher than those of the fish tested, suggesting the *Daphnia* equilibrate with aqueous dimethyl mercury concentrations within 5 minutes or less. This was confirmed in trial runs. In equilibrium uptake experiments, *Daphnia* were given at least 30 minutes exposure to test solutions before measurement.

The rapidity of dimethyl mercury uptake by *Daphnia* suggests that natural populations are always in equilibrium with aqueous dimethyl mercury concentrations. Another consequence of this rapid uptake is that the food chain is not a significant mechanism of uptake in *Daphnia.*

The high standard errors shown in Table 10 for concentration factors call for special comment. As noted above, no statistically significant difference was found for results at different temperatures. Thus it was possible to treat all 15 replicates as samples from the same statistical population. On this basis the mean concentration factor was 72, with a coefficient of variation of 18 percent.

An explanation for this high coefficient of variation may be found in radiation counting error. *Daphnia* samples were counted immediately upon removal from dimethyl mercury solutions and once again after a 30 minute rinse bath. Dimethyl mercury uptake was based on the difference between these two counts. The counts were high relative to their difference; a typical initial count was around 5000 cpm, with a final count only about 700 cpm lower.

It was possible to estimate the expected size of the counting error of this difference based on the theoretical error of an individual count (Division of Radiological Health, 1960) and on the relation

$$S_D = (S_A^2 + S_B^2)^{\frac{1}{2}}$$

where S_D is the error of the difference between two counts A and B having standard errors S_A and S_B respectively (Snedecor and Cochran, 1967).

The mean estimated error of these differences for 15 replicates was calculated to be 107.2. This estimated error was applied to the mean value of the differences to yield a coefficient of variation due to counting error of 14.7 percent. In other words, counting error alone would be expected to give a coefficient of variation for results of 14.7 percent. Since the actual coefficient of variation for concentration factors was only 18 percent, it appears that counting error was the major source of overall error in *Daphnia* uptake experiments.

Summary

Equilibrium uptake of dimethyl mercury in biota is best described in terms of the concentration factor. defined as the ratio of dimethyl mercury concentration in the organism to that in the surrounding water For fathead minnows, equilibrium concentration factors fell in the range of 3 to 9 on a wet weight basis They were found to correlate with lipid content according to the relationship:

Concentration factor = 2.52 + 224 (fraction lipid)

Equilibrium concentration factors were insensitive to temperature over the range of 18°C to 27°C.

Rates of uptake and loss of dimethyl mercury from fish were identical, and unaffected by the activity level of the fish For an empirical first-order model. the half time of equilibration was about 19 minutes. These results suggest a non-biological uptake mechanism is involved. The speed of the process indicates that practically speaking, fish are likely to be continuously in equilibrium with aqueous dimethyl mercury concentrations. This also suggests that food chains are not important in uptake or loss kinetics.

Equilibrium concentration factors for *Daphnia* were about 70 on a dry weight basis. This is within the range of values found for fish, expressed on the same basis. Temperature had no significant effect over the range of 10°C to 30°C

Rates of dimethyl mercury transfer to or from *Daphnia* were too fast to permit meaningful measurement. Surface area to volume considerations applied to kinetic data for fish suggest that the half time of equilibration is 2 minutes or less.

ALGAE STUDIES

Experimental Procedures

The purposes of studies involving algae were to determine the equilibrium uptake of dimethyl mercury and to define at least roughly the kinetics of uptake and loss.

Algal cultures

As a representative of the lowest trophic level, the green alga *Selenastrum capricornutum* was selected This organism is widely found in oligotrophic waters, and may be taken as a typical primary producer From an experimental standpoint, *S. capricornutum* was a convenient organism in that it is easy to culture under laboratory conditions. In typical cultures the cells do not attach to glass surfaces or flocculate, and do not excrete toxic substances. *S. capricornutum* has been found to grow well in NAAM medium an inorganic medium of composition similar to many natural waters. (See Appendix A.)

The algae were cultured on a semicontinuous basis, using a six-day residence time. The culture was maintained in an 18 l glass carboy filled to the 12 l mark. One-third of the culture was withdrawn every other day, and replaced with NAAM medium made up fresh from concentrate solutions. This provided 4 l of algal suspension every other day

The algae thus produced were not bacteria free, but the inorganic medium used effectively held the bacteria to low levels. The suspension thus produced had an algal concentration of about 40 mg/l dry weight. It was necessary to use algal concentrations about an order of magnitude higher in order to get acceptable results when measuring uptake Centrifuging was used to accomplish this concentration process. Algal concentrations were determined as total suspended solids (American Public Health Association, 1971).

Measurement of dimethyl mercury in algae

The measurement of dimethyl mercury concentration in algae was found to be impossible using conventional methods because of the volatility of the compound, and a new method was developed. The method was as follows: an algal suspension containing dimethyl mercury was added to a 12 ml glass centrifuge tube. A screw cap was added to prevent volatilization and

the tube was placed near the gamma detector such that only the tapered tip of the tube was actually in the well. After counting gamma emissions in this configuration the tube was placed in a centrifuge and the algal cells were concentrated into a pellet at the tip of the centrifuge tube. The tube was then recounted in the same position as previously. The radio-activity in the water gives the same count as before, but the activity in the cells gives a higher count after centrifuging because they were concentrated into a portion of the centrifuge tube wherein a greater percentage of the emissions was counted. The ratio of the counts after to counts before centrifuging was the basis for determining the portion of the dimethyl mercury concentrated in the cells.

In mathematical terms this method may be described as follows. Before centrifuging, the total count may be expressed as

$$C_B = C_W + C_C$$

where C_B is the count before centrifuging and C_W and C_C are counts attributable to dimethyl mercury in water and algal cells respectively

After centrifuging, the count from the water is unchanged, but the count from the algal cells is multiplied by some factor due to their more favorable geometrical orientation:

$$C_A = C_W + GC_C$$

where C_A is the count after centrifuging and G is the geometry factor: G is defined as the ratio of the count due to a substance concentrated at the tip of tube divided by the count due to the same amount of radioactivity dispersed uniformly throughout the tube. G may be readily determined experimentally.

If R is defined as the ratio of counts after centrifuging to counts before, then,

$$R = \frac{C_A}{C_B} = \frac{C_W + GC_C}{C_W + C_C}$$

Solving for the ratio of the activity in the algal cells to that in the water

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$$\frac{C_C}{C_W} = \frac{R - 1}{G - R}$$

A more meaningful measure of algal uptake is the concentration factor, previously defined as the ratio of dimethyl mercury per gram of cells to dimethyl mercury per gram of water (Polikarpov, 1966). This is readily obtained by dividing the ratio C_C/C_W by the concentration of algal cells in suspension. If the cell concentration is expressed as mg/l,

$$c.f. = \frac{R - 1}{G - R} \times \frac{10^6}{mg/l\ algae} \quad \cdots \cdots \quad (2)$$

where the constant 10^6 is required to convert mg/l to a weight ratio.

The experimental apparatus associated with this method is shown as Figure 17. The lead shield shown was designed especially to accommodate the centrifuge tubes used. It had the effect of increasing the geometry factor G, and served to position the centrifuge tubes precisely during counting.

The method developed for algal uptake required that the correct temperature be maintained at three locations; in the gamma radiation detector, in the centrifuge, and in the place where the centrifuge tubes were stored between steps of the procedure. For runs at 10° and 23°, it was possible to manipulate room temperature to get the desired reading.

For the 30° run, temperature in the counting well was adjusted by placing a plastic cylinder over the entire detector and adjusting heat input by varying the position of a light bulb. Temperature in the centrifuge was controlled by assemblying from plywood a small alcove in which to place the unit, and regulating temperature with a Bunsen burner. Despite their appearances these setups provided good temperature control. A water bath was used to maintain the temperature of the centrifuge tubes in between counting and centrifuging steps.

Metabolism of dimethyl mercury

The presence of non-volatile mercury residues in all experiments in which radiolabeled dimethyl mercury was added to water indicated that this residue might be the result of conversions occurring while the experiments
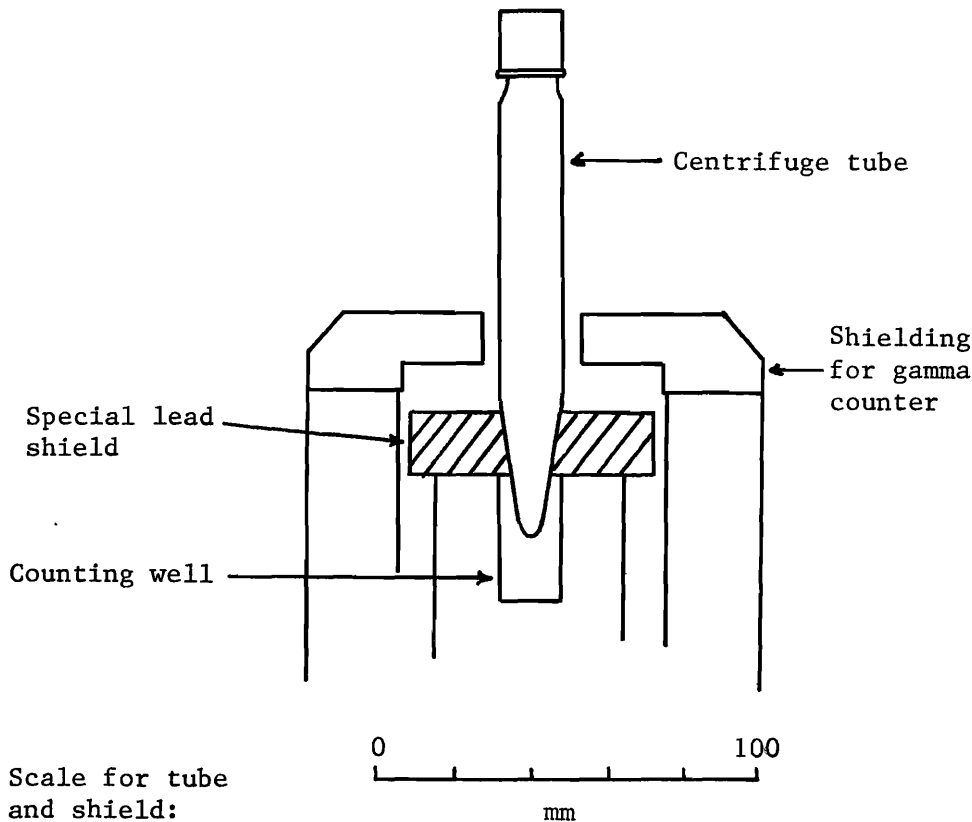
Figure 17. Experimental apparatus for measuring dimethyl mercury concentration in algae.

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were in progress, either because of radiolysis, or because of microbial degradation.

To test this hypothesis, 17 ml culture tubes were filled with dimethyl mercury solutions and incubated in the light at 24°C. The dimethyl mercury was contained in three series of tubes; one series contained distilled water, another contained bacterized dechlorinated tap water taken from tanks containing fish, and the third series contained a bacterized *S. capricornutum* suspension at about a 40 mg/l concentration. There were eight tubes in each series, for a total of 24.

Each series of tubes was filled from a common flask containing purified dimethyl mercury stock in the desired aqueous medium. Thus all the tubes in each series initially contained the same percent non-volatile mercury, although this percentage was not necessarily the same in all the three series.

To test for conversion of dimethyl mercury to non-volatile forms, the following procedure was followed at regular intervals: two tubes from each series were counted in the gamma counter, after which they were opened and the contents transferred to 100 ml beakers to allow the dimethyl mercury to volatilize over night under the hood.

The following day the contents of each beaker were carefully and completely transferred back into the original tubes and recounted. Counting of empty beakers confirmed that adsorption to the glass walls of the beakers was not occurring. The non-volatile activity was expressed as a percentage of the previous day's count. An increase in this percentage was taken as an indication of dimethyl mercury conversion.

Results and Discussion

Uptake of dimethyl mercury by algae

The method described for dimethyl mercury content in algae was applied to experiments using *S. capricornutum* at three temperatures: 10°C, 23°C, and 30°C. In all experiments the mercury concentration was about 0.4 mg/l. Results are summarized in Table 11.

Table 11. Summary of results of algal uptake experiments for dimethyl mercury.

Result	Temperature, °C		
	10	23	30
Concentration factor, dry wt. basis	53	54	54
Standard error of concentration factor	2.0	2.3	4.8
Number of replicates	5	3	5
Algal concentration, mg/l	400.5	321.4	471.8

These results show that the concentration factor for dimethyl mercury, defined as concentration of mercury in the cells divided by the concentration of mercury in the water, was virtually the same for all three temperatures. The concentration factor of about 54 implies that in nature the phytoplankton would contain a small portion of the dimethyl mercury in solution. For example, if a mixed algal population were present at a "bloom" concentration of 10 mg/l, and the mean concentration factor were 50, only 0.05 percent of the dimethyl mercury in the water would be concentrated in the algae.

This concentration factor for dimethyl mercury is low relative to other forms of mercury. Mercury in the ionic form has been reported to have concentration factors in a variety of fresh water algae of between 4100 and 7700 (Polikarpov, 1966), and monomethyl mercury produced by radiolysis in this study has been found to have a concentration factor in *S. capricornutum* of over 25,000.

There is good reason to think that somewhat different concentration factors might be obtained if other species of algae were tested, or even if *S. capricornutum* were grown under differing nutrient conditions or at a different mean residence time.

This expectation is based on the tendency of dimethyl mercury to partition itself into lipid compartments, combined with the observation that algal lipid content varies from species to species of algae (Fogg, 1965). Also, lipid content has been found to vary with conditions of growth, increasing with age and also under conditions of nitrogen limited growth (Fogg 1965).

A point made in discussing *Daphnia* data applies here also. When concentration factors are given on a dry weight basis the water content of the cell affects the answer. For example, if an algal cell were 80 percent water, the portion of the overall concentration factor due to the dimethyl mercury in the water in the cell would be 0.8/0.2 or 4. If the water content were 90 percent, the corresponding value would be 9. When the overall concentration factor is over 50 as in these experiments, this source of variation is not of major importance.

The method used for algal uptake measurement made the study of uptake kinetics impossible. However, kinetic data for dimethyl mercury uptake in fish strongly indicate that algae are able to equilibrate with a sudden change in dimethyl mercury concentration in a matter of seconds. These data also indicate that loss and uptake kinetics are identical, and occur via a non-metabolic mechanism.

Measurement of dimethyl mercury in algae

Measurement of dimethyl mercury content of algae was complicated by the volatility of the compound, by its relatively low uptake by algae, and by the presence of

mercury in forms other than dimethyl mercury. These factors precluded use of the two most common methods of measuring uptake of a radiolabeled compound. These methods are based on filtration and on centrifuging.

The filtration method involves passing an aliquot of algal suspension through a glass fiber filter, and then counting either gamma or beta emissions from the filter paper. This method could not be used because of the volatility of dimethyl mercury. In an attempt to overcome this problem, the filter paper was saturated with mercuric chloride in the hope that dimethyl mercury in algal cells would be "fixed" by instantaneous conversion to the monomethyl form.

This method was rejected because it was found that when blank dimethyl mercury solutions containing no algae were passed through the treated filter, a small fraction of the activity was retained on the filter paper, even after a distilled water rinse. This residue amounted to only about 1 percent of the radioactive mercury passing through the filter, but the exact amount varied by about + 1/2 percent. Since the percentage of mercury in the algal cells was only about 2 percent, this element of variability was too large to permit this method to be used.

Another approach tried was to measure the activity in the algal suspension, and then that of the supernatant after centrifuging. This method was ruled out when it was found that the two counts were virtually identical. The reason for this was made clear when it was found that even with an algal suspension as thick as 400 mg/l, only about 2 percent of the dimethyl mercury was in the cells. This meant that the supernatant count would theoretically be 98 percent of that for the suspension. This difference was too small to reliably measure, especially in view of the small losses of dimethyl mercury which occurred in handling samples.

The method finally developed for measuring dimethyl mercury uptake in algae proved to be virtually trouble-free. Since the entire procedure was conducted with the algae sealed in the centrifuge tube, volatilization was not a problem. Sensitivity was excellent. If a criterion were established requiring that the count after centrifuging must be at least 10 percent above the count before centrifuging, it may be shown that uptake as low as 0.5 percent by cells may be detected using the apparatus described. If the same 10 percent criterion were applied to the suspension/supernatant method developed for this study, sensitivity would be decreased by a factor of 20.

This new method is applicable to measuring the uptake of any gamma-emitting labeled compound into any suspended substance which can be separated out by centrifuging. As noted, the method's special advantages are its ability to detect low uptake and its applicability to volatile compounds.

The method has one important limitation: because at least 5 or 10 minutes elapse between the completion of

the first count and completion of the final count after centrifuging, the method cannot provide data on uptake for a discrete point in time, as can the filtration method. Thus this method is not applicable when kinetic data are needed for rapid uptake situations.

Metabolism of dimethyl mercury

Results of experiments to determine rates of dimethyl mercury conversion to non-volatile forms are summarized in Table 12, and shown graphically in Figure 18.

Table 12. Summary of results of dimethyl mercury conversion experiments.

| Days Incubation | Percent Residue | | |
	Dist. Water	Fish Water	Algal Susp.
0	5.05	9.65	9.49[a]
3	5.54[a]	-	10.34[a]
15	11.92[a]	8.77[a]	-
19	-	12.33	9.90
Rate of increase, %/day	0.458	0.141	0.0217

[a]Data for one replicate; other values are means of two replicates.

As shown in Table 12, the initial percent of non-volatile mercury varied somewhat as expected, depending on the aqueous medium used. The significant result in these experiments was the increase in this percentage with time. The distilled water series showed the highest rate of conversion, apparently due to radiolysis. This rate was less than one-half percent per day, and the conversion was even slower in tubes containing algal suspensions and water from the fish tank.

In view of the fact that each of the biological uptake experiments in this investigation had a duration of about 24 hours or less, the results of these experiments clearly refute the hypothesis that non-volatile mercury residues are due to radiolysis or microbial conversion occurring during the course of the experiment.

The most anamalous result of these experiments is that conversion occurred faster in tubes containing distilled water than in those containing "fish water" or algae; it would be expected that even if microbial conversion was not taking place that radiolysis would still proceed without impairment. One explanation is that the primary product of radiolysis occurring in water may be peroxides (Jimruska, 1972) capable of attacking the dimethyl
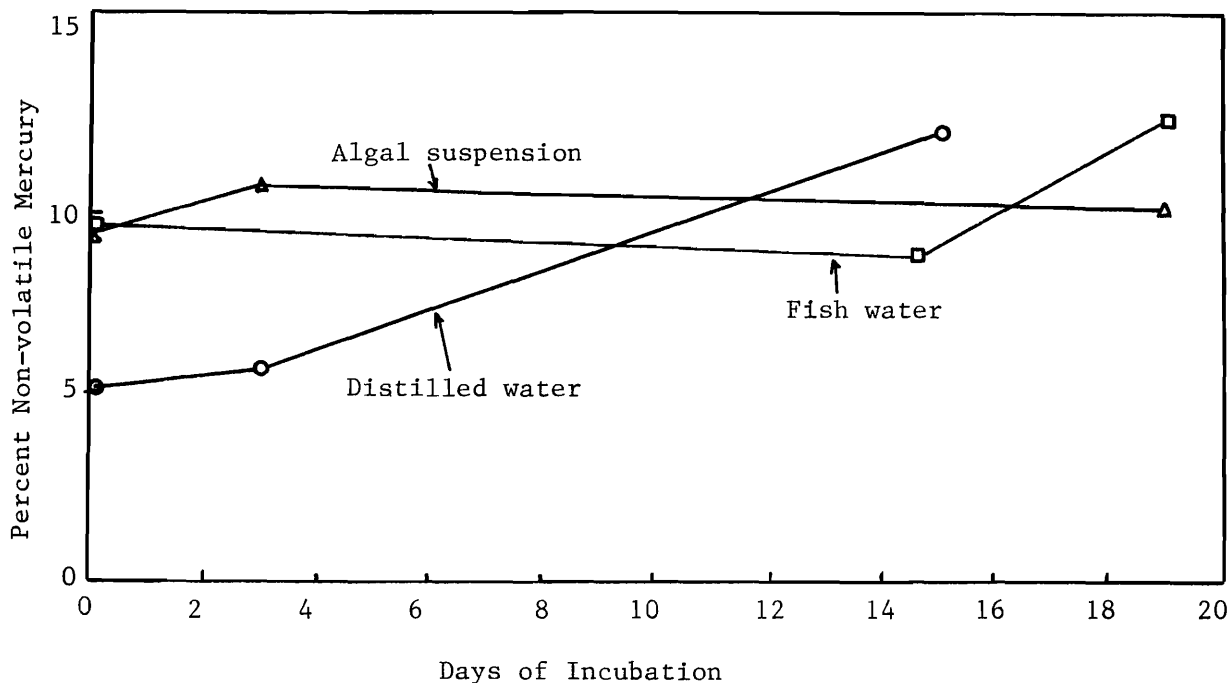
Figure 18. Conversion of dimethyl mercury to non-volatile forms with time.

mercury molecule, and that organic matter in the aqueous medium may serve as an alternative target.

These experiments were not intended to be at all definitive, but were aimed only at resolving a specific doubt. It seems likely that dimethyl mercury can in fact serve as a bacterial substrate, and the whole question of biological conversion of dimethyl mercury is worthy of further research. The initial metabolic product of such a conversion is likely to be monomethyl mercury (Ostlund, 1969), a compound far more toxic than its precursor. This suggests the interesting possibility that a natural selection process acts against organisms capable of effecting such a conversion.

Summary

The equilibrium concentration factor for dimethyl mercury in the green alga *Selenastrum capricornutum* was found to be about 54 on a dry weight basis. Temperature had no significant effect over the range $10°C$ to $30°C$. Equilibrium was attained too quickly to measure. Kinetic data for fish suggest that in algae equilibrium is attained within seconds.

Microbial conversion of dimethyl mercury to other forms could not be demonstrated in experiments which involved injecting the compound into bacterized algal suspensions or into bacterized water samples taken from fish tanks. These experiments lasted about three weeks.

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SUMMARY AND CONCLUSIONS

The purpose of these studies was to investigate a variety of ecologically significant interactions between dimethyl mercury and the aquatic environment in which it may be present.

Behavior in Water

In aqueous solution dimethyl mercury behaves in every way like a sparingly soluble gas such as oxygen. In stirred systems the first-order kinetic constant for volatilization, K_L, increased with temperature according to the relation.

$$(K_L)_T = (K_L)_{20} \, 1.027^{(T-20)}$$

where T is the temperature in degrees centigrade. Values of K_L were found to increase with the square root of water velocity, as measured by paddle speed.

Absolute values of K_L were less than those reported for oxygen under the same conditions by a factor of 2.4.

Behavior in Biota

Concentration factors, defined as the ratios of equilibrium dimethyl mercury concentrations in biota to concentrations in surrounding water, ranged from 30 to 90 for biota at three trophic levels: green algae (*Selenastrum capricornutum*), water fleas (*Daphnia magna*) and fathead minnows (*Pimephales promelas*) Comparable factors for mercury in the ionic or monomethyl form are at least 100 times higher.

Concentration factors were essentially independent of temperature for all three organisms, and data for fish indicate concentration factors are independent of dimethyl mercury concentrations at low levels. Equilibrium concentration factors in fish were found to correlate with lipid content, providing a good basis for predicting uptake in other organisms.

Studies on fish showed both uptake and loss occurred rapidly and according to the same empirical first-order model. Kinetics were not affected by the activity level of the fish. Half time of equilibration was about 20 minutes. Surface area to volume considerations suggest that *Daphnia* equilibrate with changes in aqueous concentrations within about five minutes. Algae equilibrate within seconds. The speed with which uptake and loss occur indicates a nonbiological mechanism is involved. The rapidity of dimethyl mercury uptake from water also indicates the food chain is not an important mechanism in this process.

Ecological Implications

Results of this study indicate that in the aquatic environment less than one part in a thousand of all dimethyl mercury is concentrated into living organisms. This fact, combined with the apparently low toxicity of the compound, suggests that dimethyl mercury may be biologically innocuous in the aquatic environment. However, if dimethyl mercury can be metabolized to the monomethyl form by aquatic organisms, then dimethyl mercury could supplement the food chain, and this direct absorption mechanism could result in high monomethyl mercury levels in these organisms. Preliminary experiments indicated that such metabolic conversions occur slowly if at all at the microbial level, but these studies were far from comprehensive, and no work was done with higher organisms.

Regardless of the biological interactions of dimethyl mercury, it seems clear that the tendency of the compound to volatilize from water is an ecologically valuable one. The results of this study lend support to the strategy of attempting to manipulate the methylation process to favor the synthesis of dimethyl mercury over the monomethyl form.

Mercury methylation is not a unique example of organometallic synthesis in nature. The transport and distribution properties described for dimethyl mercury in this study may serve as a model of predicting the behavior of other such volatile organometallic compounds synthesized in nature. One example is diphenyl mercury, which is formed by the microbial conversion of phenyl mercuric acetate (Matsumura et al., 1971).

Areas for Further Study

The whole area of dimethyl mercury synthesis remains unexplored. It would be useful to know the rates of dimethyl mercury synthesis in natural environments, and concentrations of dimethyl mercury in natural waters, and to know what conditions favor the synthethis of dimethyl mercury over the monomethyl form.

Another important gap in understanding dimethyl mercury interactions in nature concerns the area of biological conversion of dimethyl mercury to other forms. Preliminary experiments using microbial populations could not demonstrate any such conversion. but these studies were far from comprehensive, and no work was done with higher organisms.

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APPENDIXES

Appendix A

NAAM Medium

"NAAM" stands for New Algal Assay Medium, which is an informal designation used for the algal medium developed for use in the Provisional Algal Assay Procedure bottle test (Weiss and Helms, 1971).

NAAM medium is made up entirely of inorganic nutrients. The composition of NAAM in terms of the reagents used in its preparation is shown in Table A-1. The composition in terms of elements is shown in Table A-2.

Table A-1. Composition of NAAM medium in terms of reagents used in preparation.

Element	Concentration, µg/l
N	4200
P	186
Mg	2904
S	1911
Ca	1203
K	468
Na	11004
B	33.0 µg/l
Mn	114.0
Zn	15.0
Co	.35
Cu	.003
Mo	2.88
Fe	33.0

Source: Weiss and Helms (1971).

Table A-2. Composition of NAAM medium by element.

Compound	Concentration, µg/l
$NaCO_3$	25500
K_2HPO_4	1044
$MgCl_2$	5700
$MgSO_4 \cdot 7H_2O$	14700
$CaCl_2 \cdot 2H_2O$	4410
$NaHCO_3$	15000
H_3BO_3	185.64 µg/l
$MnCl_2$	264.27
$ZnCl_2$	32.70
$CoCl_2$	0.78
$CuCl_2$	0.009
$Na_2MoO_4 \cdot 2H_2O$	7.26
$FeCl_3$	96.0
$Na_2EDTA \cdot 2H_2O$	300.0

Source: Weiss and Helms (1971).

Appendix B

Data for Volatilization Experiments

Table B-1. Volatilization results for runs at 10°C and 40 rpm.

Replicate 1: non-volatile residue: 2900 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm
0	7845	4945
1.5	5231	2331
3.0	3936	1036
4.5	3492	592
6.0	3152	252
7.5	3079	177
9.0	2958	53
∞	2637	

Replicate 2: non-volatile residue: 2860 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm
0	7760	4900
1.5	5316	2456
3.0	4106	1246
4.5	3519	659
6.0	3135	275
7.5	2962	102
9.0	2931	71
∞	2603	1

Table B-2. Volatilization results for runs at 20°C and 40 rpm.

Replicate 1: non-volatile residue: 2880 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm	c/c$_o$
0	7537	4657	100
1.5	4813	1933	41.51
3.0	3683	803	17.24
4.5	3187	307	6.59
6.0	2980	100	2.14
7.5	2961	81	
9.0	2906	26	
∞	2549		

Replicate 2: non-volatile residue: 2740 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm	c/c$_o$
0	7035	4195	100
1.5	4359	1619	38.59
3.0	3330	590	14.06
4.5	2990	250	5.96
6.0	2846	106	2.52
7.5	2778	-	
9.0	2766	16	
∞			

Table B-3. Volatilization results for runs at 30°C and 40 rpm.

Replicate 1: non-volatile residue: 3320 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm	c/c$_o$
0	8127	4807	100
1.5	4687	1367	28.44
3.0	3776	456	9.49
4.5	3459	139	2.89
6.0	3320	0	
7.5	3389		
9.0	3327		-

Replicate 2: non-volatile residue: 3310 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm	c/c$_o$
0	7764	4454	100
1.5	4563	1253	28.13
3.0	3637	327	7.34
4.5	3371	61	1.37
6.0	3502	192	
7.5	3310	0	
9.0	3435		

Table B-4. Volatilization results for runs at 20°C and 0 rpm.

Replicate 1: non-volatile residue: 3400 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm	c/c$_o$
0	9280	5880	100
1.5	8805	5405	91.9
3.0	8257	4857	82.60
4.5	7666	4266	72.55
6.0	7460	4060	69.05
7.5	7014	3614	61.46
9.0	6514	3114	52.96
∞	2969		

Replicate 2: non-volatile residue: 3500 cpm

Time, hours	Gross cpm	Me$_2$Hg cpm	c/c$_o$
0	9570	6070	100
1.5	8984	5484	90.35
3.0	8489	4989	82.19
4.5	7940	4440	73.15
6.0	7705	4205	69.28
7.5	7269	3769	62.09
9.0	6606	3106	51.17

Table B-5. Volatilization results for runs at 20°C and 20 rpm.

Replicate 1: non-volatile residue: 1200 cpm

Time, hours	Gross cpm	Me_2Hg cpm	c/c_o
0	4584	3384	100
1.0	3289	2089	61.73
2.0	2555	1355	40.04
3.0	2134	934	27.60
4.0	1842	642	18.97
5.0	1607	407	12.03
6.0	1470	270	7.98
7.0	1364	164	4.84
∞	1081		

Replicate 2: non-volatile residue: 1100 cpm

Time, hours	Gross cpm	Me_2Hg cpm	c/c_o
0	5124	4024	100
1	3708	2608	64.81
2	2910	1810	44.98
3	2310	1210	30.07
4	2000	900	22.37
5	1632	532	13.22
6	1496	396	9.84
7	1352	252	6.26
∞	1043		

Table B-6. Volatilization results for runs at 20°C and 60 rpm.

Replicate 1: non-volatile residue: 1000 cpm

Time, hours	Gross cpm	Me_2Hg cpm	c/c_o
0	4164	3164	100
1	2592	1592	50.31
2	1786	786	24.84
3	1502	502	15.87
4	1292	292	9.23
5	1157	157	4.96
6	1050	50	1.58
7	954		
∞	405		

Replicate 2: non-volatile residue: 900 cpm

Time, hours	Gross cpm	Me_2Hg cpm	c/c_o
0	3766	2866	100
1	2298	1398	48.78
2	1585	685	23.90
3	1306	406	14.16
4	1134	234	8.16
5	1034	134	4.68
6	955	55	1.92
7	852		
∞	368		

Appendix C

Computer Programs

**Computer program for least squares
fit of first-order rate data**

```
FILE   5=KULB
$ SET  SINGLE
C         PROGRAM LOGFIT
          DIMENSION T(25),XBAR(25)
    99 READ /, NDAT,RK,XO
    51 FORMAT(1H1,I10,2F18.6)
          WRITE(6,51)NDAT,RK,XO

          DO 5 I=1,NDAT
          READ /,T(I),XBAR(I)
     5 CONTINUE
          ISTOP=0
          E=0.0
          DO 1 I=1,NDAT
          Y=XO*EXP(RK*T(I))
          E=E+(XBAR(I)-Y)*(XBAR(I)-Y)
     1 CONTINUE
    20 RK=RK-0.001
          E1=0.0
          DO 2 I=1,NDAT
          Y=XO*EXP(RK*T(I))
          E1=E1+(XBAR(I)-Y)*(XBAR(I)-Y)
     2 CONTINUE
          TEST=E-E1
          WRITE(6,50) TEST,E1,RK,E
    50 FORMAT(1H ,4E16.6)
          E=E1
          ISTOP=ISTOP+1
          IF(ISTOP.GT.500) GO TO 99
          IF(TEST) 99,99,20
          END
```

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Computer program for calculating least squares regressions and comparison of significant differences.

```
FILE  5=GRENNEY
$ SET SINGLE
      DIMENSION  X(20,50),Y(20,50),XSUM(20),YSUM(20),XYSUM(20),XXSUM(20)
     1,YYSUM(20),N(20),XBAR(20),YBAR(20),CTX(20),CTY(20),CTXY(20)
     2,XDSQ(20),YDSQ(20),XYDSQ(20),B(20),H(20),SS(20)
     3,A(20)
      READ(5,50) M
   50 FORMAT(2I5)
      DO 5 I=1,M
      WRITE(6,55)

   55 FORMAT (1H  // 1H )
      READ(5,50) N(I)
      WRITE(6,52) M,N(I)
      DO 10 J=1,N(I)
      READ(5,51)  X(I,J),Y(I,J)
   51 FORMAT(2F10.3)
   52 FORMAT(1H ,'M=',I5,5X,'N=',I5 // 1H ,11X,'X',14X,'Y' / 1H )
      WRITE(6,53) X(I,J),Y(I,J)
   53 FORMAT(1H ,2F15.3)
   10 CONTINUE
      WRITE(6,54)
   54 FORMAT(1H // 1H )
    5 CONTINUE
      NSUM1=0
      NSUM2=0
      TXYDSQ=0.0
      TXDSQ=0.0
      TYDSQ=0.0
      SSWIN=0.0
      DO 15 I=1,M
      XSUM(I)=0.0
      YSUM(I)=0.0
      XYSUM(I)=0.0
      XXSUM(I)=0.0
      YYSUM(I)=0.0
      DO 20 J=1,N(I)
      XSUM(I)=XSUM(I)+X(I,J)
      YSUM(I)=YSUM(I)+Y(I,J)
      XYSUM(I)=XYSUM(I)+X(I,J)*Y(I,J)
      XXSUM(I)=XXSUM(I)+X(I,J)*X(I,J)
      YYSUM(I)=YYSUM(I)+Y(I,J)*Y(I,J)

   20 CONTINUE
      XBAR(I)=XSUM(I)/N(I)
      YBAR(I)=YSUM(I)/N(I)
      CTX(I)=XSUM(I)*XSUM(I)/N(I)
      CTY(I)=YSUM(I)*YSUM(I)/N(I)
      CTXY(I)=XSUM(I)*YSUM(I)/N(I)
      XDSQ(I)=XXSUM(I)-CTX(I)
      YDSQ(I)=YYSUM(I)-CTY(I)
      XYDSQ(I)=XYSUM(I)-CTXY(I)
      B(I)=XYDSQ(I)/XDSQ(I)
      A(I)=YBAR(I)-B(I)*XBAR(I)
      R(I)=XYDSQ(I)/SQRT(XDSQ(I)*YDSQ(I))
      SS(I)=YDSQ(I)-(XYDSQ(I)*XYDSQ(I)/XDSQ(I))
      SSWIN=SSWIN+SS(I)
      TYDSQ=TYDSQ+YDSQ(I)
      TXDSQ=TXDSQ+XDSQ(I)
      TXYDSQ=TXYDSQ+XYDSQ(I)
      NSUM2=NSUM2+N(I)-2
      NSUM1=NSUM1+N(I)-1
   15 CONTINUE
      SMWIN=SSWIN/NSUM2
      SSCOM=TYDSQ-(TXYDSQ*TXYDSQ/TXDSQ)
      NSUM1=NSUM1-1
C
C   NSUM1 HAS BEEN CHANGED TO CALCULATE MSREG
C
      SSREG=SSCOM-SSWIN
      SMREG=SSREG/(NSUM1-NSUM2)
      F=SMREG/SMWIN
      IDFN=NSUM1-NSUM2
      IDFD=NSUM2
      WRITE(6,56)
   56 FORMAT(1H1 // 1H ,8X,'A',12X,'B',12X,'R',10X,'XBAR', 8X,'YBAR',8X,
     1'CTX',9X,'CTY',9X,'CTXY'8X,'SSI',7X,'NSUM2',3X,'NSUM1')
      DO 25 I=1,M
      WRITE(6,57)A(I),B(I),R(I),XBAR(I),YBAR(I),CTX(I),CTY(I),CTXY(I)
     1,SS(I),NSUM2,NSUM1
   57 FORMAT(1H / 1H ,9E12.5,2I8)
   25 CONTINUE
      WRITE(6,58) SMWIN,SSCOM,SSREG,SMREG
   58 FORMAT(1H // 1H ,'SMWIN = ',E13.5,'   SSCOM = ',E13.5,'   SSREG ='
     1,E13.5,'   SMREG = 'E13.5)
      WRITE(6,59) F,IDFN,IDFD
   59 FORMAT(1H , 'F = ',F7.3,'   IDFN =',I6,'   IDFD =',I6)
      CALL EXIT
      END
```

Appendix D

Data for Fish and *Daphnia* Experiments

Table D-1. Dimethyl mercury concentration factors for fathead minnows at two concentrations of dimethyl mercury in water at 23.2°C.

Fish number	1	2	3	4
Wet weight, g	1.2923	1.1837	0.8077	1.5122
Standard length, mm	48	43	39	46
Run at 0.4 mg/l me$_2$Hg[a]				
Gross count	50,039	47,864	30,538	82,750
Residue	23,028	29,299	17,756	38,050
me$_2$Hg	27,011	18,565	12,782	44,700
Water count, per g	4,043	4,043	4,043	4,043
Concentration factor	6.103	4.587	4.616	8.637
Run at 1.3 mg/l me$_2$Hg[a]				
Gross count	60,537	57,936	37,778	100,766
Residue	36,142	43,354	27,492	59,910
me$_2$Hg	24,395	14,582	10,286	40,856
Water count, per g	3,676	3,676	3,676	3,676
Concentration factor	6.057	3.957	4.090	8.679

[a]Counts expressed as cpm corrected for background.

Table D-2. Dimethyl mercury concentration factors for fathead minnows at two temperatures.

Fish number[a]	1	2	3	4
Run at 18.2°C:[b]				
Gross count	65,080	68,007	42,279	105,207
Residue	42,981	54,491	32,869	70,705
me$_2$Hg	22,099	13,516	9,410	34,502
Water count, per g	3,430	3,430	3,430	3,430
Concentration factor	5.887	3.930	4.011	7.854
Run at 27.0°C				
Gross count	70,563	75,367	46,903	115,381
Residue	49,603	61,297	36,949	79,907
me$_2$Hg	20,960	36,949	9,954	35,474
Water count, per g	3,242	3,242	3,242	3,242
Concentration factor	5.907	4.328	4.488	8.543

[a]For length and weight, see Table D-1.

[b]Counts expressed as cpm corrected for background.

Table D-3. Data for regression of dimethyl mercury concentration factors on lipid content for fathead minnows.

Fish number	Wet weight, g	Lipid content, percent	me$_2$Hg concentration factor
1	1.212	0.5485	3.781
2	1.418	0.9855	6.330
3	1.042	-[a]	17.97
4	1.276	0.5857	2.103
5	1.567	0.6932	3.037
6	1.042	3.557	9.393
7	1.741	0.9958	4.324
8	0.834	0.6448	3.630
9	0.753	0.9005	4.357
10	0.947	2.088	7.268
11	1.292	1.4246	6.103
12	1.184	0.7550	4.587
13	0.808	0.7545	4.616
14	1.512	1.819	8.637

[a]Sample lost.

Table D-4. Dimethyl mercury absorption and elimination rates for fathead minnows at 23°

Fish number	1	2	3	4
Uptake data:				
Exposure time, min.	10	10	10	10
Gross uptake, cpm	18,407	8,359	10,567	15,249
Residue, cpm	1,931	558	614	2,500
me$_2$Hg uptake cpm	16,476	7,801	9,953	12,749
Percent of eqn. uptake	61.0	71.8	84.6	51.6
Elimination data:				
Rinse time, min.	10	9	9	11
Gross initial count, cpm	30,773	12,455	13,513	28,533
Count after rinse cpm	14,393	4,039	4,061	16,145
me$_2$Hg loss, cpm	16,380	8,362	9,452	12,388
Percent of eqn. uptake[a]	60.7	85.6	89.22	45.6

[a]Corrected to 10 minutes rinse.

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Table D-5. Dimethyl mercury elimination from fathead minnows in stirred rinse water

Fish number	1	2	3	4
Standard length, mm	51	42	40	42
Weight, g	1.7405	0.8338	0.7530	0.9473
Equilibrium data:				
Initial count	30,773	12,455	13,513	28,533
Final residue count	3,772	1,596	1,742	3,836
Net me$_2$Hg ount	27,001	10,859	11,771	24,697
First test:				
Rinse time, min.	10	9	9	11
Total count	14,393	4,039	4,061	16,145
me$_2$Hg remaining	10,621	2,443	2,319	12,309
Percent remaining	39.3	22.5	19.7	49.8
Second test:				
Rinse time, min.	20	19	20	21
Total count	9,036	3,002	3,012	11,585
me$_2$Hg remaining	5,291	1,406	1,270	7,749
Percent remaining	19.6	12.9	10.8	31.4
Third test:				
Rinse time, min.	33	32	33	33
Total count	6,571	1,875	2,066	8,445
me$_2$Hg remaining	2,799	279	324	4,609
Percent remaining	10.36	2.57	2.75	18.66

Table D-6. Dimethyl mercury elimination from fathead minnows placed in special rinse apparatus.

Fish number[a]	1	2	3	4
Equilibrium data:				
Initial count	33,213	14,671	15,843	31,107
Final residue count	8,059	4,461	4,879	7,430
me$_2$Hg count	25,154	10,210	10,964	23,677
First test:				
Rinse time, min.	12	11	11	10
Total count	17,227	7,085	6,973	18,695
me$_2$Hg remaining	9,168	2,624	2,094	11,265
Percent remaining	36.45	25.70	19.10	47.58
Second test:				
Rinse time, min.	21	21	21	22
Total count	13,283	5,557	5,803	14,721
me$_2$Hg remaining	5,224	1,096	924	7,691
Percent remaining	20.77	10.73	8.43	30.79
Third test:				
Rinse time, min.	33	33	33	32
Total count	11,055	5,047	5,209	12,311
me$_2$Hg remaining	2,996	586	330	4,881
Percent remaining	11.91	5.74	3.01	20.61

[a]These are the same fish used in Table D-5.

Table D-7. Dimethyl mercury elimination from fathead minnows in stirred rinse water.

Fish number	5	6	7	8
Standard length, mm	48	43	39	46
Weight, g	1.2923	1.1837	0.8077	1.5122
Equilibrium data:				
Initial count	50,039	47,864	30,538	82,750
Final residue count	23,028	29,299	17,756	38,050
Net me$_2$Hg count	27,011	18,565	12,732	44,700
First test:				
Rinse time, min.	10	9	9	9
Total count	34,766	35,596	20,724	61,460
me$_2$Hg remaining	11,738	6,297	2,968	23,422
Percent remaining	43.46	33.92	23.22	52.40
Second test:				
Rinse time, min.	19	19	20	20
Total count	29,656	33,274	19,088	54,290
me$_2$Hg remaining	6,628	3,975	1,332	16,240
Percent remaining	24.54	21.41	10.42	36.33
Third test:				
Rinse time, min.	39	39	40	39
Total count	26,388	31,892	18,678	46,632
me$_2$Hg remaining	3,360	2,593	922	8,582
Percent remaining	12.44	13.97	7.24	19.20

Table D-8. Dimethyl mercury elimination data from fathead minnows normalized to permit test of kinetic model.

Fish[a]	c_o[b]	Rinse time, min.	$\dfrac{c - 2.52}{c_o - 2.52}$[c]
7	4.324	10	0.9447
		20	0.4712
		33	0.2490
8	3.630	9	0.7392
		19	0.4238
		32	0.844
9	4.357	9	0.4685
		20	0.2568
		33	0.654
10	7.268	11	0.7631
		21	0.4812
		33	0.2859
11	6.103	10	0.7413
		19	0.4186
		39	0.2122
12	4.587	9	0.7546
		19	0.4763
		39	0.3108
13	4.616	9	0.5126
		20	0.2300
		40	0.1560
14	8.637	9	0.7404
		20	0.5134
		39	0.2713

[a]Numbered as per Table D-3.

[b]Equilibrium concentration factor.

[c]c is concentration factor at time t.

Table D-9 Dimethyl mercury uptake in *Daphnia* at three temperatures.

Daphnia tube	1		2		3	
Replicate	1	2	1	2	1	2
Run at $10^\circ C$[a]						
Total count	4,887	4,841	7,890	8,036	11,022	10,770
Residue count	4,372	4,298	7,504	7,564	9,806	10,032
me_2Hg counts	515	543	386	472	1,215	738
Water count per g	1,660	1,620	1,660	1,610	1,650	1,600
Concentration factor	83.25	89.94	45.76	57.69	85.23	53.39
Run at $20^\circ C$[a]						
Total count	3,638	5,768	4,514	8,673	6,999	11,568
Residue count	2,948	4,566	3,612	7,884	5,715	10,350
me_2Hg counts	690	602	902	789	1,284	1,218
Water count per g	2,325	2,080	2,260	2,060	2,180	2,010
Concentration factor	79.62	77.66	78.54	75.37	68.18	70.14
Run at $30^\circ C$[a]						
Total count	5,903	–	8,689	–	14,178	–
Residue count	5,500	–	8,232	–	13,456	–
me_2Hg counts	403	–	457	–	722	–
Water count per g	1,360	–	1,350	–	1,350	–
Concentration factor	79.51	–	66.61	–	61.90	–
Dry wt. of organisms, mg		–				
Number of organisms						

[a]All counts expressed as gross cpm.

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Data for Algae Experiments

Centrifuge Tube Number	1	2	3	4	5	Mean
Run at $10^\circ C$:[a]						
Before centrifuging:						
Total count	3,119	3,377	3,364	3,414	3,267	
Residue count	26	25	34	32	27	
me_2Hg count	3,093	3,352	3,330	3,382	3,240	
After centrifuging:						
Total count	4,776	5,164	5,110	5,141	4,894	
Residue count	495	536	534	541	518	
me_2Hg count	4,281	4,628	4,576	4,600	4,376	
Ratio R	1.3841	1.3807	1.3742	1.3601	1.3506	1.3700
Concentration factor	54.77	54.28	53.36	51.35	49.99	52.75
Run at $23^\circ C$:[b]						
Before centrifuging:						
Total count	5,486	5,945	5,947	-	-	
Residue count	214	210	220	-	-	
me_2Hg count	5,272	5,735	5,727	-	-	
After centrifuging:						
Total count	10,835	11,292	11,606	-	-	
Residue count	3,959	3,894	4,072	-	-	
me_2Hg count	6,876	6,105	7,534	-	-	
Ratio R	1.3042	1.2900	1.3155	-	-	1.3032
Concentration factor	54.062	51.539	56.891	-	-	53.891
Run at $30^\circ C$:[c]						
Before centrifuging:						
Total count	8,636	9,468	9,287	10,022	9,740	
Residue count	331	329	304	332	406	
me_2Hg count	8,305	9,139	8,983	9,690	9,334	
After centrifuging:						
Total count	18,068	18,596	18,813	20,201	20,756	
Residue count	6,287	5,767	5,759	6,128	6,740	
me_2Hg count	11,781	12,829	13,054	14,073	14,016	
Ratio R	1.419	1.404	1.453	1.452	1.506	1.4468
Concentration factor	50.72	48.90	54.83	54.71	61.25	54.08

[a]All counts expressed as net cpm. Algal concentration was 400.5 mg/l.

[b]All counts expressed as net cpm. Algal concentration was 321.4 mg/l.

[c]All counts expressed as net cpm. Algal concentration was 471.8 mg/l.