BIOACCUMULATION OF METHYL MERCURY THROUGH A FOOD CHAIN

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ABSTRACT

The bioaccumulation of mercury through the food chain has received much attention in Arkansas during the last three years. The discovery of mercury contamination and subsequent fish consumption advisories in southwest Arkansas have increased public awareness of the potential for bioaccumulation of different toxic compounds. It is postulated that the mercury problem in Arkansas is a result of methylation and resulting bioaccumulation of mercury as methyl mercury. This study was designed to derive a Bioaccumulation Factor (BAF) for mercury in a simple food chain under controlled conditions. A BAF is calculated by dividing the concentration found in the organism by the concentration found in the available food. The "Producer" trophic level was represented by the green alga, Selenastrum capricornutum. The "Consumer" trophic levels were represented by the water flea, Daphnia magna, and the Fathead Minnow, Pimephales promelas. Growth of the alga in media containing methyl mercury, followed by feeding the contaminated algae to the water fleas, and finally by feeding contaminated water fleas to the Fathead Minnows resulted in the derivation of BAF for each trophic level.

Key Words: Bioaccumulation, Trophic Levels, Methyl Mercury

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Introduction

The bioaccumulation of mercury through the food chain has received much attention in Arkansas during the last four years. The discovery of mercury contamination and subsequent fish consumption advisories in southwest Arkansas have increased public awareness of the potential for bioaccumulation of different toxic compounds. It is postulated that the mercury problem in Arkansas is a result of the methylation and resulting bioaccumulation of mercury as methyl mercury.

Elemental mercury (Hg) is water insoluble, while inorganic mercury (Hg^{++}) has a low solubility in water and therefore will not accumulate through the food chain. However, inorganic mercury attached to sediment particles is available for microbial methylation. The most bioavailable form of mercury according to Spry (1) is methyl mercury (CH_3Hg^+). The literature suggest that the bioaccumulation of methyl mercury has several potential routes of uptake through the trophic levels. Rucker and Amend (2) suggest that the concentration of mercury may be accomplished via the food chain or by direct assimilation from the surrounding medium. This provides two routes of exposure: contaminated water and/or food.

This study was designed to trace methyl mercury accumulation through a specific food chain, under controlled

conditions. Additionally, this study proposes to confirm the potential for bioaccumulation of methyl mercury in a south Arkansas oxbow lake. The "Producer" trophic level was represented by the green alga, Selenastrum capricornutum. Selenastrum was grown in media containing appropriate levels of methyl mercury. The primary "Consumer" level was represented by the water flea, Daphnia magna. The secondary "Consumer" was represented by the Fathead Minnow (Pimephales promelas).

The daphnids were fed a diet of contaminated Selenastrum. The minnows were then fed contaminated Daphnia. A Bioconcentration Factor (BCF) was then calculated for the uptake of methyl mercury by the alga (Phase 1). A Bioaccumulation Factor (BAF) was calculated for the accumulation of methyl mercury by the daphnids from the alga (Phase 2). A BAF was also calculated for the methyl mercury accumulation from the daphnids to the fish (Phase 3).

The objective of the final phase (Phase 4) was to test the Daphnia and fish *in situ*. Caged Daphnia and caged fish were placed in Woodard Lake near Camden, Arkansas. Woodard Lake is an oxbow lake off of the Ouachita River and is a known area of methyl mercury contamination.

Objectives

The problems addressed in the this study were the bioaccumulation of mercury in a food chain and the verification of mercury accumulation in a south Arkansas lake *by in situ* exposure. The objectives were three fold. The first objective was to trace the accumulation of methyl mercury through a specific food chain under controlled conditions. The second objective was to derive a Bioaccumulation Factor (BAF) for each trophic level. Three organisms, an alga, an aquatic invertebrate and a fish, were used to represent the three trophic levels of the food chain.

The third objective was to test the results of the food chain test and the derived BAF's in a field situation. The field site (Woodard Lake) was chosen based on known fish contamination with mercury.

Related Research

The literature on mercury pollution is quite overwhelming. According to Bjornberg (3), there are over 50,000 publications on mercury as a pollutant. Therefore, the literature presented here has been limited to that deemed pertinent to the study.

Many investigators have collected evidence for the

magnification of mercury through trophic levels (4, 5, 6). However, considerable uncertainty exists in the biological behavior of mercury in the lower trophic levels (7).

As stated in the Introduction, there are two routes of exposure for mercury to aquatic organisms; through the diet and direct exposure from the water. Zillioux (8) states that the importance of uptake pathways (diet vs water) depends on trophic level, duration and intensity of exposure, and other environmental factors. Obviously, for a producer such as algae, direct contact with the contaminant will be the primary route of exposure. However, for higher organisms such as zooplankton and fish, the primary uptake route for mercury will vary from system to system. Selenastrum was one of the algal species used in the early 1970's in the National Eutrophication Research Program of the Environmental Protection Agency (9). This program utilized Selenastrum in the Algal Assay Bottle Test as a standardized algal growth test. During recent years, Selenastrum has been utilized as a toxicity test species (10) and as a food source for Cladocerans (10, 11).

Daphnia have been used in toxicity testing for several decades. Several species of Daphnia have been cultured for study and testing. These include D. magna, D. pulex as well as Ceriodaphnia dubia. D. magna is one of the largest of this genera. It is easily cultured on a variety of diets and in a variety of conditions (12-14). The literature concerning D.

magna is extensive.

The Fathead Minnow, (Pimephales promelas) has become the most utilized fish species in toxicity testing (15). It is ubiquitous to North America and is also easily cultured in the lab.

Materials and Methods

The green alga, Selenastrum capricornutum, was utilized as the producer in this study. S. capricornutum has been grown continuously in this laboratory for over five years. It is used in this laboratory as a food source for the culture of three species of water flea: D. magna, D. pulex, and Ceriodaphnia dubia.

As stated previously, Selenastrum has traditionally been utilized as a eutrophication test species by the EPA. The media utilized in the EPA Bottle Test protocol is now widely used by laboratories interested in growing the alga as a food for Cladocerans (10, 11).

The protocol used in this laboratory follows that outlined by Knight and Waller (16). Several changes were incorporated to facilitate the exposure of the alga to methyl mercury during the log growth phase. Data collected in this laboratory indicate that the algal growth curve resembles an idealized growth curve under the conditions provided. The conditions utilized here include a 16:8 hour photoperiod,

constant temperature of 24 ± 1^0 C, and the addition of a vitamin solution on day two of the seven day growth cycle.

Additionally, the algal growth procedure utilized in this lab calls for the harvesting of equal amounts of three-day old and seven-day old algae. The purpose of this is to provide a wider range of cell sizes to larval Cladocerans (16). However, for this study the Selenastrum was grown for 5 days and harvested in preparation for feeding to the daphnids.

Algal Exposure to Methyl Mercury: Phase 1

A concentration of < 0.1 ug/L of total mercury has been found in the Ouachita River and the average, total mercury concentration detected in most of the contaminated waters in south Arkansas is approximately 0.05 ug/L (17). Using this data as a guide, methyl mercury (as methyl mercury chloride) was added to the algal media in concentrations of 0.1, 0.2 and 0.5 ug/L during Phase 1 of this study to determine if the amount in the water column (media) is related to the amount of uptake by the algae. These concentrations are higher than concentrations suggested in the literature (18), however, to meet the proposed objectives the concentrations were increased in order to determine a BCF.

After harvesting, the algal suspensions were centrifuged to separate the algal cells from the mercury contaminated media. If the algal cells were to be used in a feeding experiment for Daphnia, then the cells were resuspended in

reconstituted hard water to achieve a cell count of 20 x 10^6 cells/ml.

Daphnid Culture:

Three species of Cladoceran have been cultured in this laboratory for more than six years. D. magna was chosen for this study because of the greater size the adult organisms achieve compared to D. pulex or Ceriodaphnia dubia.

Currently, D. magna are routinely cultured in reconstituted hard water at a temperature of 25^0 C in this laboratory. The protocol utilized here for feeding mass cultured D. magna is outlined in Table 1. Neonates were not used from the first brood produced by adult organisms to initiate experiments in phase 2.

TABLE 1

Day	Food (ml)
0	3
1	3
2	3
3	3
4	3
5	4
6	4
7-14	4 / day

Phase 2: Daphnids Exposed to Contaminated Algae

Less than 24-hour old neonates of D. magna were utilized to initiate tests in Phase 2 of this study. Fifty neonates

were added to a 600 ml beaker containing 400 ml of hard water. The Daphnia were harvested after 4 days (prior to releasing their first broods). Immediately after harvesting, the organisms were frozen in preparation for analysis or used immediately in Phase 3, feeding of Fathead minnows. As much water as possible was removed prior to weighing by siphoning with a disposable, Pasteur pipet. The resulting weight is therefore a "wet weight" for the daphnids. This method of weighing increases the margin of error when calculating a BAF, however a dry weight would likely result in a loss of methyl mercury, interfering with the analysis.

Phase 3: Exposure of Fathead Minnows to Contaminated Daphnia

Fathead minnows (Pimephales promelas) are currently cultured in a flow through system consisting of eight brood tanks. The flow through water is carbon-filtered tap water. Temperature and light are monitored to achieve suitable conditions for reproduction. Newly hatched minnows are fed brine shrimp nauplii and\or trout chow.

Three minnows were placed in a two liter test beaker containing one liter of reconstituted hard water. The minnows were approximately three months of age. After allowing time for the fish to acclimate, each culture was fed with either 50 contaminated or un-contaminated (control) Daphnia. In each experiment, the fish consumed all of the daphnids in less than 30 minutes. The fish were fed once daily for four consecutive

days. On day five, the fish were harvested and frozen for later analysis.

Phase 4: Field Testing

The final phase of this study was conducted in Woodard Lake. Small cages were built with the intention of exposing Daphnia *in situ*. The Daphnia were contained within the cages in an effort to protect from predators and to keep track of the known age individuals. The organisms were allowed to feed on native algal species for five days (as in Phase 2). At the end of the test period, Daphnia were harvested and prepared for tissue analysis.

Cages were constructed of PVC pipe and nylon netting. After construction, the cages were allowed to soak in a local pond to remove any contamination. The cages were suspended approximately one meter below the surface when positioned at Woodard lake.

Three-month-old Fathead Minnows were also caged and tested in the field. As with the Daphnia, the cages were designed to protect from predators and allow for tracking of known age individuals. There were approximately five minnows per cage, and the exposure time was five days.

The primary criteria for field site selection will be reducing the chance that the cages will be disturbed. Woodard Lake was chosen based on data collected that indicates mercury contamination.

Mercury Analysis:

Tissue samples from each of the three organisms (alga, daphnids and fish) were frozen immediately after collection. Digestion of the tissues was performed immediately prior (or the day before) to analysis.

The analytical method for mercury followed that of the manufacturer (LDC Analytical). The appropriate changes in methodology were incorporated for tissue analysis.

Results

The results of the first algal experiment (Figure 1) illustrate an inhibition of growth of algae with an increase in the concentration of methyl mercury. As stated in the Materials and Methods, the concentrations utilized in this study are higher than those found in the Ouachita River in south Arkansas. The concentrations detected in the Ouachita River were < 0.1 ug/L throughout the Ouachita basin (17). A statistically significant difference was detected by ANOVA between the 0.1 ug/L and the 0.2 ug/L concentrations of methyl mercury in the algal media.

Figure 2 illustrates an experiment conducted to validate the results in the first algal experiment. Again, an inhibition of growth of algae with an increase in the

Figure 1

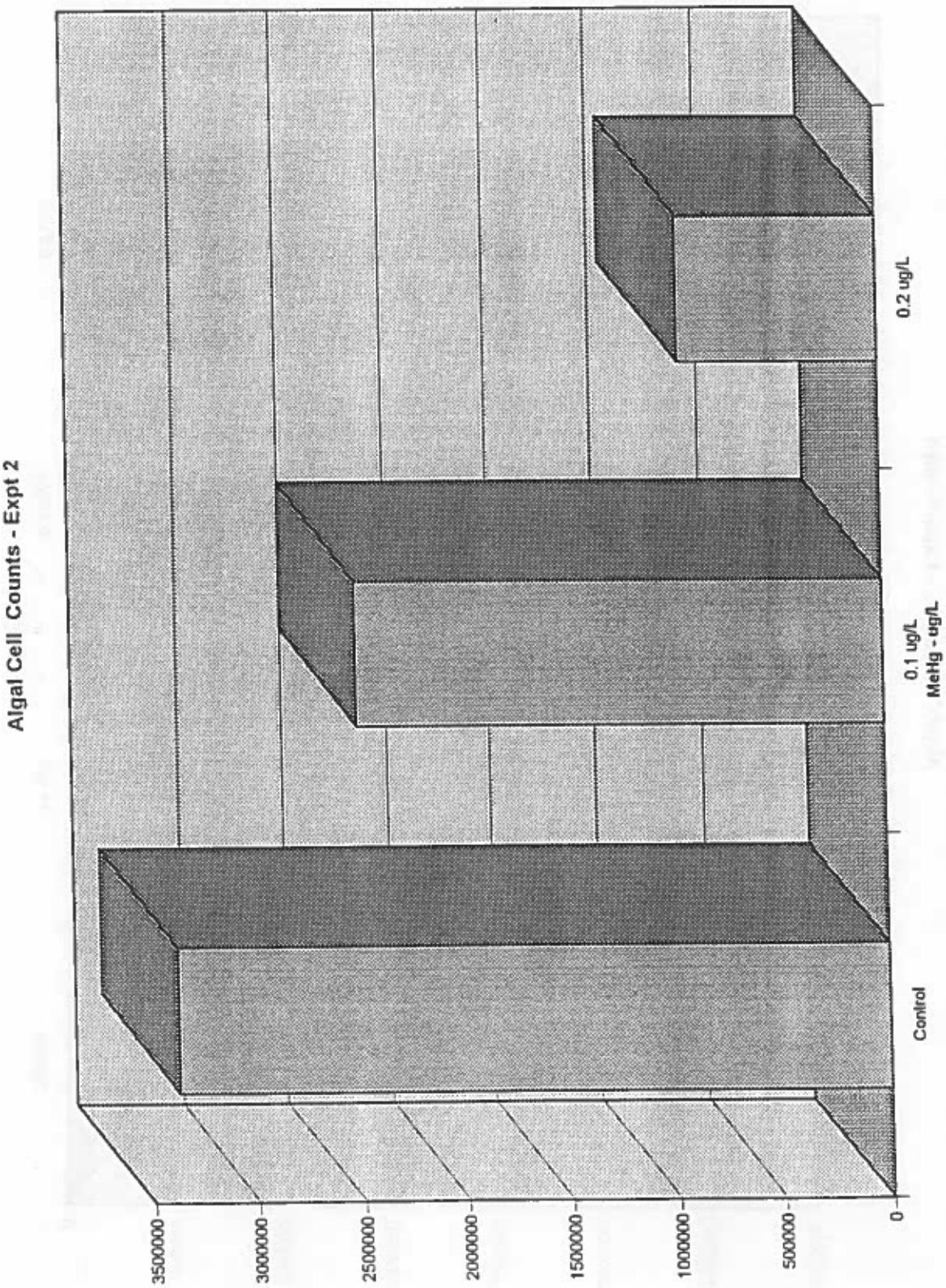
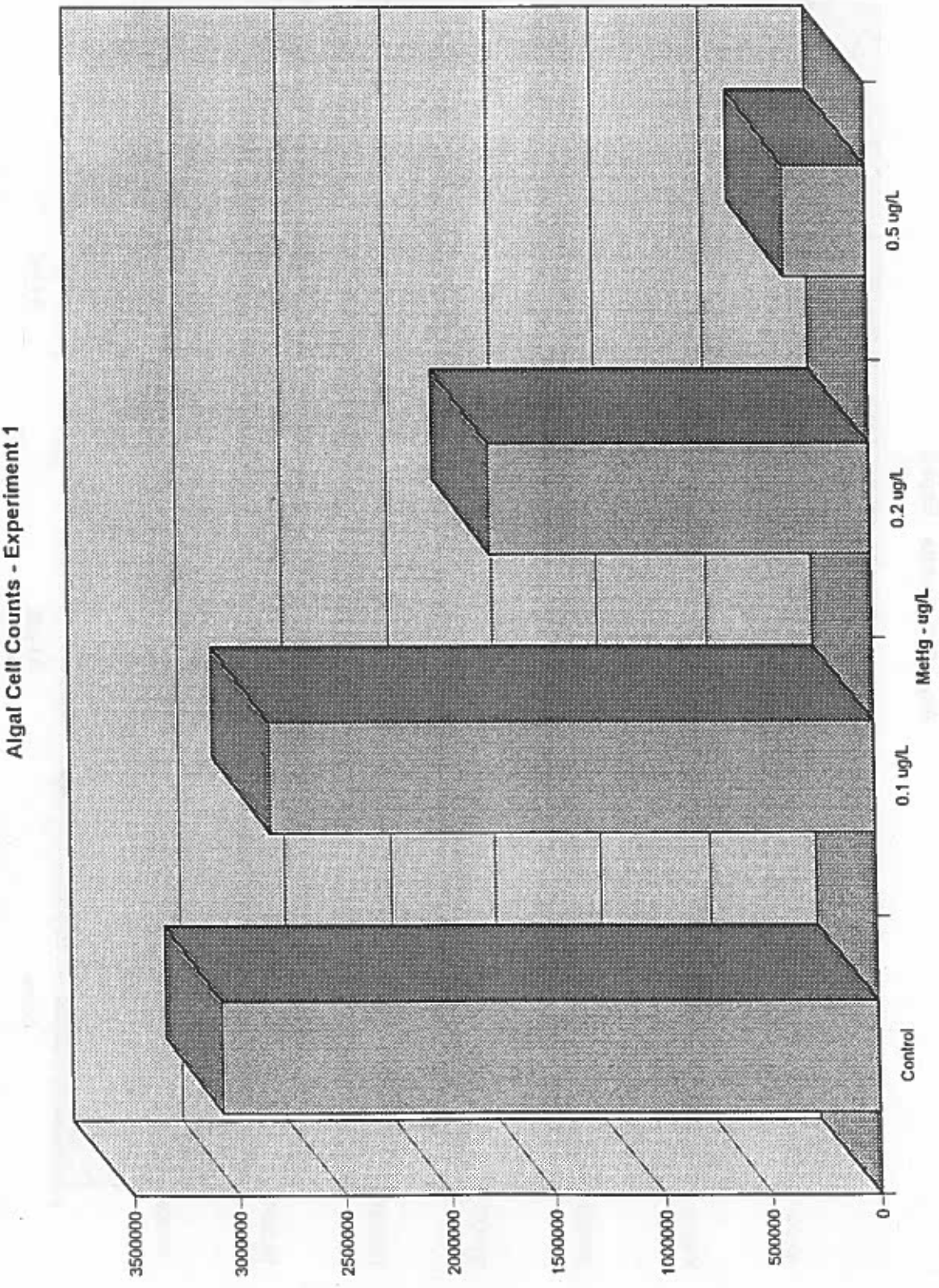

Algal Cell Counts - Expt 2
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Control
0.1 ug/L
0.2 ug/L
MeHg - ug/L

Figure 2


Algal Cell Counts - Experiment 1
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Control
0.1 ug/L
0.2 ug/L
0.5 ug/L
MeHg - ug/L

concentration of methyl mercury was observed. A statistically significant difference was detected in this experiment between the control (0.0 ug/L) and 0.1 ug/L concentrations.

The calculation of a Bioconcentration Factor (BCF) for these data results in an average of 2.4 and 5.2. The BCF is calculated by dividing the amount of methyl mercury in the algae by that available in the water - a direct exposure. The BCF of 2.4 is a result of the calculation for the algae exposed to 0.1 ug/L methyl mercury while the 5.2 is the mean for the algae exposed to 0.2 ug/L methyl mercury. This indicates that an increase in the concentration of methyl mercury detected in the water results in a greater uptake / bioconcentration in the algae.

The toxic response of Selenastrum to methyl mercury in the laboratory does not necessarily indicate that higher levels of methyl mercury would cause a similar toxic response to native species. The toxic response by Selenastrum should be interpreted as, "if there is a native algal species as sensitive as Selenastrum, it would be expected to respond in a similar fashion". Additionally, synergistic effects between the methyl mercury and other environmental components may increase or decrease the toxicity of the methyl mercury.

In phase 2, D. magna were fed algae grown in media containing methyl mercury. The results of this experiment indicate that the daphnids bioaccumulate the methyl mercury via the diet of algae (Table 2). A "control" was also

employed in which daphnids were fed "clean" alga (alga grown in mercury free media).

The data in Table 2 indicate that the algae grown in the media containing no methyl mercury (control) actually accumulated a trace amount of methyl mercury. The concentration of 0.110 ug/L methyl mercury in the control algae is actually a concentrated amount due to the adjustment

Table 2

Algal Feeding Suspension MeHg conc ug/L		Conc ug/L MeHg in 50 D. magna	BAF
Control	0.110	0.326	0.059
0.1	0.290	1.829	0.126
0.2	0.546	3.817	0.14

of the feeding suspension. The algal feeding suspension is prepared such that there are 20 x 10^6 cells per milliliter. In this way, the cell numbers have been increased approximately 7 fold per milliliter above the number of cells in the algal media at the end of the five day growth period.

The accumulation of methyl mercury from the algae to the daphnids, unlike the concentrating step from water to algae, is more uniform when the BAF is calculated. From Table 2, the BAF (0.126) for the daphnids fed the "0.1 ug/L" algae is very similar to the BAF (0.14) for the daphnids fed the "0.2 ug/L"

alga. These BAF's are based on the number of Daphnia instead of the actual weight of the daphnids.

The data in Table 3 represent an experiment performed exactly as the experiment in Table 2. However, two changes were significant and should be noted. The first change is that there were no daphnids fed alga from the "0.2 ug/L" because there was not enough left from the original experiment. Secondly, the algae fed (control and 0.1 ug/L)

Table 3

AlgalFeeding Suspension MeHg conc ug/L		Conc ug/L MeHg in 50 D. magna	BAF
Control	0.110	0.012	0.002
0.1	0.290	0.074	0.005

to the daphnids in this particular experiment had been stored in the refrigerator for over three weeks. The data indicate a loss of methyl mercury from the original analysis. However, this could not be checked because there was not enough algal feeding suspension left to perform the analysis. Dimethyl mercury is extremely volatile, however it has been assumed by most investigators that mono-methyl mercury binds to tissue more readily and should not be lost to the atmosphere.

The data presented in Table 4 result from the feeding of daphnids to Fathead minnows. There are two potential

methods to calculate the BAF for this particular experiment. If the number of daphnids (50) is utilized the resulting BAF for the control organisms is 9.75 (0.117/0.012), while for the mercury exposed organisms the BAF is 3.45 (0.255/0.074). If the BAF is calculated utilizing the wet weight of the daphnids, the results are 0.49 and 0.21 for the controls and mercury exposed organisms, respectively. The resulting concentrations of mercury do indicate an accumulation when compared to the initial cohort fish.

Table 4

Conc. ug/50 D. magna	Conc. ug/g D. magna	Conc. ug/g fish
*		0.069
0.012	0.24	0.117
0.074	1.23	0.255

* Concentration of methyl mercury from a cohort fish that was sacrificed prior to the initiation of the experiment. This fish was never exposed to mercury.

The same problem (number of daphnids vs wet weight of daphnids) is exhibited in the results in Table 5. If the number of daphnids (50) is utilized the resulting BAF for the control organisms is 6.53 (0.098/0.015), while for the mercury exposed organisms the BAF is 2.92 (0.111/0.038). If

the BAF is calculated utilizing the wet weight of the daphnids, the results are 0.81 and 0.34 for the controls and mercury exposed organisms respectively. However, the resulting concentrations of mercury in the fish of this experiment do not indicate an accumulation when compared to the initial cohort fish, however. This may be due to the fact that the alga used to feed the daphnids in the experiment outlined in Table 5 had been stored for over three weeks, allowing for volatilization of the mercury. This would result in less mercury in the daphnids (0.326 ug/g) than in the previous experiment.

However, for both of these experiments, there seems to be a greater accumulation of mercury in the "controls" than in the mercury exposed organisms. The possible explanations for this are the volatilization of the mercury in the room where the test is occurring, and contamination of the water the fish are being cultured in. Cross-contamination of the water from methyl mercury should be investigated thoroughly since this would indicate that a significant amount of accumulation / bioconcentration can occur across tissue membranes.

In phase three of this study, organisms (daphnids and Fathead minnows) were placed in cages and positioned in Woodard Lake near Camden, Arkansas. The netting utilized in the construction of the cages for the water fleas was chosen based on work by other investigators from the literature (500 um mesh). The netting size should be chosen using two primary

Table 5

Conc. ug/50 D. magna	Conc. ug/g D. magna	Conc. ug/g fish
*		0.092
0.015	0.121	0.098
0.038	0.326	0.111

* Concentration of methyl mercury from a cohort fish that was sacrificed prior to the initiation of the experiment. This fish was never exposed to mercury.

criteria; 1) large enough mesh size to allow adequate exposure of the organisms to water and food, and 2) small enough mesh size to prevent predators from consuming the organisms and also to prevent the daphnids from escaping.

Several problems arose with the *in situ* exposure of the daphnids. When placing them in a new area, care must be taken not to "shock" the organism by an abrupt change in temperature. In this study, a temperature change of greater than two degrees centigrade was considered too much. Additionally, because of the construction of the cage, "floaters" (daphnids that are floating due to a change in water temp) cannot be detected.

Upon retrieval of the daphnids after the *in situ* exposure, there were too few organisms to analyze for mercury. It is felt that this is a result of organisms escaping, and

floaters that had died during the exposure. Upon testing the cages in the lab (in a large beaker), the smaller organisms were capable of passing through the mesh. If larger organisms (older) are utilized, the complicating factor of offspring being produced becomes a problem if the females partition compounds such as methyl mercury into the developing eggs. At a temperature of 20° C, D. magna should begin to produce their first brood by the end of day 5 and release them by the end of day 6. Little or no testing has been done to determine if compounds such as methyl mercury accumulate in eggs, or other tissues for daphnids primarily because the amount of tissue required for analysis is greater than can be feasibly obtained.

The cages for the fish were similar in structure with the exception that they are larger and the mesh size is larger. The size of the fish as well as the longer life span allow larger mesh size and increased exposure time. Additionally, the larger fish provide ample tissue for analysis.

The fish were placed in Woodard Lake and remained there for seven days. The results of the analysis are presented in Table 6. The data indicate that there was no increase in the amount of mercury from the original un-exposed fish. There are several potential problems with this method of *in situ* exposure. The time required to "see" an accumulation of mercury and the limiting of food sources by caging the fish. Further studies will have to be done to determine is a longer

exposure will allow for potential accumulation.

Since the design of the cage limits the food sources to only those items that can pass through the netting, this may also affect the ability to "see" mercury accumulation in the fish. Especially if there are differences in mercury accumulation in the food sources themselves and if there are seasonal changes in the availability of different food sources for minnows.

Table 6

Concentration of Methyl Mercury (ug/g fish)	
Initial fish*	0.092
7 day exposure **	0.077

* Concentration of methyl mercury from a cohort fish that was sacrificed prior to the initiation of the experiment. This fish was never exposed *in situ*.

** Mean of 3 fish.

Conclusions

Several conclusions can be drawn from this study. The routine analysis of mercury is one of the most difficult analysis to perform because it is easily contaminated. With this in mind the routine analysis of samples with trace amounts (at or near the minimum detection limit) of mercury is questionable. Without a clean room dedicated entirely to the analysis of mercury, the data collected in this range is subject to question.

The logistical planning utilized in this study could be improved upon based on the data collected. The exposures beginning with the media / alga, followed by the alga / daphnids, followed again by the daphnids / fish, should all be performed within a short time period. Ideally this would be performed in a two week period. The results presented here indicate that the methyl mercury my be volatilized from the algae, even under refrigeration.

The data collected here indicate that both a BCF and BAF can be derived in a simulated food chain. Further testing is needed to confirm BAF/BCF values determined in this study. Additionally, confirmation of the "volatilization" of methyl mercury from algal tissue must be exhibited.

Determining a BCF in the field with surrogate organisms is a possibility, however there are several factors that must be worked through to make this a useful test. The minimum

exposure time must be worked through *in situ*. The difference between the minimum exposure time necessary in the field as compared to the lab will be different primarily because the concentration of methyl mercury in food sources *in situ* may be not be the same as the concentration in Selenastrum. Also, if mercury (organic or inorganic) is being accumulated across the membranes, such as the gill membranes, then the overall accumulation / concentration would be expected to be different between lab exposure and field exposure.

The potential use for *in situ* exposures to delineate areas of mercury contamination and levels of mercury contamination in specific areas is significant and should receive further attention.

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