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Measurement of Mercury Methylation in Lake Water and Sediment Samples

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Biological mercury methylation was assayed by a new radiochemical technique in the water column and sediments of a mercury-contaminated lake. In 24 weeks during 1979, there were three episodes of methylating activity in surface floc and in water, each lasting 3 to 5 weeks. Periods of methylation in the water column coincided with surface sediment methylation and appeared to be related to overall microbial activity. Mercury was actively methylated in the presence of bound sulfide.

Sites of biological mercury methylation in contaminated lakes have been of interest for a number of years. Most studies have concluded that methylation occurred in the sediment (2, 22, 25, 29).

One of the major limitations of methylation studies has been the low sensitivity of the methodology to measure in situ methylmercury production (8, 34). Long incubation times of several weeks, increased mercury concentrations, or both have been necessary to detect methylation (15, 20, 23, 28). These conditions have an unknown effect on the natural microbial community and limit the usefulness of this approach as an indicator of in situ mercury methylation.

As an alternative to the direct measurement of methylation rates in the sediments, live fish have been utilized as bioaccumulators of methylmercury (16, 23, 27). However, this indirect laboratory method also requires long incubation times and provides limited information on the in situ activities of methylating bacteria. In addition, other methylation sites such as the water column, fish intestinal contents and slime, and other intermediate steps of the food chain cannot be investigated individually (24, 31).

This paper presents methylmercury production data obtained by using a very sensitive radiochemical technique on natural lake samples. The samples were taken from Clay Lake, Ontario, which has been severely contaminated with mercury for the past 17 years (3). Methylation in the water column, in the layer of recently deposited organic floc at the sediment-water interface, and in the gastrointestinal tract of several fish species has been investigated. Methylation data for the first two sites are described in this paper. The intestinal methylation data are presented by Rudd et al. in the following article (31).

MATERIALS AND METHODS

Sampling. Samples were collected from the unstratified eastern and the stratified western basins of Clay Lake (Z_{max}, 3 and 18 m, respectively). Water samples from the aerobic eastern basin were taken approximately 10 cm below the surface by submerging an 8-liter polyethylene bottle. From the deeper western basin, depth profile samples for methylating activity were taken by a Van Dorn sampler and transferred into three heat-sterilized 270-ml reagent bottles for each depth sampled. These bottles were overflowed to minimize atmospheric oxygen contamination.

Much of Clay Lake has a heavy gray-colored clay bottom with a thin overlying layer of recently deposited organic-rich flocculent material, which is brown in the eastern basin but black in the deeper, anoxic western basin. This organic-rich layer (sediment-floc) was collected by an Ekman grab, which sampled 233 cm^2 of surface area (11). For the samples from the aerobic eastern basin, the Ekman grab was rocked until the sediment-floc was suspended in the overlying water. The suspension was then transferred with a 50-ml syringe to a 1-liter plastic bottle. For the anoxic western basin samples, the floc was withdrawn through the undisturbed overlying water with a syringe and transferred into a glass reagent bottle until completely filled. This procedure minimized exposure to atmospheric oxygen.

Methylation incubation. This method used a bottle incubation of environmental samples in the presence of $^{203}Hg(NO_3)_2$ (1 mCi/mg, New England Nuclear Corp.). More than 95% of the ^{203}Hg was immediately adsorbed by the particulate matter in the samples. After incubation, radioactive methylmercury produced during the incubation period was extracted using a modified solvent extraction technique and assayed by scintillation spectrometry. All methylation incubations were in preignited (450°C) 270-ml glass reagent bottles. Each test used three incubation bottles: duplicate tests and a control. The control samples were fixed before incubation by 1 ml of 4 N HCl. Incubation was started within 2 h of sampling. The test samples were acidified to terminate incubation. The incubation period was 4 days at in situ tempera-

tures in the dark. Water samples from the anoxic hypolimnion were directly spiked with 0.41 μCi of ^{203}Hg, an equivalent concentration of ^{203}Hg.

The sediment-floc samples from the eastern basin were transferred in 100-ml portions into the incubation bottles and spiked with 0.3 μCi of ^{203}Hg. The 270-ml samples of anaerobic hypolimnetic sediment-floc were directly spiked with 0.80 μCi of ^{203}Hg (an equivalent ^{203}Hg concentration).

[^{203}Hg]methylmercury extraction. After acidifying the samples, the $CH_3{}^{203}Hg^+$ could be stored for up to 5 days before extraction without loss of activity. The solvent extraction method developed by Uthe et al. (37), intended to extract methylmercury from fish tissues, was modified to extract radioactive methylmercury produced during incubation of sediment-floc and water column samples. A 2-ml volume of 0.5 M copper sulfate and 10 ml of 3 M sodium bromide in 11% sulfuric acid were added to the samples. After mixing and standing, the water column samples and the supernatant of the "settled" sediment-floc samples (usually 60 to 80 ml) were transferred into 125-ml glass separatory funnels. A 20-ml volume of glass-distilled toluene (Caledon) was added and shaken for at least 3 min. The aqueous phase was removed, and the remaining toluene phase was dried with about 1 g of anhydrous sodium sulfate. The toluene phase was decanted into a 50-ml Erlenmeyer flask and further dried with about 0.5 g of sodium sulfate. A 10-ml sample of the dried extract was placed in a clean separatory funnel, to which 5 ml of 0.0025 M sodium thiosulfate in 20% ethanol was added. Then 3 ml of the lower aqueous phase was pipetted into a glass-stoppered test tube to which 1 ml of 3 M potassium iodide and 1 ml of glass-distilled benzene (Caledon) were added. Subsamples (250 μl) of the extracted monomethylmercury iodide concentrated in the benzene phase were assayed for radioactivity by liquid scintillation counting in 10 ml of PCS cocktail (Amersham) and, if required, for total methylmercury concentration by gas chromatography using an electron capture detector (37). The extraction efficiency was 97 to 100%. The scintillation counting efficiency was 85% based on comparison with a gamma counter of a known efficiency.

Extraction confirmation. Benzene extracts were analyzed for organic ^{203}Hg before and after addition of equal volumes of saturated silver sulfate to bring organic ^{203}Hg into the aqueous phase (21).

For specific identification and confirmation of $CH_3{}^{203}Hg^+$ production, two different thin-layer chromatography systems were used. The first system used was Baker-flex silica gel 1B-F 4463 plates (Machrey and Nagel Polygrams) with ethyl ether-petroleum ether (3:7) as the solvent. The second system used silica gel plates (Kodak 13179 with 6061 fluorescent indicator) with hexane-acetone (9:1) as the solvent (36). The extracted radiomercury in the benzene was supplemented with cold monomethylmercury iodide, which permitted visualization with 0.05% dithiazone in chloroform. After partitioning, 1-cm-interval scrapings of the silica gel were assayed by scintillation counting and were compared with the R_f value of a simultaneously run cold monomethylmercury iodide control.

The method was also tested to confirm that non-biological isotopic exchange was not responsible for $CH_3{}^{203}Hg^+$ production. Samples of lakewater were treated as described in Table 1. The methylmercury was filter sterilized, and the $^{203}Hg^{2+}$ was sterilized and rendered free of organic matter by photooxidation (6). The autoclaved samples were completely cooled before the sterilized spikes were introduced, to avoid isotopic exchange induced by high temperature (18). Bacterial growth was measured turbidimetrically on day 4 of incubation. The above described incubation, extraction, and measurement procedures for radioactive methylmercury were used.

Other analyses. Samples for methylmercury and total mercury concentrations as well as for oxygen depth profiles were obtained by the same methods as used in methylmercury production sampling. Methylmercury samples were preserved with sulfuric acid at 0.3% final concentration and transported in 8-liter polyethylene bottles. Dissolved methylmercury was extracted using the 5-liter continuous extractor method of Chau and Saitoh (12) and analyzed by gas chromatography. Total mercury samples, preserved with final concentrations of 0.05% chromate and 1% nitric acid, were transported in preignited glass reagent bottles. Total mercury was determined by flameless atomic absorption spectrometry (A. Lutz, manuscript in preparation). Sediment-floc samples dried at 60°C were assayed for total mercury (5) and for organic carbon (33).

Dissolved oxygen profiles were assayed according to the Hach analytical method. Biological oxygen demand measurements were conducted on 40-ml epilimnetic samples contained in 50-ml glass syringes. Four replicates were analyzed after 0, 6, 12, and 24 h of

TABLE 1. *Test for non-biological isotopic exchange*[a]

Sample treatment	dpm/ml on day of incubation:			Optical density[b] (day 4)
	0	1	4	
No MeHg[c] added	0	0	207	0.260
Autoclaved	0	0	7	0.025
MeHg (0.01 μg/ml)	0	5	304	0.290
Autoclaved	0	1	5	0.016
MeHg (0.02 μg/ml)	0	0	18	0.130
Autoclaved	0	6	2	0.018
MeHg (0.10 μg/ml)	0	5	96	0.220
Autoclaved	0	3	9	0.018
MeHg (0.02 μg/ml) + HCl	0	0	0	0.016
Autoclaved	0	0	3	0.018

[a] Samples were 60 ml of lake water with 0.6 g of nutrient broth (Difco) and 3 μCi of $^{203}Hg(NO_3)_2$. The ^{203}Hg concentration was increased 10-fold to enhance possible isotopic exchange.

[b] λ = 450 nm.

[c] MeHg, Methylmercury.

incubation by the semi-micro method of Rudd et al. (30).

RESULTS

Confirmation of $CH_3^{203}Hg^+$ production. On every occasion (11 times) when silver sulfate was added to the benzene extract, the methylmercury peaks on the chromatograms and the radioactive counts disappeared. This indicated that the radioactivity was in an organic mercury compound.

More than 90% of the radioactivity in the thin-layer chromatograms was found in the same area as the monomethylmercury iodide control. The R_f values were 0.55 for the Kodak system and 0.4 for the Bakerflex system.

The results of bacterial growth and isotopic exchange experiment are shown in Table 1. At concentrations up to 0.1 μg of methylmercury per ml, no significant isotopic exchange occurred under these conditions in the sterilized controls. Acidification of samples provided a satisfactory control since it effectively stopped bacterial growth and mercury methylation, and it did not enhance isotopic exchange. The amount of methylation (disintegrations per minute) paralleled the amount of bacterial growth (optical density) in this test.

Jacobs and Keeney (20) found it necessary to test for recovery of methylmercury with different sediment types. Three methylmercury recovery tests were conducted on sediment-floc of the eastern basin during the year. A 10-ng sample of methylmercury was added to 100 ml of sediment-floc suspension (~1.5 g, dry weight). In each case, all of the methylmercury was recovered (95 to 103%).

Eastern basin. Samples of the sediment-floc from the eastern basin were tested during the summer of 1979 to determine whether methylation occurred and, if so, to obtain some impression of the variation of methylating activity with time. Averages of duplicate analyses of sediment-floc samples are presented in Fig. 1. Mean percent coefficient of variation for these samples was 9.9 ± 10.2% for 14 duplicates. Methylating activity occurred in three separate peaks. The first and largest peak coincided with the spring run-off, when the water residence time in Clay Lake was reduced from about 50 to 10 days.

Mercury methylation also occurred in the water column (Table 2 and Fig. 1). The peaks of activity in the water column samples coincided with the peaks of activity of the sediment-floc samples.

On 10 July 1979, lake water and sediment-floc samples were enriched with 2 mg of tryptic soy broth (Difco) per ml to determine whether the methylating process was limited by bacterial substrate. Tryptic soy broth contains organic

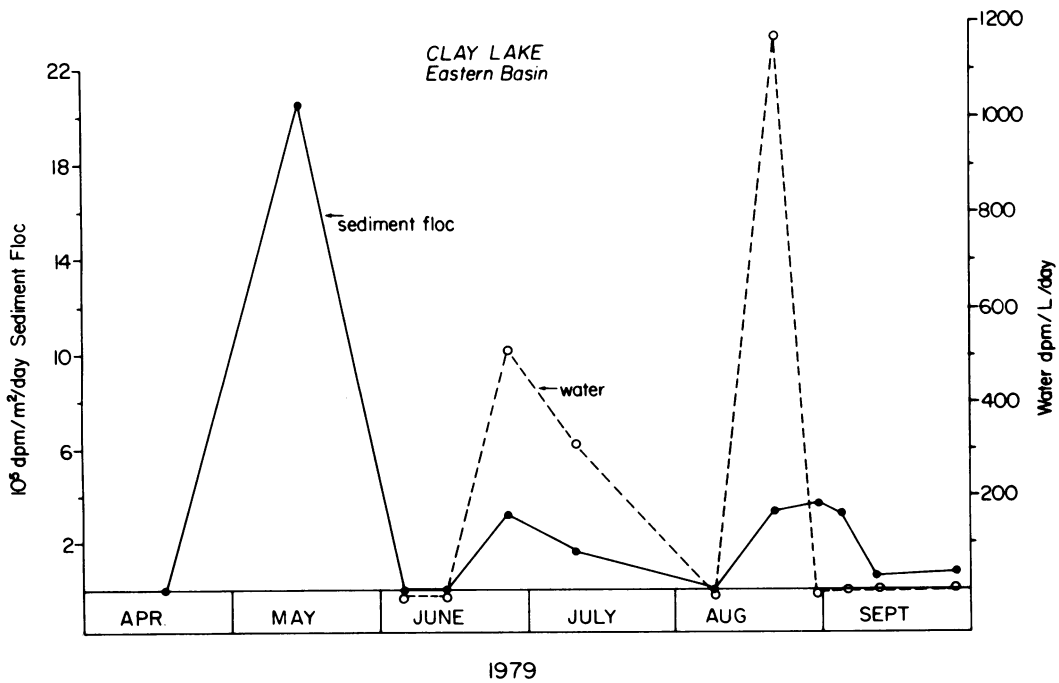

FIG. 1. *Seasonal variation of methylmercury production in water column and sediment-floc samples.*

TABLE 2. *Mercury methylation in the eastern and western basins of Clay Lake with their corresponding organic carbon and total mercury concentrations*

Location	Date	Lake water		Sediment-floc		
		MeHg[a] produced (dpm/liter per day)	Total Hg concn (μg/liter)	MeHg produced (10^3 dpm/m^2 per day)	Total Hg concn (μg/g, dry wt)	Organic carbon (mg/g)
Eastern basin	17 April			UD[b]		
	14 May			2,046		
	5 June	UD		UD		
	14 June	UD	24	UD		
	27 and 28 June	512	14	320	2.5	
	10 and 11 July	314	27	168	1.9	19
	10 July	4,397[c]		52,956[c]		
	8 and 9 Aug.	UD	24	UD	1.6	34
	21 and 22 Aug.	1,176	25	331	1.7	25
	29 Aug.	UD	27	364	4.0	27
	5 Sept.	UD	32	320		
	11 Sept.	UD		43	2.4	20
	28 and 29 Sept.	UD	64	75		
Western basin	10 July	104, 887[d]				
	11 Sept.			113		
	4 Oct.	UD, UD[d]		394	2.5	32

[a] MeHg, Methylmercury.
[b] UD, Undetectable.
[c] Tryptic soy broth added.
[d] First number, quantity in epilimnion; second number, quantity in hypolimnion. In samples taken on 8, 21, and 29 August, mercury methylation was undetectable in both epilimnion and hypolimnion in the western basin.

carbon, energy, and nutrient sources which are easily utilized by a wide variety of bacterial species. The methylating activity of the water column samples increased 15 times, and that of the sediment-floc samples increased 315 times (Table 2).

On four occasions the role of bacterial substrate was further investigated in the water column samples by monitoring the biological oxygen demand over a 24-h period. In Fig. 2 the methylating activity of the water column samples is plotted against the rate of oxygen consumption. These data suggest a linear relationship between overall microbial activity (i.e., community respiration) and the rate of methylation. Total mercury concentrations of these water samples were found to be unrelated to changes in methylating activity (Table 2). Similar oxygen demand measurements were attempted on sediment-floc samples, but rapid chemical uptake of oxygen masked the biological oxygen consumption. In this case methylation was found to be not correlated with the organic carbon content of the sediment samples (Table 2). This may have been because the organic carbon was not readily available as a bacterial substrate.

Western basin. During the summer of 1979, 1-m epilimnion and composite hypolimnion mercury methylation samples were taken from the stratified western basin. After August, no methylation in the water samples was detected (Table 2), although hypolimnetic methylmercury concentrations increased (Fig. 3; a similar increase was noted in 1978 by J. W. Parks [personal communication]). The hypolimnetic sediment-floc at this site was, however, actively methylating (Table 2). Thus increased methylmercury concentrations probably resulted from diffusion away from the sediment-floc.

Late in the summer the sediment-floc of the anoxic portion of the hypolimnion of the western basin was black. When these samples were acidified, the black color rapidly changed to the same brown color of the eastern basin and there was a strong hydrogen sulfide odor, indicating that the sulfide was bound as insoluble iron sulfide. Despite the presence of sulfide, which is known to tightly bind mercury (K_{sp}, 10^{-53}), $^{203}Hg^{2+}$ was actively methylated on each occasion (Table 2).

DISCUSSION

The production of $CH_3{}^{203}Hg^+$ from inorganic $^{203}Hg^{2+}$ was confirmed by the silver sulfate test and by the two thin-layer chromatography

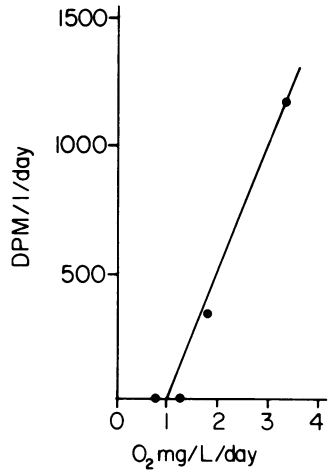

FIG. 2. *Correlation between the oxygen consumption and the methylation activity in water column samples in the eastern basin of Clay Lake. r = 0.995; P < 0.01.*

methods. It was concluded that chemical isotopic exchange was not interfering with the measurement of biological methylation (Table 1); this agrees with Kudo et al. (26), who found insignificant isotopic exchange under expected environmental conditions. We conclude that biological mercury methylation was being assayed by the technique described.

There are several advantages and some limitations in using this radioactive technique. Use of the isotope allowed mercury methylation to be detected with much greater sensitivity than the chemical methods. The incubation period could be reduced from weeks to 4 days, enabling the detection of short-term fluctuations in in situ activity. It also decreased the effects of enclosing a microbial community in a bottle. There is evidence (24a) that surface sediments from Frain's Lake, Michigan, could be incubated in the laboratory for at least 4 days without changing the overall microbial activity of the sediments.

Another advantage of this technique is that because only a small amount of mercury is added to the samples, in comparison to previous incubation techniques with stable mercury, the selection of a more mercury-tolerant bacteria would probably be minimized (38). This increases the likelihood that the methylating activity observed was from the same microbial population that was active in the lake sediment and water at the time the samples were collected. The amount of mercury added in our technique was the carrier mercury of ^{203}Hg used for the spikes, about 0.3 to 0.6 μg of Hg per spike.

A limitation of the method is that it is not a conventional radiotracer method. A tracer method requires that the mercury isotope be in equilibrium with all mercury species in the sample (9). In these experiments, free $^{203}Hg^{2+}$ was added to samples containing mercury naturally complexed to clays and organic materials, and isotopic equilibrium was probably not achieved during the incubation period. It is also necessary to know the concentration of the bioavailable mercury species, which we were not able to measure. Therefore, absolute methylation rates, i.e., micromolar per liter per hour, cannot be calculated. Although the quantity of free carrier mercury added to the samples was small, the total mercury concentrations in the water samples were increased about 300-fold. For sediment-floc samples the total mercury concentrations were increased only about a tenth, but the in situ bioavailable mercury was probably increased significantly, since, in experiments using mild mercury extraction techniques, i.e., calcium chloride, extractable mercury was less than 0.01% of the total mercury in Clay Lake sediments (19). Thus if the in situ bioavailable mercury pool was significantly increased with labeled Hg^{2+}, and because mercuric ion is the mercury species that is the substrate for biological methylation (7, 14), then methylation rates could be expressed as a relative rate (i.e., disintegrations per minute per liter per hour). This appears to be a valid approach since we have

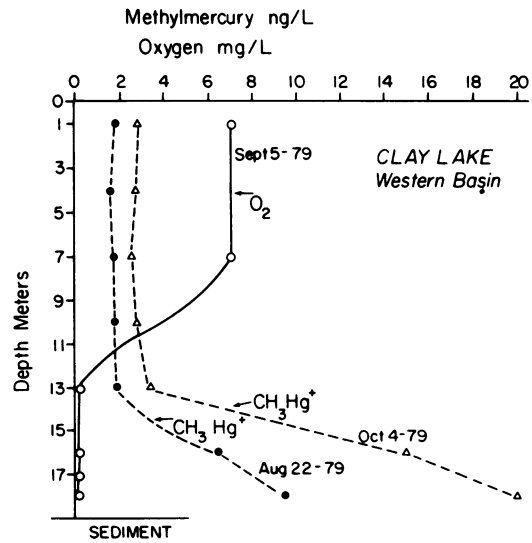

FIG. 3. *Depth profiles demonstrating the increase in methylmercury concentration that occurred during a 6-week interval. There was no detectable methylation occurring in the water column during this period. The sediment-floc was actively methylating in the presence of sulfide.*

observed progressively increasing disintegrations per minute (radioactive methylmercury) concentration in time course experiments in the laboratory (Table 1) and in field samples (31).

Although methylating activity can be compared within the same compartment of a lake, comparison between compartments (e.g., water versus sediments) may not be meaningful because of the large differences in mercury concentrations. Differences in ability of sediment type to complex mercury should also be considered. For example, production of $CH_3{}^{203}Hg^+$ by sediment-floc of non-mercury-contaminated Lake 114 in the Experimental Lakes Area (10) was about 10 times more rapid than that in mercury-contaminated Clay Lake. The most obvious differences between these sediments were the lower mercury concentration and smaller quantities of clay in Lake 114 sediments.

Despite its limitations, the method can be successfully applied to a wide variety of environmental research problems. These data are the first reported for biological mercury methylation in the water column of a lake (Fig. 1, Table 2), a possible significant source because of the lake's large volume. Armstrong and Scott (4) suggested, when evaluating mercury concentration changes in sediment, water, and fish, that biological mercury methylation might occur in Ball Lake, which is downstream in the same polluted system as Clay Lake, and Rudd et al. (32), in limnocorral experiments, concluded that it probably occurred in Clay Lake. Photochemical mercury methylation at the lake surface is another possibility, although this has only been demonstrated in the laboratory (1).

Coincident peaks of cyclic methylation activity in the sediment-floc and the water column of the eastern basin (Fig. 1, Table 2) suggest that there was a common factor controlling methylation. It is not known whether the cause of this cyclic behavior is the same as that noted by Hamdy and Noyes (17) when they investigated methylation by pure bacterial batch cultures. In Clay Lake, it appears from experiments on nutrient addition (Table 2) and community oxygen consumption (Fig. 2) that nutrient supply and micobial activity are the important factors controlling in situ methylation (23, 27). If this is the case, then the cyclic nature of methylation could be caused by the cyclic supply of phytoplankton-derived bacterial substrates (34) or periodic resuspension of sediments.

It is generally conceded that mercury methylation is reduced by about 10^{-3} in the presence of sulfide (15, 20) because Hg^{2+} is precipitated ($K_{sp} = 10^{-53}$) as bio-unavailable HgS. Jacobs and Keeney (20) accounted for the observed slow methylation in the presence of sulfide by suggesting that complexing organics competed with the sulfide to provide bioavailable organically complexed Hg^{2+}.

In Clay Lake the $^{203}Hg^{2+}$ was actively methylated in sulfide-containing sediment-floc samples taken from the anoxic western basin. Jackson and Woychuk (19) in their study of the same basin in 1978 found sediment pH of 6.6 with about 30 μg (dry weight) of bound sulfide per g. The sulfide was present as amorphous FeS (G. J. Brunskill, personal communication), which has a dissociation constant of about 10^{-16} to 10^{-17} (13). Probably the geochemical dissociation of FeS and the sulfide binding of mercuric ion as HgS did not occur quickly enough to severely inhibit methylation, since the activity of the anoxic western basin samples and that of the aerobic eastern basin sediment-floc samples were not dissimilar (Table 2).

This observation may have important consequences for the mercury methylation process and movement of mercury through aquatic ecosystems. In the case of the western basin of Clay Lake, some of the mercury entering the anoxic sediments on particulates was probably methylated before it could be bound as mercuric sulfide. The result was the gradual accumulation of hypolimnetic methylmercury in the presence of sulfide as shown in Fig. 3. Thus, instead of being permanently sequestered in the sediments as HgS, some of the newly sedimented mercury was released back into the lake water as methylmercury.

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