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STUDIES OF CONCENTRATIONS OF METHYL MERCURY IN SEDIMENTS FROM THE St. CLAIR SYSTEM AND RATE OF BIOLOGICAL METHYLATION IN INCUBATED SAMPLES OF SEDIMENTS

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The levels of methyl mercury in lake and river sediment from the St. Clair system have been investigated in relation to total-mercury concentrations and to levels of ethyl-lead compounds which were detected during the work.

The co-occurrence of ethyl-lead and methyl mercury indicate a connection probably in the form of a chemical methylation of mercury from methyl groups among the ethyl ones in alkyl-lead.

In the incubated river and lake sediment from the St. Clair system no biological methylation of mercury could be statistically certified.

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Introduction

In 1970 high concentrations of mercury in fish was found in Lake St. Clair and the adjacent parts of St. Clair River, Detroit River and Western Lake Erie. Further investigations have described patterns of mercury contamination of fish more in detail and together with analyses of mercury concentrations in sediment these investigations suggested the outfall from DOW Chemical in Sarnia as a possible main source for the contamination. In an attempt to evaluate the importance of this source in relation to others, a series of investigations have been performed by OWRC, Dr. John Wood, University of Illinois, and the Swedish Water and Air Pollution Research Institute.

The present study on "Concentrations of Methyl Mercury in Sediments from the St. Clair System and Rate of Biological Methylation in Incubated Samples of Sediments" is one in this series.

The results are published in this report after a long delay due to the previously unclear legal situation. The reason for publishing it is that many references are made to some of the findings without the report being available.

CONCENTRATIONS OF METHYL-MERCURY IN SEDIMENT FROM THE St. CLAIR SYSTEM

Materials and Methods

I. Analytical methods

A. Determination of methyl-mercury by gas chromatography

Principle

The methyl-mercury-(MeHg) complexes in the sample are transferred into the bromide complex by acidification and addition of sodium bromide. Addition of copper sulphate is made to mask any sulfhydryl groups present in the sample. The resulting MeHg bromide is extracted into benzene.

The benzene extract is then treated with aqueous sodium thiosulphate. This separates certain constituents which could interfere with the GLC analysis. The MeHg will be transferred to the aqueous phase as the thiosulphate complex. Addition of Cupric bromide is followed by extraction of MeHg bromide into benzene.

Procedure

The incubated samples were analysed in the following manner: Immediately upon opening each flask 10 ml of acidic NaBr-solution and 1 ml of $CuSO_4$-solution were added. The flask was closed, gently shaken, and then allowed to stand for approx. 30 minutes. The content was transferred to a 30 ml test tube and 10 ml of toluene was added, extracted 2 min. and centrifuged. The toluene layer was transferred with a pipette into a centrifuge tube. The transferred volume was noted. 2 ml of 0.010 M thiosulphate was added

and followed by extraction. The mixture was centrifuged. The aqueous phase was transferred to an 8 ml test tube by means of a Pasteur pipette.

1.5 ml of Cupric bromide and 1.0 ml of benzene was added to the extract and followed by extraction during 1 minute. The benzene extract was analysed by GLC.

Instrument conditions

Chromatograph: Varian Aerograph 204 B

Detector: Electron capture, Tritium

Column: 150 cm glass column with ID 0.2 cm. 5 per cent Carbowax 20 M on Varaport-30 100-120 mesh.

Carrier gas: N_2, purified by passing through molecular sieve 5 A, 20-40 ml/min. The carrier gas was virtually free from O_2.

Temp. of injector block: 190° C

Temp. of column: 190° C

Temp. of detector: 220° C

Retention time: MeHgBr c. 3 min.

Volume sample: 1-10 µl

B. Determination of organo-lead compounds and ethyl-mercury

When the sediment samples from St. Clair River were analysed for methyl-mercury, two peaks appeared that, according to experience from analyses performed in Sweden, showed the characteristics for $(Et)_3Pb^+$ and $EtHg^+$. As these compounds were not available in the laboratory, high-octane gasoline containing $(Et)_4Pb$ was mixed with an aqueous solution of $HgCl_2$. In

this procedure $(Et)_3PbCl$ and EtHgCl is formed. These compounds are extractable in the same way as $MeHg^+$ and after extraction the samples were injected into the GC. The retention times of the two peaks thus obtained were in accordance with those for the two unknown samples. Later, Mr. John Sager at the laboratory of OWRC, performed the same test on both Carbowax 20 M column and BDS column with the same results. Furthermore, it was shown which of the peaks that emanated from $(Et)_3Pb$ and EtHg resp. for the two columns. It was also shown, that $MeHg^+$ was formed in these experiments; probably due to the presence of (Me)Pb-compounds in the gas line.

The high levels of organo-lead compounds in the sediment appeared be due to discharge of such compounds from among others Ethyl Corporation. When the sewage water from this plant was treated with $HgCl_2$-solution, $(Et)_3Pb^+$, and $MeHg^+$ was formed.

C. Determination of total mercury content, dry weight, and ignition loss

These analyses were performed by the OWRC laboratory.

II. Investigation area

The investigation area in the St. Clair River system was divided into five sub-areas with regardto their content of total mercury in the surface sediment, which was mapped down, in 1970, by OWRC.

The total mercury content in the different parts of the investigation area.

A. Lake Huron and St. Clair River, upstream the outlet point of DOW Chemical	<0.1	mg tot-Hg/kg dry weight
E. St. Clair Lake, Detroit River	0.1-1	do.
D. St. Clair Lake. Delta area	1-10	do.
C. St. Clair River	10-100	do.
B. Outlet area of DOW Chemical	>100	do.

III. Methods of sampling

25 stations were distributed on the 5 sub-areas as follows:

Area A, 2 stations
Area B, 2 do.
Area C, 4 do.
Area D, 10 do.
Area E, 7 do.

On each station, 3 samples were collected with a Shipek dredge. The dredge was washed with detergent and rinsed with alcohol just before a sample was collected. Each sample was transferred from the dredge into a sterile 500 ml glass stoppered bottle with a porcelain scoop, which had been sterilized by dipping into alcohol.

The bottles were transported to the laboratory and the content was analysed for total- and methyl mercury, dry weight and per cent loss on ignition.

Results and discussion

The results obtained showed very low concentrations of methyl-mercury in sediments from the background areas with total mercury concentrations below 0.1 ppm in Lake Huron and the upper part of St. Clair River.

As soon as the area with extremely high concentrations of total mercury (> 100 ppm) was reached downstream the outfall of DOW Chemical Corporation the concentrations of methyl-mercury was found to be extremely high too.

Together with the high concentrations of methyl-mercury strong indications (same retention time on the column of the gaschromatograph) were found on the presence of ethyl mercury and alkyl-lead compounds.

Thus the presence of methyl-mercury in the sludge was established and the occurrence of ethyl-mercury and ethyl-lead compounds was strongly indicated.

However, the relation between total mercury and methyl-mercury concentrations in the sediment did not conform to the pattern normally observed when methyl-mercury is formed through the activity of microorganisms. Furthermore, the co-occurrence of ethyl-mercury and ethyl-lead compounds suggested a chemical alkylation of mercury (giving methyl- and ethyl-mercury) from alkyl-lead compounds as described earlier by Beijer, Jernelöv and Rudling.

One statistical approach to these results was to determine the single and multiple correlation between concentrations of methyl mercury in sediment on one hand and total-mercury and ethyl-lead concentrations on the other. This was done by means of different kinds of linear regression analyses.

This method does not allow any firm conclusions in this situation as the conditions in several aspects deviate from the ideal ones.

1. Ethyl-lead concentrations are classified only to magnitude and sub-divided into only four groups (<5, 5-50, 50-400, >400 represented by 5, 25, 200, 400 respectively).
2. The occurrence of total-mercury and ethyl-lead might not be stochastically independent.
3. The non-random distribution of the sampling stations.
4. Unidentified factors might cause a part of the variation in methyl mercury concentration.

Furthermore, although methyl mercury is assumed to be formed from methyl-lead compounds, the concentration of ethyl-lead has been used in the statitical tests under the assumption that occurrences of methyl- and ethyl-lead are strongly correlated.

However, the results from these statistical analyses can be used as indicators as to the relative ability of the variation in concentrations of total-mercury and alkyl-lead compounds to explain variations in methyl mercury concentrations in sediment.

The following table shows some of the results of the statistical tests. In the table coefficients of correlation and proportions of variance in methyl mercury concentrations attributable to regression are shown.

		Sub-areas		
		A+B+C+D+E	B+C	A+D+E
Coefficients of correlation	MeHg-Tot Hg;$(Et)_3Pb^+$	0.85	0.78	0.90
	MeHg-Tot Hg	0.83	0.78	0.59
	MeHg-$(Et)_3Pb^+$	0.27	-0.40	0.80
	Tot Hg-$(Et)_3Pb^+$	0.09	-0.49	0.23
Proportion of variance due to regression on:	Tot Hg	0.68	0.60	0.35
	$(Et)_3Pb^+$	0.07	0.16	0.64
	Tot Hg;$(Et)_3Pb^+$	0.72	0.60	0.81

When the whole area is considered, a significant positive multiple correlation is found between MeHg and Tot Hg; $(Et)_3Pb^+$ where the correlation MeHg-Tot Hg is stronger and contributes more to the multiple correlation than does MeHg-$(Et)_3Pb^+$. However, from the table of residuals it can be seen that the samples within the sub-areas B and C deviate considerably in both directions from the regression model, Therefore, independent calculations were performed on data from these two sub-areas on one hand and A, D, and E on the other. Then, for sub-areas A, D, and E taken together a clearly significant multiple correlation was found between MeHg and Tot Hg $(Et)_3Pb^+$ to which $(Et)_3Pb^+$ contributes more than does Tot Hg.

In sub-areas B and C on the other hand no effect of $(Et)_3Pb^+$ on MeHg - or even a negative one - could be indicated. This might be attributable to the extremely high contamination in the near-source-area (outside DOW Chemical and Ethyl Cooperation resp.) where also the crued classification of $(Et)_3Pb^+$ is of special importance.

RATE OF BIOLOGICAL METHYLATION OF MERCURY IN INCUBATED SAMPLES OF SEDIMENT FROM THE St. CLAIR SYSTEM

Incubation technique

The samples arrived at the laboratory in glass stoppered bottles. The incubations were done in the evening of the day of sampling.

From each sample five aliquots of approx. 10 g sediment were transferred to sterilized 250 ml glass stoppered Erlenmeyer flasks and the exact sediment weight was noted. The sediment in the original sampling bottle was not homogenized before the aliquot was removed. The reason for this was the technical difficulties to homogenize samples consisting of a mixture of easily suspendible organic matter and gravel, sand or clay - a two phase system - under sterile conditions. A clean sterilized glass spoon was used when the sediment was transferred from the original sample.

From each station in the investigation area bottom water was sampled with a sterilized rubber-bulb. 10 ml of this water was added

to the sediment and the mixture of sediment and water was shaken before incubation. The incubation temperature was +16° C. After 1, 6, 13, and 20 days resp. the five sediment suspensions were analysed for methyl mercury.

When the samples were taken in the St. Clair system, five sub-areas were defined according to the order of size of mercury content in the sediment, that had appeared in earlier investigations. However, the analyses came out to form a somewhat different picture with respect to total mercury content of sediments. On the basis of these new data and the content of methyl mercury in the sediments the sub-areas were modified prior to the statistical evaluation of the results.

Furthermore, due to the variations within parallel samples, which in turn is a consequence of the type of sediment in question, in one statistical evaluation, all the single samples were considered to be randomly distributed within the whole sub-area. This means, that the number of variates used in the statistical tests in this case equalled three times the number of stations within the sub-area.

The results from consecutive, as well as extreme, times of incubation have been compared by means of Student's t-test. Degrees of freedom, t-values, and resulting p-values have been calculated.

Results

No regular patterns in the changes of mean concentrations of methyl mercury after different times of incubation can be discovered in the diagrams. The situation is the same whether methyl mercury concentrations or the ratios between methyl- and total-mercury concentrations are considered.

Apparently, for each station, the variations between the parallel samples, as indicated by the plotted standard errors, are so large compared to the differences between mean values that no statistical significance can be found for any change with time.

The statistical comparison between consecutive times of incubation within each sub-area showed no significant difference except for one case.

Discussion

Thus, in these experiments, there is no significant evidence of any biological methylation of mercury.

In the experiments where laboratory substrate was infected with microorganisms from the sediments, methyl mercury was found after addition of inorganic divalent mercury and the amounts increased with time of incubation. The rate of methylation was found to be higher after anaerobic incubation than after aerobic.

Disregarding - for the following discussion - differences in substrate, form or association of mercury and relative abundance of different microorganisms there are two striking differences, that

have been found to be of importance for the methylation of mercury, between the two series of experiments.

1) pH was much higher in the incubated river and lake sediments than in the laboratory substrate. A high pH is known to increase the formation of volatile methyl mercury compounds.

2) Efforts were made to keep the incubated river and lake sediments as aerobic as possible. The experiments with laboratory substrate gave higher methylation rates under anaerobic conditions.

The failure to demonstrate biological methylation of mercury in the incubated river and lake sediments can mean that

1) no biological methylation occurred;
2) the rate of methylation was so low that the experimental design did not allow for its detection;
3) the end product was a volatile methyl mercury compound that was not trapped with the acid-extraction procedure that was used.

The second and third of these possibilities need some further discussion.

Due to the high concentrations of alkyl-mercury compounds (methyl- and ethyl-) presumably originating from chemical alkylation as indicated by the co-occurrence of alkyl-lead compounds, in some of the sampling areas, the possibilities to detect a small increase against the background is substantially reduced.

In this situation the choice of parallel series from the same station rather than conventional replicates from one homogenized sample further reduced the possibilities to statistically certify a small change.

On the other hand it can be argued that a biological methylation,

which is undetectable against the background of chemically methylated mercury and the variation in the parallel samples, could not occur at a very high rate and should thus - if it takes place - be without very large significance.

Previous experiments have shown that the formation of volatile dimethyl-mercury is favoured by high pH. Dimethyl-mercury is known to have a tendency to evaporate from the water system - if not dissolved in fat-tissues of organisms on its way to the surface - and thus only to a limited extent contribute to the contamination of fish in the lake or river where it is formed.

It is tempting to try to explain the lack of significant evidence of biological methylation in the incubated lake and river sediment samples with the hypothesis that the end products were volatile methyl mercury compounds that were not caught in the acid extraction. This would explain the differences between this series of experiments and the one where synthetic substrate was used.

It would also bring the results in agreement with those obtained in similar studies in Scandinavia and Southern USA and allow the use of similar mathematic-ecological models for the turnover of mercury in the St. Clair system.

On the other hand the acid extraction used in the experiment should according to previous experience, trap the main part of dimethyl-mercury present in the E-flask. Thus, at the moment, there is nothing to support the hypothesis of formation of persistent volatile methyl mercury compounds in the lake and river sediment incubation experiment, but the fact that it would fit established models

Summary and Conclusions

The levels of methyl mercury in lake and river sediment from the St. Clair system have been investigated in relation to total-mercury concentrations and to levels of ethyl-lead compounds which were detected during the work.

The co-occurrence of ethyl-lead and methyl mercury indicate a connection probably in the form of a chemical methylation of mercury from methyl groups among the ethyl ones in alkyl-lead.

In the incubated river and lake sediment from the St. Clair system no biological methylation of mercury could be statistically certified.