EPA-600/3-75-014
October 1975

Ecological Research Series

METHYLATION OF MERCURY IN A TERRESTRIAL ENVIRONMENT

Environmental Monitoring and Support Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Las Vegas, Nevada 89114

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/3-75-014
October 1975

METHYLATION OF MERCURY IN A TERRESTRIAL ENVIRONMENT

by

R. D. Rogers
Monitoring Systems Research and Development Division
Environmental Monitoring and Support Laboratory
Las Vegas, Nevada 89114

ROAP No. 21BKN
Program Element No. 1AA006

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
LAS VEGAS, NEVADA 89114

DISCLAIMER

This report has been reviewed by the Environmental Monitoring and Support Laboratory-Las Vegas, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS

LIST OF TABLES

INTRODUCTION

Since the time Fujiki (1963) first proposed the possibility that the Minamata Disease was caused by natural methylmercury formation, the majority of reports on this subject have dwelled on the occurrence of methylmercury in anaerobic aquatic systems (Jensen and Jernelov, 1969; Wood *et al.*, 1968; Lander, 1970; Jernelov, 1972). However, there has also been some work on aerobic aquatic systems. Fagerstrom and Jernelov (1971) indicated that under aerobic aquatic conditions mercuric sulfide can be shown as the initial substrate for methylmercury synthesis. Methylmercury has also been found in aerobic microbial cultures (Parks *et al.*, 1973). Bisogni and Lawrence (1973) found that methylation rates for aerobic aqueous systems were higher than those for corresponding anaerobic systems.

While many hypotheses on mercury cycling have become prevalent as a result of these findings, the methylation of mercury in terrestrial environments has only recently been found. Beckert *et al.*, (1974) found methylmercury in desert soils which had been amended with mercuric nitrate containing mercury-203. The presence of methylmercury was discovered using thin-layer chromatography, but the amount was not quantified. Methylmercury has also been found in the atmosphere above a soil amended with mercuric chloride (Braman and Johnson, 1974).

Coal and other fossil fuels earmarked for use in the nation's energy program are known to contain elevated levels of the element mercury. As a result, concern has been expressed over the lack of understanding of mercury cycling in terrestrial environments, but it should also be noted that there are many additional natural and man-made terrestrial mercury exposure pathways which are not fully understood. This study was undertaken to confirm the findings of occurrence of methylmercury in terrestrial soil systems, and to study the kinetics involved in its production.

CONCLUSIONS

The following conclusions were drawn from the current study.

1. Methylation of mercury does occur in a terrestrial environment.

2. There is a mechanism available for the decrease in methylmercury concentration with time.

3. It is possible that the methylation process could, in part, be abiotic.

4. Non-sterile systems have a net loss of methylmercury such that there is less methylmercury in non-sterile soils than in the sterile soils.

5. Standing water on the surface of the soil reduces the loss of methylmercury.

6. The rate of conversion of mercury into methylmercury is dependent upon ionic mercury concentration, soil texture, temperature, and soil moisture.

These conclusions open exciting possibilities for future research.

RECOMMENDATIONS FOR FUTURE RESEARCH

1. Determine the total mercury budget. It will be possible to do this with the use of semi-closed systems. Soil amended with mercuric ion would be maintained in a flask. At some predetermined rate, the flask atmosphere would be flushed through a series of selective absorption tubes which will separate the mercury in the air into mercuric ion (Hg^{2+}) compounds, methylmercury (CH_3Hg^+) compounds, metallic mercury, and dimethylmercury (Braman and Johnson, 1974). It will also be possible, by conventional methods, to determine the total mercury and synthesized methylmercury remaining in the soil environment. This work will help in understanding not only the fate of applied mercuric ion, but also the fate of synthesized methylmercury. The important fact is that this system of analysis has been developed and is ready for use.

2. Conduct field work at terrestrial sites known to contain high background levels of mercury. Many areas fall into this category. For example, a belt of mercury deposits runs through southern California, Nevada, Idaho, and into Canada. Soils sampled from one part of the belt in Nevada were found to contain a mercury concentration of 4,240 parts per billion. McKeague and Kloosterman (1974) report finding soils in Canada containing mercury up to 14,000 parts per billion. Further work in such areas will be of great interest.

3. Use of a microwave emission spectrometric detector system (Talmi, 1975), which will increase the detection limits for methylmercury by at least a factor of 10, will make it possible to study the kinetics of methylmercury formation using reduced quantities of initial mercury substrate.

4. Work should be expanded in the areas of mercury fixation in soils and the effect of such variables as pH, temperature, moisture content, clay content, and various soil elements.

5. There should be an attempt to isolate the postulated biotic system responsible for methylation and the subsequent loss of methylmercury.

6. Because the possibility for abiotic methylation has been shown, further investigation of this possibility is recommended.

MATERIALS AND METHODS

Soils used for this investigation were obtained from an area around Logandale in the Moapa Valley of Nevada. The Valley is approximately 60 miles

northeast of Las Vegas and is primarily an agricultural locality. The soils were collected in December 1974 and had supported crops during the previous growing season. Depth of the soil collections was limited to the upper 10 centimeters (cm) of the Ap horizon*. The moist soil was processed through a 2-millimeter sieve and stored at room temperature in plastic bags. The physical and chemical properties of the soils are found in Table 1.

Table 1. PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

Soil (Texture Classification)	Series	% Sand	% Clay	% Organic Carbon	meq per 100 g CEC*	pH
Sand (Loamy sand)	Bluepoint - a member of the mixed, thermic family of Typic Torripsamment	79.8	3.5	.53	4.3	9.0
Loam (Fine sandy loam)	Calico - a member of the coarse-loamy over clayey, mixed (calcareous), thermic family of Aquic Xerofluvents	53.9	10.8	1.30	12.7	8.6
Clay (Silty clay loam)	Overton - a member of the fine montmorillonitic, calcareous, thermic family of Mollic Haplaquepts	14.7	50.0	3.44	29.0	7.8

* CEC = cation exchange capacity

In all cases, mercuric nitrate ($Hg(NO_3)_2$) was used for the ionic mercury (Hg^{2+}) soil amendment and each treatment was carried out in triplicate. Because of the restricted amount of moisture which could be added to the soils under some soil moisture regimes, the mercury amendments needed to be in a highly concentrated solution. So that all studies would remain uniform with respect to the volume of mercury amendment, all soils were amended with the same volume of concentrated mercury solution. The volume of mercury solution added to the soils was 2 milliliters (ml). Unless specified otherwise, the concentration of the mercury solution was ~~500~~ 12,500 parts per million (ppm) mercury (Hg) as $Hg(NO_3)_2$, for a total addition of 25,000 micrograms (µg) Hg per 50 grams (g) of soil. In order to solubilize the $Hg(NO_3)_2$ in water, it was found necessary to add 2 or 3 ml of concentrated hydrochloric acid (HCl) per 100 ml Hg solution.

The amendment and incubation process was carried out in the following manner: 50 g of soil was spread thinly on a sheet of acetate and then

* "A" horizon, plow layer, *i.e.*, agriculturally disturbed topsoil

sprayed with 2 ml of the mercury solution using an atomizer. The amended soil was mixed with a spatula and poured into a 250-ml flask. The amount of water necessary to adjust the moisture content of the soil to the desired level was then added dropwise. The flask and soil were weighed and capped with a loose fitting aluminum foil cap. On alternate days during the period of incubation, the flask and its contents were reweighed and brought back to the initial weight by the addition of distilled water. All soils were incubated in the dark at 24 degrees Celsius (°C).

A modified Westoo (1966) method was used to extract methylmercury (CH_3Hg^+) from the soils. It was found that 50 ml of 6*N* HCl per 50 g of soil resulted in the best extraction of standard methylmercury chloride (CH_3HgCl) solution. The soils were highly calcareous and unless care was exercised effervescence, as a result of the acid addition, forced much material from the flask. Following the addition of acid, the flasks containing the soil solutions were shaken for 1 hour on a reciprocating shaker. The resulting mixture was then filtered under vacuum through Whatman No. 1 paper. The extracted soil was then rinsed with three additional 5-ml acid washes. Next, nanograde quality benzene was used to extract CH_3Hg^+ from the soil leachate. The soil leachate was extracted twice with two separate 50-ml quantities of the benzene using 250-ml separatory funnels.

A 1% cysteine solution was used to extract CH_3Hg^+ from the combined benzene washes. It was found that the two separate extractions with 6-ml quantities of the cysteine solution were necessary. The cysteine solution was made in the following manner: dissolve 1.000 g of cysteine hydrochloride ($HSCH_2CH(NH_2)COOH \cdot HCl \cdot H_2O$), 0.775 g of sodium acetate ($CH_3COONa \cdot 3H_2O$), and 12.500 g anhydrous sodium sulfate (Na_2SO_4), in that order, in 75 ml distilled water. Additional distilled water was added to make up the volume to 100 ml. This solution was adjusted to pH 8.3 with 5% sodium hydroxide just before use.

The two cysteine extracts were combined in a 60-ml separatory funnel, acidified with 10 ml of 6*N* HCl (the resulting mixture must have a pH of 1 or less), and extracted with 10 ml of benzene. This final benzene extract was analyzed by gas chromatography.

In order to evaluate the effectiveness of the extraction procedure, standard solutions consisting of 1 µg Hg as CH_3HgCl in distilled water were added to the three soils. The soils were extracted using the above method and the quantity of the extracted CH_3Hg^+ was compared to the amount initially added. From an average of nine replications for each soil, it was determined that with the sand soil there was a 57.1% recovery of applied CH_3Hg^+, loam soil 52.2%, and clay soil 38.9%. These findings were in general agreement with those reported by Krenkel (1974) who indicated that a soil's affinity for CH_3Hg^+ increases with soil clay content. By knowing the percent recovery of CH_3Hg^+ from these soils it was possible to calculate the amount of methylmercury contained in the soil by applying the appropriate correction factor for that soil.

The gas chromatograph used for these studies was a Hewlett-Packard Model 5713A with a nickel-63 linear electron capture detector. The attenuation of the chromatograph was adjusted so that 1 µl of 0.1 ppm Hg as CH_3HgCl in benzene caused a 3/4-scale deflection on the recorder. Figure 1 shows a typical

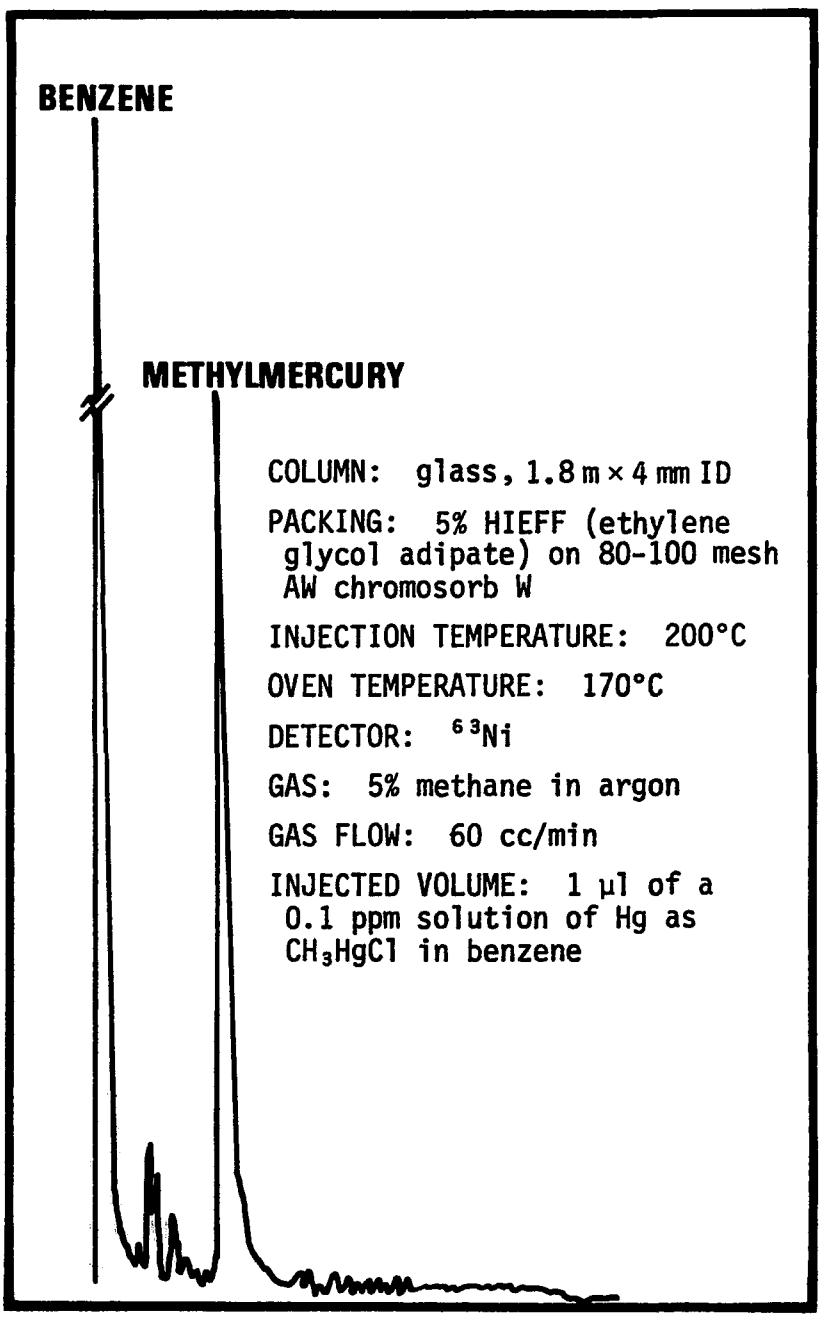

Figure 1. Typical gas chromatograph tracing for methylmercury

chromatograph for CH_3Hg^+ under the specified conditions.

Because of the hazard involved in using high concentrations of mercury, special masks were worn. These masks were 3M mercury vapor respirator masks, number 8707.

RESULTS

STERILE AND NON-STERILE SOIL

Biological mechanisms have been hypothesized as the causative agents for the synthesis of methylmercury. In order to verify this premise in a terrestrial environment, soils were sterilized by autoclaving and then amended with 25,000 micrograms of mercury as mercuric nitrate. Non-sterile soils amended with 25,000 micrograms of mercury as mercuric nitrate were used as controls. Both sets of soils were incubated in the dark at 24°C for 1 week.

Analyses of these soils produced striking results (Table 2). In every case, the autoclaved soils produced substantially more methylmercury than did the non-autoclaved soils. To substantiate these findings, the experiment was repeated. This time, in an effort to produce more effective sterilization, the soils were autoclaved at 4-hour intervals every other day for a period of 5 days. In addition, to enhance microbial growth, those soils used as non sterile controls were amended with a 20% glucose solution at 4 milliliters per 50 grams of soil. The mercury amendment and conditions of incubation were the same as those used in the initial experiment. Analytical results obtained from these soils (Table 3) were similar to those reported in Table 2; however, some differences were noted between the two sets of experiments. Those soils which received the extended autoclaving, except for sand, appeared to have increased concentrations of methylmercury over those found initially, while the glucose-amended soils when compared to the other non-sterile soils, except for sand, showed a decrease in methylmercury.

Table 2. METHYLMERCURY OCCURRENCE IN STERILE AND NON-STERILE SOIL SYSTEMS AFTER 1 WEEK OF INCUBATION

Soil	Concentration (ng CH_3Hg^+/50 g soil)	
	Sterile	Non-Sterile
Sand	105	74
Loam	223	169
Clay	318	215

Table 3. METHYLMERCURY OCCURRENCE IN STERILE AND GLUCOSE-AMENDED NON-STERILE SOIL SYSTEMS AFTER 1 WEEK OF INCUBATION

Soil	Concentration (ng CH_3Hg^+/50 g soil)	
	Sterile	Non-Sterile
Sand	108	81
Loam	307	127
Clay	420	190

These results appear to indicate the abiotic conversion of mercuric ion into methylmercury. In addition, it was possible that there was also a biological mediation occurring in the non-autoclaved soils leading to a reduction in methylmercury. This was indicated because those non-sterile soils amended with glucose, except sand, contained only about 40% the amount of methylmercury found in the autoclaved soil. However, the non-sterile soils without glucose amendment contained 70% as much methylmercury (Tables 2 and 3). Because soils amended with glucose showed an increase in the metabolic activities of many microbial species, it was possible that this resulting activity was responsible for the methylmercury loss.

It is tempting to speculate on the presence of a mercury cycle involving the methylation of mercury coupled with demethylation into other unknown forms of mercury. It was possible that biotic methylmercury was being produced as an intermediate whose gross occurrence was not observed because of a subsequent biotic mediated loss. Because there is much evidence for biotic demethylation (Magos *et al.*, 1964; Tonomura *et al.*, 1968; Frissel *et al.*, 1971: Tonomura *et al.*, 1972; Spangler *et al.*, 1973; Alberts *et al.*, 1974) this cycle would appear to be a biotic possibility. This possibility was further enhanced by the observation that the CH_3Hg^+ in these soils decreased with time and increased with temperature. These results will be discussed in later sections of this report. The possibility of a methylation-demethylation cycle occurring in soil, similar to that reported for aqueous systems, would greatly expand the understanding of mercury transformation.

MOISTURE CONTENT

A prevalent hypothesis has been that anaerobic conditions are necessary for the maximum formation of methylmercury. Other evidence is available indicating that methylmercury formation occurs at a higher rate in aerobic aqueous systems than in anaerobic ones (Bisogni and Lawrence, 1973). Similar work with soils is not available at this time.

Soils under varying oxygen tensions were examined for the production of methylmercury. Oxygen content was adjusted by varying the amount of moisture content in the soil. The higher the moisture content the lower the oxygen tension. Sets of the three soils were developed which contained 25%, 50%, and 75% of the soil's moisture-holding capacity. Under this regime, the higher the percentage of moisture in the soil, the lower the percentage of air spaces in the soil. Therefore, at 100% moisture holding, the soil should contain no air spaces, and it would be considered to be under a favorable anaerobic condition. In addition to the three different moisture conditions described, one set of the loam soil was maintained with 1 to 2 centimeters of standing water over a period of 3 weeks. All soils were amended at a rate of 25,000 micrograms of mercury as mercuric nitrate.

Results of this work (Table 4) indicate that during the first week of incubation there was little difference in the amount of methylmercury produced in any soil regardless of the soil moisture content. At the end of the third week, apparent differences were seen between treatments. The decrease in methylmercury coincides with improving conditions for anaerobic microbial growth. As a rule of thumb, optimum moisture-holding capacity for aerobic microbial growth is about 60%. This information, coupled with the results from the

sterile, non-sterile soils study of the preceding section, gives credence to the hypothesis that biologically initiated loss of methylmercury can occur and is, in fact, enhanced when conditions for biological proliferation are provided.

Table 4. METHYLMERCURY OCCURRENCE IN SOILS WITH VARIOUS MOISTURE CONTENTS

Soil	Moisture-Holding Capacity %	Concentration (ng CH_3Hg^+/50 g soil) 1 Week	3 Weeks
Sand	25	88	sample lost
	50	98	sample lost
	75	105	sample lost
Loam	25	223	130
	50	188	62
	75	212	54
Clay	25	277	144
	50	256	108
	75	195	51

The loam soil with standing water was analyzed after 3 weeks. Under these conditions, the system contained from 4 to 10 times more methylmercury (537 ng CH_3Hg^+ per 50 g of soil) than the soil under the other moisture regimes. Because this information seemed contradictory (the trend had been to increase the loss of methylmercury with increased moisture content), studies using mercury-203 tracer were carried out in order to determine what effects soil moisture had on the loss of methylmercury from the loam soil. Low quantities of mercury-203 labeled methylmercury were mixed into the soil. Fifty grams of the soil was placed into wide-mouth jars. This soil was then adjusted to 10%, 50%, and 100% moisture content in order to give even greater differences in moisture content than used previously. In addition, one set of the saturated soil contained 2 to 3 centimeters standing water. The mouth of each jar was covered with a charcoal filter. Jars containing samples were then incubated in the dark at 24°C. At the end of each week, the filters were replaced and the used filter analyzed by means of a gamma radiation detector for the presence of mercury-203. By the end of the second week, the mercury-203 content in the filters increased as the moisture content of the soils increased, while the soil covered with standing water showed relatively little loss.

This information indicated that decreasing aerobiosis increased the loss of methylmercury. The presence of standing water moderates this loss. It is possible that more methylmercury was found in the loam soil with standing water because regardless of the form of the volatile mercury, it is not easily lost through the water. Of course, it is also possible that the

mechanism for volatilization does not occur under standing water. In either event, systems under standing water appear to be producing more methylmercury because of a reduced mercury loss.

INCUBATION TEMPERATURE

The soils were incubated at various temperatures to ascertain the effect of this variable upon the methylation process. Temperatures selected were 4°C, 24°C, and 36°C. Results of this work are shown in Table 5.

Table 5. METHYLMERCURY OCCURRENCE IN SOILS INCUBATED AT VARIOUS TEMPERATURES

Soil	Incubation Temperature °C	Concentration (ng CH_3Hg^+/50 g soil)	
		1 Week	3 Weeks
Sand	4	42	60
	24	67	sample lost
	36	123	109
Loam	4	65	196
	24	169	62
	36	300	46
Clay	4	128	174
	24	179	107
	36	195	36

Incubation of the soils for 1 week produced the expected results; *i.e.*, the production of methylmercury was directly proportional to the temperature. After 3 weeks, however, except for sand, the concentration of methylmercury was inversely proportional to the temperature. These data indicated that both the formation and loss of methylmercury were temperature dependent. The soils maintained at 4°C increased in methylmercury content over the first week. Evidently, the mechanism for methylation is more active at a lower temperature than the mechanism for mercury loss.

MERCURY CONCENTRATION

In order to understand the effect that mercury concentration has on the kinetics of methylmercury production, the soils were amended with three increasing concentrations of divalent mercury ion. These concentrations were 5,000, 12,500, and 25,000 micrograms of mercury as $Hg(NO_3)_2$ per 50 grams of soil. The soils were incubated in the dark for 1 week. The results from this experiment

Table 6. METHYLMERCURY OCCURRENCE IN SOILS INCUBATED FOR 1 WEEK WITH AMENDMENTS OF VARYING MERCURIC NITRATE CONCENTRATION

Soil	$Hg(NO_3)_2$ Added (μg Hg/50 g soil)	CH_3Hg^+ Detected (ng/50 g soil)
Sand	5,000	28
	12,500	56
	25,000	98
Loam	5,000	38
	12,500	85
	25,000	188
Clay	5,000	41
	12,500	67
	25,000	256

indicated that the amount of conversion of divalent mercury ion to methylmercury was dependent upon the amount of applied mercury ion (Table 6). These same findings were reported by Parks *et al.* (1973) and Jensen and Jernelov (1969). While the loam soil showed a direct relationship between methylmercury produced and the amount of mercuric nitrate used, such a relationship for the other two soils was not as pronounced. These data indicated that the methylation process is also controlled by a rate-limiting step dependent upon substrate concentration.

DISCUSSION

The salient findings from this study are the confirmation of methylmercury synthesis from applied divalent mercury ion in terrestrial systems. In addition, there is a strong indication that a mechanism exists which prevents the accumulation of quantities of methylmercury. It is not known whether the loss of methylmercury is due to demethylation or volatilization, but this loss from soil systems was influenced by time, temperature, soil moisture, available carbon in the soil, and soil texture.

The site of methylmercury synthesis in soil has not been determined. From work with apparently sterile soils, there was evidence that the process could have been abiotic (Tables 2 and 3), but this evidence is circumstantial. The absolute sterilization of soil systems by a gas procedure has yet to be used, but absolute sterilization would not rule out the possibility that extracellular enzyme systems and organic substrates could account for the occurrence of methylmercury (Wood *et al.*, 1968; Imura *et al.*, 1971; Bertilsson and Neujahr, 1971). Whatever the process of methylation in this study, it was apparently dependent upon the concentration of mercuric nitrate applied to the soil (Table 6.)

Because only about 1×10^{-5} of the mercuric ion applied was detected as methylmercury, there appeared to be an unfavorable equilibrium for methylmercury production. However, it is possible that the mercury applied to the soil was fixed in such a way that most of it was not available for methylation. In support of this, it was found that after 1 week an acid loam soil amended with 25,000 micrograms of mercury as mercuric nitrate per 50 grams of soil contained three times more methylmercury than the alkaline loam soil treated in the same manner (612 ng Hg per 50 g of soil versus 188 ng Hg per 50 g of soil). The higher acidity could have increased the availability of mercury ion.

Temperature also had an effect upon the rate of methylation. Soils incubated at lower temperatures contained less methylmercury at the end of 1 week than soils incubated at elevated temperatures (Table 5). These findings were in agreement with McArthur and Sommers (1974) who found that methylation rates in two calcareous lake sediments were doubled by increasing the temperature from 4°C to 25°C. At the end of 3 weeks, the soils at lower temperatures showed a net increase in methylmercury, while soil maintained at higher temperatures had a net decrease.

An increase in moisture content and the amount of available carbon also increased the net loss of methylmercury with time (Tables 2, 3, and 4). An increase in available carbon has also been shown to increase methylmercury loss in calcareous lake sediments (McArthur and Sommers, 1974). Such losses under alkaline conditions could partially explain the findings of D'Itri (1972). He found that neutral and alkaline environments favor the formation of dimethylmercury, which is more volatile than monomethylmercury. These factors all indicate that the loss of methylmercury from soil was mediated by biological systems. It is important to further define this pathway for methylmercury loss from soil since the presence of such a pathway reduces the probability of methylmercury buildup in the terrestrial environment.

Soil texture was found to be related to the occurrence of methylmercury in both sterile and non-sterile soils. The clay soil contained the most methylmercury, followed by the loam soil, and finally the sand. The cause for this phenomenon has not yet been investigated. It is possible that methylmercury production depends upon available surface area, since the same increase in methylmercury content with increase in clay content was also noted for the autoclaved soil. Also, the biological synthesis of methylmercury could be expected to be the greatest under conditions favorable for microbial growth. Bacterial counts of the three soils used in this study showed that microbial numbers increased with clay content. In addition to this, Van Faassen (1973) found that in soils treated with mercuric chloride, microbial processes were inhibited more strongly in sand than in clay soil. It was reasonable, then, to expect the clay soil would have an elevated methylmercury content. The discrepancies between the sand and the other soils, with respect to the loss of methylmercury over time and increasing temperature (Tables 2, 3, and 4), are further evidence of the biological amelioration of clay content and the detrimental effect that mercury has on microbial populations associated with the sand.

REFERENCES

Alberts, J. J., J. E. Schindler, R. W. Miller and D. E. Nutter, Jr. "Elemental Mercury Evolution Mediated by Humic Acid," *Science, 184*, pp 895-897 (1974).

Beckert, W. F., A. A. Moghissi, F. H. F. Au, E. W. Bretthauer and J. C. McFarlane. "Methylmercury: Evidence for Its Formation in a Terrestrial Environment," *Nature, 249*, pp 674-675 (1974).

Bertilssen, L., and H. Y. Neujahr. "Methylation of Mercury Compounds by Methylcobalamin," *Biochemistry, 10*, pp 2805-2808 (1971).

Bisogni, J. J., Jr., and A. W. Lawrence. "Kinetics of Microbially Mediated Methylation of Mercury in Aerobic and Anaerobic Aquatic Environments," USDA Publication No. PB-222025 (1973).

Braman, R. S., and D. L. Johnson. "Ambient Forms of Mercury in Air," Proceedings of the Second Annual NSF RANN Trace Contaminants in the Environment, pp 75-78 (August 29-31, 1974).

D'Itri, F. M. *The Environmental Mercury Problem*, CRC Press, Cleveland, Ohio, pp 63-67 (1972).

Fagerstrom, T., and A. Jernelov. "Formation of Methylmercury from Pure Mercuric Sulphide in Aerobic Organic Sediment," *Water Research, 5*, pp 121-122 (1971).

Frissel, M. J., P. Poelstra, P. Reiniger and H. A. Das. "Contamination of the Soil with Mercury," *Radioecology Applied to the Protection of Man and His Environment*, Commission of the European Communities International Symposium, pp 7-10 (1971).

Fujiki, M. "Studies on the Course that the Causative Agent of Minamata Disease Was Formed, Especially on the Accumulation of the Mercury Compound in the Fish and Shellfish of Minamata Bay," *J. Kumamoto Med. Soc., 39*, p 494 (1963).

Imura, N., E. Sukegawa, S. Pan, K. Nagae, J. Kim, T. Kwan and T. Ukita. "Chemical Methylation of Inorganic Mercury with Methylcobalamin, A Vitamin B_{12} Analog," *Science, 172*, pp 1248-1249 (1971).

Jensen, S., and A. Jernelov. "Biological Methylation of Mercury in Aquatic Organisms," *Nature, 223*, pp 753-754 (1969).

Jernelov, A. "Factors in the Transformation of Mercury to Methylmercury," *Environmental Mercury Contamination*, edited by R. Hartung and B. D. Dinman, p 167 (1972).

Krenkel, P. A. "Mercury: Environmental Considerations, Part II," *Critical Reviews in Environmental Control, 4*, pp 251-339 (1974).

Lander, L. "Biochemical Model for the Biological Methylation of Mercury Suggested from Methylation Studies *in vivo* with *Neurospera crassa*," *Nature, 230*, pp 452-453 (1970).

Magos, L., A. A. Tuffery and T. W. Clarkson. "Volatilization of Mercury by Bacteria," *Brit. J. Industr. Med., 21*, pp 294-298 (1964).

McArthur, F., and L. E. Sommers. "Mercury Transformation in Lake Sediments," Agronomy Abstracts Annual Meetings, Chicago, Illinois (November 10-15, 1974).

McKeague, J. A., and B. Kloosterman. "Mercury in Horizons of Some Soil Profiles in Canada," *Can. J. Soil Sci., 54*, pp 503-507 (1974).

Parks, G. A., F. W. Dickson, J. O. Leckie, P. L. McCarty, P. Berendson and K. L. Pering. "Part of Trace Elements in Water: Origin, Fate, and Control," Progress Report, Stanford University, March 1972 to February 1973, pp 247 (1973).

Spangler, W. J., J. L. Spigarelli, J. M. Rose and H. M. Miller. "Methylmercury: Bacterial Degradation in Lake Sediments," *Science, 180*, pp 192-193 (1973).

Talmi, V. "The Rapid Sub-Picogram Determination of Volatile Organo-Mercury Compounds by Gas Chromatography with a Microwave Emission Spectrometric Detector System," *Analytica Chimica Acta, 74*, pp 107-117 (1975).

Tonomura, K., K. Madeda and F. Futai. "Studies on the Action of Mercury Resistant Microorganism on Mercurials, (II) The Vaporization of Mercurials Stimulated by Mercury-Resistant Bacterium," *J. Ferment. Technol., 46*, pp 685-692 (1968).

Tonomura, K., K. Furukawa and M. Yamada. "Microbial Conversion of Mercury Compounds," *Environmental Toxicology of Pesticides*, edited by F. Matsumura, G. M. Bores and T. Misate (1972).

Van Faassen, H. G. "Effects of Mercury Compounds on Soil Microbes," *Plant and Soil, 38*, pp 485-487 (1973).

Westoo, G. "Determination of Methylmercury Compounds in Foodstuffs, (I) Methylmercury Compounds in Fish, Identification and Determination," *Acta Chemica Scandivica, 20*, pp 2131-2137 (1966).

Wood, J. M., F. S. Kennedy and C. G. Rosen. "Synthesis of Methylmercury Compounds by Extracts of a Methanogenic Bacterium," *Nature, 220*, pp 173-174 (1968).

GPO 967-357

TECHNICAL REPORT DATA *(Please read Instructions on the reverse before completing)*		
1. REPORT NO. EPA-600/3-75-014	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE METHYLATION OF MERCURY IN A TERRESTRIAL ENVIRONMENT		5. REPORT DATE October 1975 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Robert D. Rogers		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Monitoring and Support Laboratory Office of Research and Development U.S. Environmental Protection Agency Las Vegas, Nevada 89114		10. PROGRAM ELEMENT NO. 1AA006 21BKN 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as above		13. TYPE OF REPORT AND PERIOD COVERED Annual FY-75 14. SPONSORING AGENCY CODE EPA-ORD, Office of Monitoring and Technical Support
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

Methylation of applied divalent mercury ion was found to occur in terrestrial soil systems. The production of methylmercury was affected by soil texture, soil moisture content, soil temperature, concentration of the ionic mercury amendment, and time. Methylation was directly proportional to percent clay content, moisture content, temperature, and mercury concentration. After an initial buildup of methylmercury in the soil, there appeared to be a mechanism that decreased the methylmercury content with increasing time.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
*Mercury (metal) *Mercury organic compounds *Soils	Methylmercury	07B 07C 06F
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED 20. SECURITY CLASS *(This page)* UNCLASSIFIED	21. NO. OF PAGES 20 22. PRICE

EPA Form 2220-1 (9-73)