Degradation of Methylmercury by Bacteria Isolated from Environmental Samples

WILLIAM J. SPANGLER, JAMES L. SPIGARELLI, JOSEPH M. ROSE, REID S. FLIPPIN, AND HOPE H. MILLER

Midwest Research Institute, Kansas City, Missouri 64110

Received for publication 6 November 1972

A total of 207 bacterial cultures, isolated from environmental samples, was screened for ability to degrade methylmercury. Of these, 30 were found positive for aerobic demethylation. Twenty-two of these were shown to be facultative anaerobes and 21 of these degraded methylmercury anaerobically. All positive species volatilized methylmercury aerobically, and methane was produced as a degradation product. Although methylmercury degradation was complete in most cases, material balances indicated some of the inorganic mercury formed was not volatilized and is presumed bound to the cells. All positive isolates were tolerant to at least 0.5 µg of methylmercury per ml, and the extent of volatilization of mercury increased with concentration to the threshold value. The results indicate that demethylating species are prevalent in the environment and may be important in suppressing the methylmercury content of sediments.

The recent discovery of methylmercury contamination in North American fish has resulted in the recognition of environmental mercury pollution as a contemporary problem. The methylmercury found in fish has been generally considered to be the culmination of a complex food chain beginning with methylation of inorganic mercury in sediments. The work of Jensen and Jernelev (5), Wood et al., Imura et al., and Bertilsson and Nejuahr (1, 4, 6) has indicated that mercury can be methylated either by enzymatic (microbial) or chemical (nonenzymatic methylation of mercury by methylcobalamin) mechanisms. Microbial methylation of mercury has recently been shown in a pure culture of Clostridium cochlearium isolated from soil (7) and methylation by organosilicons (trimethylsilyl salts) has been suggested by DeSimone (2) as a probable source of methylmercury in sediments. Whether any or all of these processes occur to a significant extent in the aquatic environment is yet to be demonstrated conclusively. The inability to find even traces of methylmercury in most sediments taken from areas highly polluted with inorganic mercury leads one to question whether significant methylation occurs in sediment under environmental conditions or whether the turnover rate of any methylmercury formed is such that significant concentrations do not accumulate.

In previous studies, we have shown that, in sediments incubated in the laboratory, concomitant methylation of inorganic mercury and demethylation of methylmercury can occur. Results of our current investigation indicate that several microbial species, capable of degrading methylmercury, are easily isolated from environmental samples and could be responsible for rapid turnover of methylmercury formed in sediments.

MATERIALS AND METHODS

Collection of samples. Samples of fish and sediment were taken from the delta area of Lake St. Clair (Mich.) in early spring and late summer. Four sites were used for collection of sediment at each sampling. Sediments were obtained by using an Ekman dredge sampler. A portion of each sediment sample was inoculated into aerobic and anaerobic medium (tryptic soy broth [TSB, Difco] and TSB plus 0.025% cysteine, nitrogen-flushed, respectively) at the site of collection. The sediment samples were placed in Whirl-pak bags, flushed with sterile nitrogen, and placed in a thermos bottle which was, in turn, flushed with nitrogen before sealing. All samples were returned to the laboratory on the day of collection for immediate isolation of organisms, initiation of long-term methylation experiments, and analysis of fish and sediment samples for total mercury and methylmercury.

Isolation of organisms. Organisms used in this study were obtained by direct isolation or isolation
from enrichment cultures. Organisms were isolated from sediments by direct isolation on tryptic soy agar (TSA) containing 0 to 320 μg of Hg²⁺ (as HgCl₂) per ml. Serial dilutions were made and plated to 10⁻¹ g of sediment by using the spread plate technique. Representative organisms were picked from high dilution plates containing 20 to 200 organisms. Mercury-resistant organisms were enriched by inoculation into TSB containing 0, 10, 20, 40, 80, 160, and 320 μg of Hg²⁺ per ml. Organisms were isolated from flasks showing growth by plating serial dilutions on spread plates containing the corresponding mercury concentration of each flask. Organisms from fish scrapings were isolated similarly.

In a separate study, fish and sediment samples were incubated in flasks with various media and gaseous atmospheres for long-term studies to select organisms or groups of organisms which could methylate inorganic mercury. Some of the organisms used in this study were obtained from these flasks by direct isolation after various periods of incubation. After discarding a number of cultures which appeared to be duplicates, 207 isolates were saved and maintained on TSA slants containing 10 μg of Hg²⁺ per ml.

**Screening of isolates for aerobic and anaerobic degradation of methylmercury.** Each organism to be tested for aerobic methylmercury degradation was grown through two transfers in shaker flasks containing TSB without inorganic mercury or methylmercury. A 1% inoculum of a 18- to 24-h culture was transferred to a 500-ml incubation flask containing 100 ml of TSB. One-half milliliter of a 50 μg/ml solution of methylmercury bromide (MMB) in ethanol was added to each flask after autoclaving to yield a final concentration of 0.25 μl of MMB per ml. The stock solution of MMB contained approximately 2.4 × 10⁶ dpm of ²⁰³Hg/MMB per ml. Each flask was connected to an HgBr₂-KBr trap containing 10 ml of trapping solution (15 g of HgBr₂/liter, 100 g of KBr/liter) as shown in Fig. 1. The traps were added to scavenge any inorganic mercury or organomercurials volatilized from the flask. Trapping of mercury vapor or organomercurials (dimethylmercury) is quantitative in the HgBr₂-KBr trapping solution. Any dimethylmercury volatilized to the trap is converted to monomethylmercury and detected as such in the trapping solution. A Hg(NO₃)₂-HNO₃ trapping solution was found to be equally effective in trapping mercury vapor or organomercurials; however, the very low extraction efficiency for organomercurials precluded its use.

Flasks were incubated in series of 35 flasks each at ambient temperature for 83 to 119 h. Head gases were flushed to traps with a stream of vapor-saturated, sterile air. For anaerobic demethylation, O₂-free N₂ was used as the flushing gas, and 0.025% cysteine was added to the TSB.

**Analytical procedures.** Trapping of volatile radioactive species was monitored by direct insertion of the traps into the detector of a Packard model 2001 gamma scintillation spectrometer with a 2-inch (ca. 5.08 cm) NaI (Th) crystal. Counting efficiencies were obtained by comparison of a standard calibrated Hg²⁺ reference source with equivalent geometry standards prepared from stock solutions of MMB-²⁰³Hg-MMB. The counting efficiencies for traps were determined with traps containing equal volumes (10 ml) spiked with known activity of ²⁰³Hg-MMB. Decay corrections were made for each dpm value calculated.

Methane in head gases was analyzed by flame ionization gas chromatography by using an OV-1 column. The presence of methane was confirmed by direct introduction of head gases from control (containing equivalent microbial growth but no methylmercury) and positive flasks, into a Varian MAT CH-4B mass spectrometer coupled with a Varian 620i laboratory computer. A peak at mass 15.997 (oxygen) for 10 mass spectra of the control and peaks at masses 15.997 and 16.032 (oxygen plus methane) for 10 mass spectra of the positive flask verified the presence of methane.

Traps and flask contents were analyzed for MMB after extraction of samples, pretreated with 5 ml of an acidic KBr solution, with 35 ml of toluene. Additional clean-up was accomplished by extraction of the toluene with 1.5 ml of buffered cysteine. The cysteine was treated with 0.6 ml of 3 M potassium iodide and extracted with 2 ml of benzene.

Methylmercury iodide was determined in the benzene extracts by using a Microtek 2000 R gas chromatograph with an electron capture (tritium) detector and a glass column (4 ft by ¼ inch; ca. 121.9 by 0.84 cm) packed with 1.5/1.7% OV-17/QF-1 on Supelcoport (100/120 mesh). The operating conditions were: temperature—column 125 C, inlet 180 C, detector 165 C; carrier flow—70 ml per min, nitrogen. A linear calibration curve was obtained for the 0- to 1.0-ng methylmercury iodide range.

**RESULTS**

To investigate the possibility that organisms capable of degrading methylmercury are present in the aquatic environment, 207 organisms which were isolated from sediments and fish were screened for demethylation of MMB. Each isolate was incubated aerobically in TSB containing 25 μg of MMB. Volatilization of
mercury, to a trap attached to each flask, was monitored by periodical insertion of the traps into the well of a gamma scintillation counter. Table 1 shows that 30 isolates were positive for aerobic degradation of methylmercury with volatilization of labeled mercury as the criterion for a positive test as compared to no volatilization from sterile controls containing methylmercury. The sterile controls consisted of uninoculated media containing 25 µg of MMB and 203Hg-MMB. The controls were connected to traps and incubated under the same conditions as inoculated flasks.

To show that methylmercury is degraded to methane and inorganic mercury (Hg0 or Hg2+), it was necessary to detect methane in positive cultures and to demonstrate that the trapped activity was not an organomercurial (3).

All cultures which appeared to be positive for demethylation were examined for methane production. The organisms were incubated overnight in 50 ml of TSB containing 25 µg of MMB (0.5 µg/ml). To prevent escape of methane, the flasks were sealed with rubber stoppers containing Bunsen valves for gas relief. Methane was determined by flame ionization gas chromatography using 1 cc of head gas from each flask. All 30 cultures were positive for methane production. Head gases from control flasks and three negative species were negative for methane. One isolate (no. 168) was grown with 0 and 5.0 µg of MMB per ml. Methane production in the 5 µg/ml flask was verified by mass spectrometry. No methane was detected in control flasks by mass spectrometric analysis.

To show that the trapped activity is inorganic mercury rather than volatile organomercurials, a series of control trapping experiments was run with 203HgCl2, 203Hg-MMB, and 203Hg2 (203HgCl2 reduced to 203Hg0 with stannous chloride or hydroxylamine). Volatility was achieved only in flasks containing reduced mercury (Hg0). An additional possibility remains that dimethylmercury (DMM) is formed from methylmercury and is volatilized to the trap. The DMM would be converted to MMB in the HgBr2-KBr trapping solution and could be detected by gas chromatography as methylmercury in the trap extract. All traps showing activity were, therefore, extracted and analyzed for methylmercury to exclude this possibility.

Selected flasks were also analyzed for residual MMB. The results (Table 2) show that very

---

**Table 1. Aerobic and anaerobic methylmercury degradation**

| Flask no. | Isolate | Aerobic CH4, a | MMB recovered as volatile mercury b (%) | Aerobic | Anaerobic |
|-----------|---------|---------------|----------------------------------------|---------|----------|
| Control   | Sterile | -             | 0.0                                   | 0.0     |          |
| 18        | IM1-45  | +             | 14.9                                  | 56.6    |          |
| 20        | IM1-47  | +             | 21.3                                  | 62.7    |          |
| 26        | IM1-55  | +             | 10.1                                  | 68.9    |          |
| 35        | IM2-1   | +             | 10.1                                  | 68.9    |          |
| 36        | IM2-2   | +             | 4.6                                   | 57.9    |          |
| 39        | IM2-5   | +             | 31.3                                  | 57.9    |          |
| 42        | IM2-9   | +             | 19.5                                  | 57.9    |          |
| 43        | IM2-10  | +             | 2.0                                   | 18.4    |          |
| 44        | IM2-11  | +             | 3.3                                   | 7.9     |          |
| 47        | IM2-14  | +             | 1.6                                   | 50.9    |          |
| 67        | IF3-9   | +             | 1.6                                   | 47.2    |          |
| 68        | IF3-10  | +             | 1.0                                   | 47.1    |          |
| 71        | IF3-13  | +             | 30.4                                  | 15.6    |          |
| 83        | IF2-1   | +             | 27.2                                  | 22.2    |          |
| 89        | IF2-7   | +             | 18.9                                  | 17.2    |          |
| 90        | IF3-1   | +             | 18.4                                  | 59.3    |          |
| 94        | IF3-5   | +             | 3.5                                   | 64.5    |          |
| 169       | III F1-16| +            | 10.0                                  | 58.6    |          |
| 167       | GF2-5   | +             | 30.7                                  | 83.8    |          |
| 168       | GF3-1   | +             | 51.1                                  | 45.4    |          |
| 170       | III F1-2| +             | 29.3                                  | 45.4    |          |
| 171       | III F1B-2| +           | 6.1                                   | 45.4    |          |
| 172       | III F1B-3| +           | 26.0                                  | 45.4    |          |
| 173       | III F1B-4| +           | 14.8                                  | 15.3    |          |
| 174       | III F3-1| +             | 28.0                                  | 13.1    |          |
| 175       | III F2-2| +             | 22.1                                  | 14.2    |          |
| 177       | III F3-4| +             | 16.1                                  | 45.9    |          |
| 185       | GF4-3   | +             | 31.8                                  | 45.9    |          |
| 190       | GF5-5   | +             | 25.4                                  | 78.4    |          |
| 206       | III F4-9| +             | 40.2                                  | 15.3    |          |

*From MMB.
*a A 0.25-µg amount of MMB and 203Hg-MMB added per ml per flask.
*b Indicates inability to grow anaerobically.

---

**Table 2. Chromatographic analysis of isolates for residual methylmercury**

| Flask no. | Isolate | MMB Recovered as volatile mercury (%) | Residual MMB in incubation flask (%) |
|-----------|---------|--------------------------------------|-------------------------------------|
| Control   | Sterile | 0                                    | 92                                  |
| 35        | IM2-1   | 10.1                                 | 0                                   |
| 39        | IM2-5   | 16.1                                 | 0                                   |
| 42        | IM2-9   | 23.8                                 | 0                                   |
| 118       | II F5-1 | 0.1                                  | 100                                 |
| 128       | II F7-2 | 0.1                                  | 101                                 |
| 160       | III F1-16| 9.2                                 | 3.4                                 |
| 167       | GF2-5   | 30.7                                 | 0                                   |
| 168       | GF3-1   | 31.0                                 | 0                                   |

*Percent of 25 µg of Hg added.
*Negative demethylator.
little, if any, methylmercury remained in the flasks positive for demethylation, whereas complete recovery of MMB was obtained in the control and flasks negative for volatilization of mercury (flasks no. 118 and 128). The trap values, however, do not indicate complete degradation of MMB as reflected by far less than 100% volatilization in most cases. Tables 3 and 4 show results of material balances which were run to resolve this discrepancy. Excellent total recovery of label was obtained in most cases. The activity remaining after volatilization is found in the flask contents or adsorbed to the flask itself and is not present as methylmercury. The remaining activity must, therefore, be present as inorganic mercury. Similar conclusions have been reached by using a strain of Pseudomonas isolated from soil (9).

Controls were included in the material balance experiments, containing heat-killed cells (Table 3). No volatilization of mercury was observed in these, implying that living cells, or enzymes therefrom, are necessary for demethylation.

All positive aerobic demethylators were tested for ability to degrade MMB under anaerobic conditions. The organisms were grown under oxygen-free nitrogen in sealed flasks containing TSB plus 0.025% cysteine. The flasks were fitted with Bunsen valves to relieve excessive gas pressure during growth. Incubation flasks, preflushed with nitrogen, received a 1% inoculum of each culture that grew under anaerobic conditions. Incubation proceeded for 113 h while being flushed continuously with O₂-free N₂. Of the 30 positive aerobic isolates, 22 proved to be facultative anaerobes. Of these, 21 were capable of degrading methylmercury anaerobically (Tables 1 and 4). In fact, most showed a greater degree of volatilization of mercury anaerobically. This is probably a reflection of the more reduced conditions anaerobically which would favor complete reduction of the Hg²⁺ formed during demethylation. To determine tolerance to MMB, positive isolates were incubated aerobically for 70 h in TSB containing 0 (control), 0.5, 1.0, 5.0, and 10.0 μg of MMB per ml. The results (Table 5) show that all positive isolates were capable of growth comparable to the control at 0.5 μg of MMB per ml. Some isolates were capable of growth and degradation at 10 μg of MMB per ml. It is interesting that the extent of volatilization generally increases with increasing MMB concentration up to the threshold point of tolerance.

### Table 3. Material balances for aerobic demethylation

| Flask no. | Recovery of label (%) | Flasks | Acid added to Flask | Total |
|-----------|-----------------------|--------|--------------------|-------|
| 5*        | 101.9                 | 0.1    | 1.7                | 103.7 |
| 18        | 54.3                  | 21.2   | 12.1               | 87.6  |
| 20        | 49.4                  | 34.5   | 8.3                | 92.2  |
| 26        | 34.7                  | 50.2   | 6.8                | 91.5  |
| 37*       | 101                   | 0.1    | 0.9                | 102   |
| 39        | 45.9                  | 51.0   | 1.5                | 98.4  |
| 42        | 54.4                  | 17.4   | 14.3               | 86.1  |
| 65*       | 94.1                  | 0.1    | 0.7                | 94.9  |
| 67        | 88.2                  | 8.8    | 1.0                | 98.0  |
| 83        | 51.4                  | 33.2   | 5.6                | 90.2  |
| 89        | 54.1                  | 33.5   | 2.8                | 92.9  |
| 160       | 75.2                  | 10.4   | 2.2                | 87.8  |
| 168       | 15.7                  | 71.5   | 5.2                | 92.4  |
| Control sterile | 100           | 0.02  | 0.03               | 100   |
| Control (heat-killed 39) | 99.2 | 0.13 | 0.05               | 99.4  |
| Control (heat-killed 42) | 100 | 0.09 | 0.03               | 100.1 |

* Recovered as volatile mercury (inorganic).

### Table 4. Material balances for anaerobic demethylation

| Flask no. | Recovery of label (%) | Flasks | Acid added to Flask | Total |
|-----------|-----------------------|--------|--------------------|-------|
| 20        | 29.8                  | 56.6   | 10.2               | 96.7  |
| 26        | 30.6                  | 62.7   | 1.3                | 94.6  |
| 35        | 26.7                  | 68.9   | 1.1                | 96.7  |
| 39        | 23.5                  | 57.9   | 12.0               | 93.4  |
| 43        | 78.8                  | 18.4   | 1.2                | 98.4  |
| 44        | 81.2                  | 7.9    | 0.7                | 89.8  |
| 47        | 35.5                  | 50.9   | 6.0                | 92.4  |
| 67        | 38.0                  | 47.2   | 2.3                | 87.5  |
| 68        | 44.9                  | 47.1   | 1.4                | 93.4  |
| 71        | 45.3                  | 15.6   | 18.9               | 79.8  |
| 83        | 61.0                  | 22.2   | 2.2                | 85.4  |
| 89        | 76.3                  | 17.2   | 1.0                | 94.5  |
| 90        | 33.4                  | 59.3   | 1.1                | 93.8  |
| 94        | 12.0                  | 83.8   | 3.5                | 99.3  |
| 167       | 19.9                  | 74.5   | 2.1                | 96.5  |
| 168       | 87.1                  | 13.1   | 1.3                | 101.5 |
| 174       | 79.5                  | 14.2   | 0.7                | 94.4  |
| 175       | 104.4*                | 0      | 0                  | 104.4 |
| 177       | 5.0                   | 2.5    | 78.4               | 85.9  |
| 190       | 27.8                  | 64.5   | 1.5                | 93.8  |
| Sterile control | 100            | 0     | 100                | 100   |

* Recovered as volatile mercury (inorganic).

* Growth with no demethylation.
**DISCUSSION**

It has been demonstrated that bacteria capable of degrading methylmercury are easily isolated from environmental samples. Of 207 cultures isolated from fish and sediment, taken from Lake St. Clair, 30 isolates were positive for aerobic demethylation without adaption to methylmercury. Of the 30 positive isolates, 22 were facultative anaerobes, and 21 of these were capable of anaerobic demethylation. Two of the isolates were gram-positive cocci, two were gram-positive rods, and the remainder were gram-negative rods with characteristics typical of *Pseudomonas*. Further characterization of these isolates is currently in progress.

Distribution of the organisms in various samples is as follows: three isolates from sediment enrichment cultures, seven direct isolates from sediments, nine isolates from long-term incubation studies with sediment, four isolates from long-term incubation studies with fish tissues, three direct isolates from Lake St. Clair fish, and four isolates from goldfish exposed to mercury-containing sediments for up to 5 weeks. It is not known whether the latter could be isolated from goldfish before exposure to mercury.

It was shown that the degradation is a reductive demethylation reaction resulting in the formation of methane and inorganic mercury. That the methane formed is produced from methylmercury is conclusive in that methane cannot be formed aerobically by the reduction of CO₂, methane is not formed by positive cultures in the absence of methylmercury, and, of three nondegrading cultures tested, none produced methane in the presence of MMB.

Previous work by Tonomura et al. (Abstr. U.S.-Japan Sem. Environ. Toxicol. Pesticides, Oiso, Japan, 25–29 Oct. 1971) indicated that demethylation is an aerobic process; however, the facultative organisms we isolated readily degrade MMB under both aerobic and anaerobic conditions.

All positive demethylators were tolerant to at least 0.5 µg of MMB per ml, and demethylation rates tended to increase with increasing mercury concentration of MMB.

The isolations discussed in this study were made from environmental samples taken from a lake with a known methylmercury problem (in fish). Since methylmercury is regarded by many to originate through methylation of inorganic mercury in sediments, it should not be surprising to find detectable concentrations of methylmercury in sediments of this lake. Although inorganic mercury was found in concentrations ranging from 0.09 to 14.8 µg/g (wet weight), no methylmercury was found. The paucity of methylmercury data may be, at least partially, explained by the widespread distribution of demethylating species found in these sediments. Although it would be presumptuous to assume, generally, that methylation does not take place in sediments, it would not be surprising if demethylating species abound and could suppress the accumulation of methylmercury in sediments.

---

**Table 5. Volatilization of MMB added at various levels**

| Flask no. | MMB tolerance (µg/ml) | Total MMB volatilized (µg/50 ml of medium) |
|-----------|-----------------------|------------------------------------------|
|           |                       | 0.5 µg per ml i | 1.0 µg per ml i | 5.0 µg per ml i | 10.0 µg per ml i |
|           |                       | concn          | concn          | concn          | concn          |
| 18        | 0.5                   | 22.0           | 2.7            | 0.0            | 0.0            |
| 20        | 0.5                   | 23.3           | 0.0            | 0.0            | 0.0            |
| 26        | 5.0                   | 23.0           | 47.3           | 241            | 0.0            |
| 35        | 1.0                   | 22.0           | 39.6           | 0.0            | 0.0            |
| 36        | 10.0                  | 23.0           | 40.6           | 220            | 452            |
| 39        | 5.0                   | 19.4           | 41.8           | 231            | 0.0            |
| 42        | 0.5                   | 18.8           | 6.8            | 0.0            | 0.0            |
| 43        | 1.0                   | 20.2           | 40.6           | 0.0            | 0.0            |
| 44        | 1.0                   | 19.7           | 41.8           | 0.0            | 0.0            |
| 47        | 10.0                  | 21.8           | 45.6           | 238            | 483            |
| 67        | 5.0                   | 21.9           | 44.6           | 0.0            | 0.0            |
| 68        | 1.0                   | 20.5           | 44.0           | 0.0            | 0.0            |
| 71        | 1.0                   | 23.1           | 44.7           | 0.0            | 0.0            |
| 83        | 1.0                   | 20.3           | 43.6           | 0.0            | 0.0            |
| 89        | 1.0                   | 18.4           | 40.8           | 0.0            | 0.0            |
| 90        | 5.0                   | 23.0           | 46.6           | 238            | 0.0            |
| 94        | 5.0                   | 21.5           | 46.0           | 38             | 0.0            |
| 160       | 1.0                   | 2.3            | 3.2            | 0.0            | 0.0            |
| 167       | 10.0                  | 20.8           | 45.3           | NG             | 450            |
| 168       | 10.0                  | 21.7           | 48.0           | 241            | 452            |
| 170       | 10.0                  | 21.1           | 43.6           | 225            | 456            |
| 171       | 1.0                   | 22.7           | 45.0           | 0.0            | 0.0            |
| 172       | 1.0                   | 22.6           | 44.6           | 0.0            | 0.0            |
| 173       | 1.0                   | 21.8           | 44.8           | 0.0            | 0.0            |
| 174       | 1.0                   | 17.7           | 43.8           | 0.0            | 0.0            |
| 175       | 1.0                   | 20.1           | 42.7           | 0.0            | 0.0            |
| 177       | 1.0                   | 13.9           | 42.1           | 0.0            | 0.0            |
| 185       | 1.0                   | 23.6           | 46.6           | 0.0            | 0.0            |
| 190       | 5.0                   | 24.2           | 48.6           | 221            | 0.0            |
| 206       | 1.0                   | 22.5           | 46.8           | 0.0            | 0.0            |

*a A 25-µg amount per flask.
*b A 50-µg amount per flask.
'c A 250-µg amount per flask.
'd A 500-µg amount per flask.
* Highest level tested that supports growth equivalent to the control without MMB.
' No growth.

---

Downloaded from http://aem.asm.org/ on March 21, 2020 by guest
ACKNOWLEDGMENTS

This work was supported by a research contract funded by 10 contributing member companies of the Manufacturing Chemists Association and by the National Paint and Coatings Association. We acknowledge the assistance of Jarl Hiltunen, Wayne Wilford, John Carr, and other personnel of the Great Lakes Fishery Research Laboratory in the collection of environmental samples used in this study.

LITERATURE CITED

1. Bertilsson, L., and H. Y. Neujahr. 1971. Methylation of mercury compounds by methylcobalamin. Biochemistry 10:2805-2808.
2. De Simone, R. E. 1972. Methylation of mercury by common nuclear magnetic resonance reference compounds. J. Chem. Soc. Chem. Commun. 13:780-781.
3. Furukawa, K., T. Suzuki, and K. Tonomura. 1969. Decomposition of organic mercurial compounds by mercury-resistant bacteria. Agr. Biol. Chem. 33:129-130.
4. Imura, N., E. Sukegawa, S. K. Pan, K. Nagao, J. Y. Kim, T. Kwan, and T. Ukit. 1971. Chemical methylation of inorganic mercury with methylcobalamin, a vitamin B12 analog. Science 172:1248-1249.
5. Jensen, S., and A. Jernelov. 1969. Biological methylation of mercury in aquatic organisms. Nature (London) 223:753-754.
6. Wood, J. M., F. S. Kennedy, and C. G. Rosen. 1968. Synthesis of methylmercury compounds by extracts of a methanogenic bacterium. Nature (London) 220:173-174.
7. Yamada, M., and K. Tonomura. 1972. Formation of methylmercury compounds from inorganic mercury by Clostridium cochlearium. J. Ferment. Technol. 50:159-166.