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Determination of Methylmercury in Fish of South Carolina

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INTRODUCTION

A good portion of the world's diet consists of fish in one form or another. Many of the world's great gastronomic materpieces are based on fish from both freshwater and saltwater.

For centuries the waters of the world have been a receptacle for man's affluent waste - sewage sludge, industrial waste, Mining, Agriculture etc. During this century, in the United States, about 75 million kilograms of mercury have been used; little information is available on its final disposition or on concentration of the element at the specific points in the environment (ABELSON, 1970).

Many potentially dangerous chemicals ultimately find their way into waters which are natural habitats for fish. The fish which live in polluted water may accumulate pollutants from the water via their food chains. The fish feeding on small marine organism, on algae, and on bottom sediments readily accumulate methylmercury.

In some waterways large quantities of mercury salts have formed complexes with biologically active sediments to form a substrate for the microbial synthesis of methylmercury. Elemental mercury is first converted into methylmercury and dimethylmercury in anaerobic sediments (JENSEN AND JERNELOV, 1969). This biological methylation is accomplished by bacteria called Methanobacterium Omelanskii living in the bottom mud sediment (Fig. 1). These bacteria are then eaten by Zooplankton which in turn are eaten by fish (EYL, 1970).

In addition to microbial synthesis of methylmercury, fish liver is capable of methylmercury synthesis from Hg^{2+} (WESTOO, 1967). Also it appears that methylmercury can be directly concentrated from the water through the Gills (MIETTENEN, 1970).

So fish accumulate methylmercury through food chains, through liver synthesis and through the Gills. Pratically all the mercury present in fish is in the form of methylmercury, CH_3-Hg^+ and CH_3-Hg-CH_3, which has been found to be readily absorbed by humans upon ingestion and is considerably more hazardous to humans. Methylmercury is insidious poison and it enters human body through the algae-fish-human food chain (EYL, 1970).

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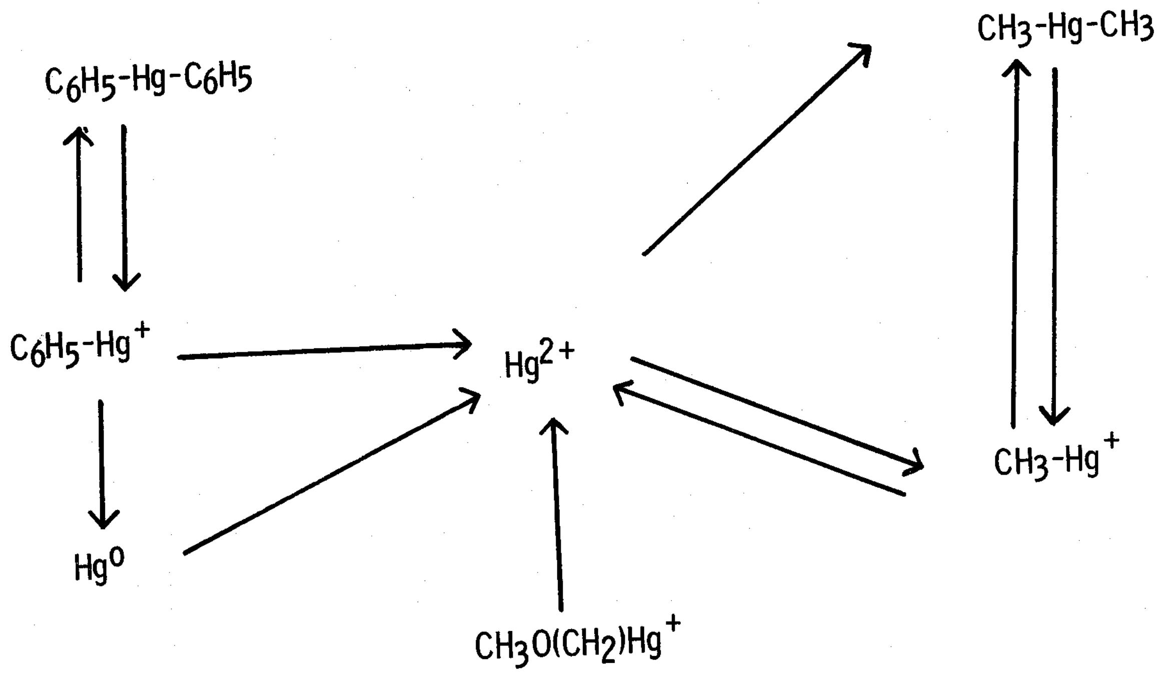

FIG. I. METHYLMERCURY CONVERSION.

MATERIALS AND METHODS

Extraction of Methylmercury

Brown Trout (2 kg whole body weight) were used for methylmercury extraction and analysis. We selected this species as an ideal, since it had more than 0.5 ppm mercury content which is the upper limit according to Food and Drug Administration guideline.

Sample of fish muscle tissue (40g) with an equal volume of Cysteine-Borate buffer (2g $Na_2B_4O_7 \cdot 10H_2O$ + 0.8g cysteine$\cdot$HCl in 40 ml of distilled water) was homogenized in a Waring blender. It was extracted with 20 ml of toluene by adding toluene to the blender, homogenized, and separated by centrifugation. It was repeated one more time. This supernatant toluene layer I probably contains CH_3-Hg-CH_3 and R-Hg-R.

To the residue, containing CH_3-Hg-S-R and other R-Hg-Cysteine, an equal volume of concentrated HCl and 20 ml of toluene were added, homogenized, and separated by centrifugation. The supernatant toluene layer II was collected and dried over Na_2SO_4. This toluene layer probably contains CH_3-Hg-Cl.

To the supernatant toluene layer I of the original centrifugations containing CH_3-Hg-CH_3 and other nonpolar R-Hg-R, 20 ml of concentrated HCl was added and was stirred for 30 minutes for acid hydrolysis, which could result in one mole of CH_3-Hg-Cl for every mole of CH_3-Hg-CH_3. The toluene layer was separated and dried over Na_2SO_4. This toluene layer probably contains CH_3-Hg-Cl.

Both the toluene layers after drying over Na_2SO_4 were combined.

Gas Chromatography

The combined toluene layers were analyzed using gas chromatographic procedure with a Tracor MT 220 GC, according to the conditions described in Table 1. Also Gas Chromatographic tracing of 1 ppm standard solution of CH_3-Hg-Cl in n-hexane were recorded for comparison and interpretation.

TABLE 1

Gas Chromatography Apparatus Conditions for Determination of Methylmercury Chloride

Column: 5 Feet Pyrex Column ¼" diameter
Liquid Phase: 1.5% OV 17/1.95% QF-1
Oven temperature: 90°C Isothermat
Injector temperature: 225°C
Detector temperature: 325°C
Detector: Ni 63 Electron Capture
Detector flow: 15 ml/min.
Gas flow: 35 ml N_2/min.

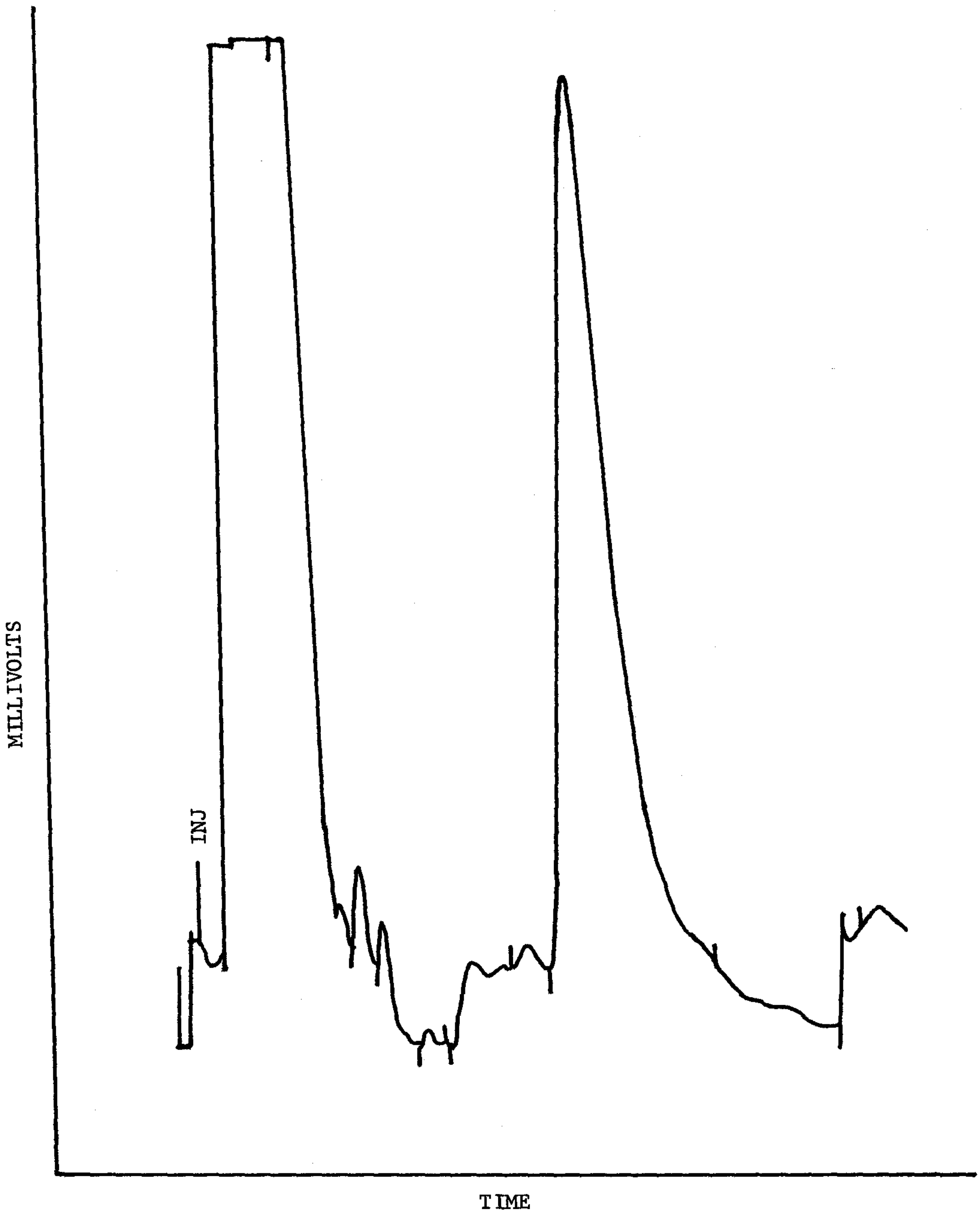

Fig. 2. Gas Chromatographic Tracing of Fish Sample After Extraction

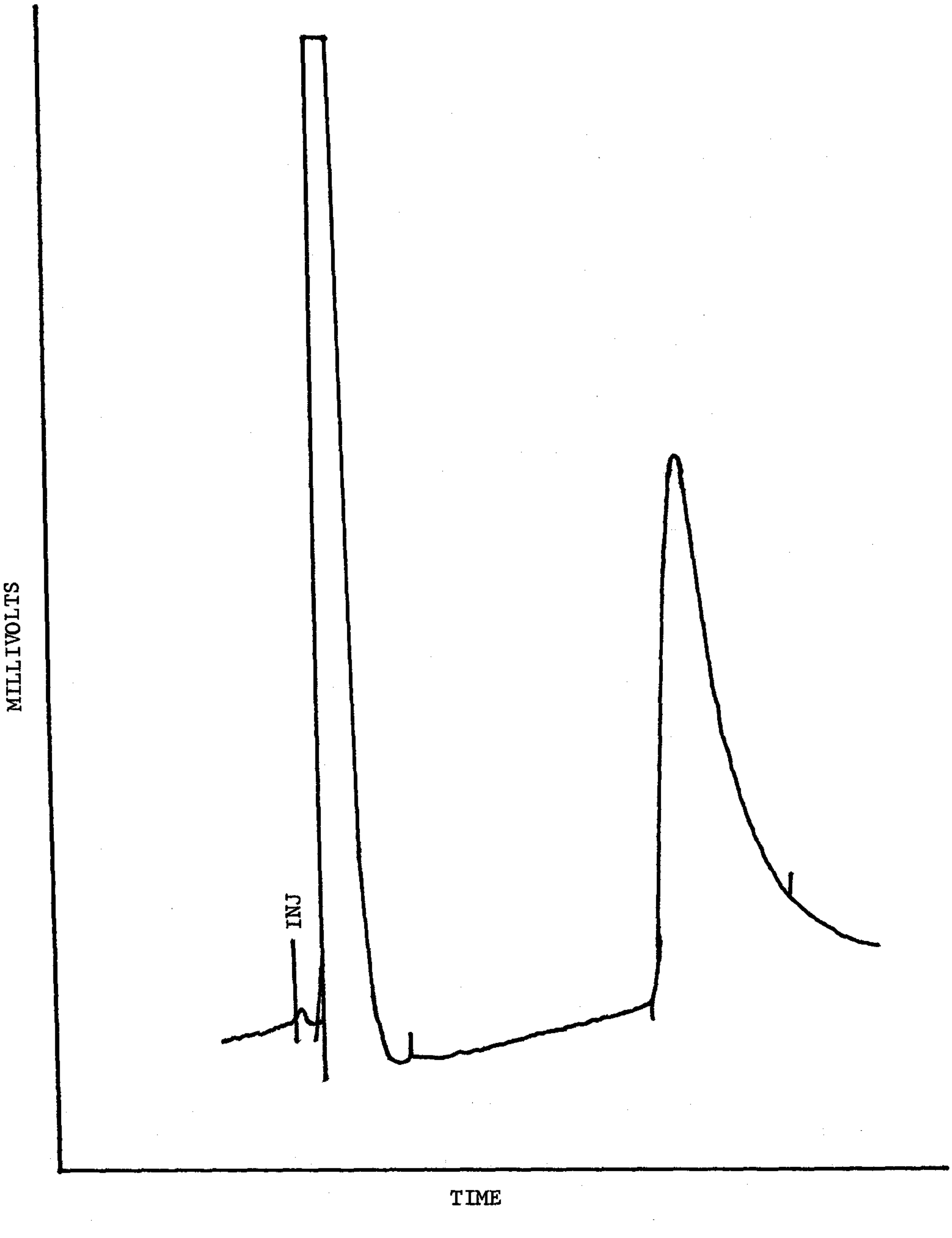

Fig. 3. Gas Chromatographic Tracing of Standard Solution

RESULTS AND DISCUSSION

Definite evidence that fish contain monomethyl mercury and dimethylmercury has been established.

The analytical technique used separated the organic mercury by means of solvent extraction, a procedure was used by Westoo (WESTOO, 1966) and Hartung (HARTUNG, 1974). Monomethylmercury and Dimethylmercury were extracted by toluene from the fish muscle tissue. Both these compounds were converted to methylmercury chlorides and then extracted with toluene.

These essential step of this method for the determination of both monomethyl and dimethylmercury was the initial addition of a cystenine-borate buffer at pH 8.2 to the sample. The purpose of this buffer was to stabilize the diemthylmercury, and to combine with any free monomethylmercury to form the water-soluble cysteine adduct. The sample which has been extracted for diemthylmercury was charged with concentrated HCl for acid hydrolysis to convert it into methylmercury chloride.

Toluene, n-hexane and concentrated hydrochloric acid were of high degree of purity.

The gas chromatographic analysis was conducted with a Tracor MT 220 GC at an oven temperature of 90°C isothermat. Since the CH_3-Hg-Cl peak was analyzed for both monomethyl and dimethylmercury, the only differences seen are caused by materials which are not removed during the clean-up (Fig. 2). The gas chromatographic tracing of 1 ppm standard solution of CH_3-Hg-Cl in n-hexane was recorded for comparison and interpretation (Fig. 3). The retention time was same in both the tracings - standard solution and sample solution (Fig. 2 and 3).

Concentration of the sample solution was calculated using the formula:

$$\text{Concentration of methylmercury in sample solution} = \frac{\text{Concentration of standard solution} \times \text{Area of sample peak}}{\text{Area of standard solution peak}}$$

$$= \frac{1\text{ ppm} \times 883655}{504517}$$

$$= 1.75\text{ ppm}$$

The area produced for each peak is proportional to that peak's concentration. This has been used to determine the exact concentration of the sample solution. It turns out that the concentration of the sample solution is 1.75 ppm.

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