The Determination of Methyl Mercury in Urine

by
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Urinary and fecal (both bile and intestinal mucosa) routes of excretion are the most important for the elimination of mercury. Elimination via the sweat and salivary glands although a minor excretory pathway may be of importance diagnostically (INTERNATIONAL COMMITTEE 1969, SUZUKI et al. 1966, JOSELOW et al. 1968). For monitoring levels of mercury in man, urinary and fecal routes of excretion are the most important. However, from a practical point of view, the urinary excretion of mercury is of special interest, and if urine analyses are to be used to monitor exposure, an analytical method specific for alkyl mercuries is desirable (INTERNATIONAL COMMITTEE 1969).

Several methods are reported in the literature for the rapid determination of total mercury in urine (MAGOS and CERNIK 1969, LINDSTEDT and SKARE 1970, LINDSTEDT and SKARE 1971). These methods are both sensitive and specific but do not have the versatility to differentiate between inorganic and organic forms of mercury.

To date, the most popular and widely used method for determining alkyl mercuries in tissue samples is the WESTÖÖ procedure (WESTÖÖ 1966, WESTÖÖ 1967, WESTÖÖ 1968). This method involves a benzene extraction of the acidified tissue homogenate, a cleanup of the benzene extract with an aqueous solution of cysteine acetate and a final extraction of the alkyl mercury salt from the cysteine solution with benzene. The benzene extract is analyzed using gas liquid chromatography (GLC) equipped with an electron capture (EC) detector system. This procedure has been used extensively by Kamps and McMahon for determining alkyl mercuries in fish tissues (KAMPS and McMAHON 1972).

This paper describes a modification of the above procedure for the determination of methyl mercury in urine employing a cysteine acetate cleanup procedure and analysis using the GLC-EC technique.

Bulletin of Environmental Contamination & Toxicology,
Vol. 10, No. 3 © 1973 by Springer-Verlag New York Inc.

EXPERIMENTAL

Reagents

Benzene (pesticide quality), the purity of benzene is such that an injection of 10 µl from a 100 ml aliquot concentrated to 10 ml shows no response other than the benzene peak.

Hydrochloric acid (Ultrex, J.T. Baker Chemical Co.), Ultra high purity concentrated hydrochloric acid was extracted in a separatory funnel several times with Nanograde benzene until injections of the organic layer did not exhibit any GLC peaks after the solvent front.

Water, glass distilled, doubly deionized, is extracted in a separatory funnel several times with benzene.

Cysteine acetate solution, (K & K Laboratories Inc., Plainview, N.Y.), was prepared by the procedure described by Westöö (WESTÖÖ 1966, WESTÖÖ 1967, WESTÖÖ 1968).

Methyl Mercuric Chloride, MMC (K & K Laboratories Inc., Plainview, N.Y.), 98% purity.

Apparatus

Gas chromatograph-Microtek, Model MT 220, equipped with an EC detector and U-shaped glass GC columns 1/4" x 6' packed with 5% HIEFF-2AP on Chromosorb W 80/100 mesh.

Temperatures: Inlet 200°C; column 170°C; transfer lines and outlet block 200°C. The polarizing voltage is set to produce a maximum response for an injection of 1.0 ng of methyl mercuric chloride.

Centrifuge, Model CL, International Clinical Centrifuge.

Procedure

All glassware is washed according to standard laboratory procedure, and then rinsed with ammonium hydroxide, deionized-distilled water, ethanol and benzene.

A 15 ml urine sample is pipetted into a 50 ml stoppered centrifuge tube and acidified to pH 1 with concentrated hydrochloric acid. To the mixture is added 15 ml of benzene, and the tube is shaken vigorously for 5 minutes. The emulsion is broken by centrifugation, and the organic layer is removed by means of a disposable pipette. The urine is extracted again as described above with another 15 ml portion of benzene and centrifuged. The organic layers are combined, and a 25 ml aliquot is removed and extracted with 5 ml of the cysteine acetate solution. The mixture is shaken vigorously and the layers separated. To a 10 ml stoppered centrifuge tube is added a 4 ml aliquot of the aqueous cysteine solution which is then acidified to a pH 1 with hydrochloric acid and extracted with two 2.5 ml portions of benzene. The benzene layers are combined, dried over anhydrous sodium sulfate and stored in a glass stoppered test tube until GLC analysis can be performed.

A calibration curve is prepared for each set of samples from data obtained by substitution of water for urine and using identical volumes of reagents and benzene for the extraction.

Recovery data were obtained by fortifying samples of urine with MMC.

RESULTS AND DISCUSSION

Recovery data obtained for urine samples which had been fortified with MMC are shown in Table 1.

TABLE 1

Recovery Data of Methyl Mercuric Chloride from Urine

No.of Samples	Level (ppb)	Recovery %
4	36	103.7
2	18	104.0
2	9	99.0

Analysis of the benzene extract afforded quantitative recovery of MMC. Direct benzene extraction of unacidified urine samples which had been fortified with MMC resulted in no recovery of MMC. The failure to detect MMC from unacidified urine samples indicates

that the binding between MMC and the constituents of urine take place rapidly and completely (SUMINO 1968a, SUMINO 1968b).

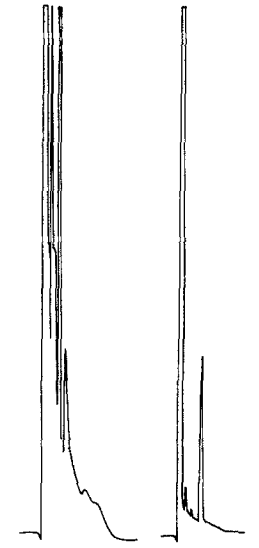


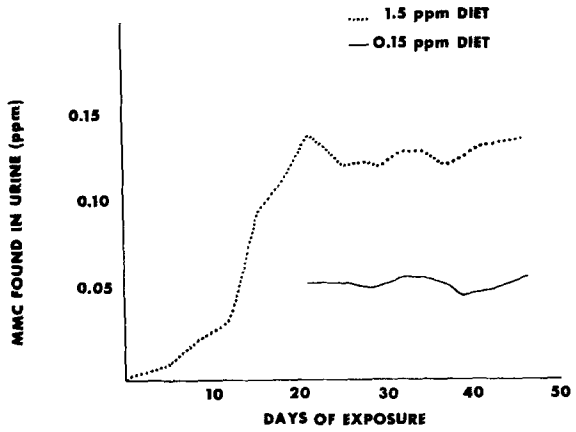
FIGURE 1

Figure 1 shows chromatograms of a benzene extract from acidified urine samples before and after cysteine cleanup. The chromatograms illustrate the need for the cleanup procedure since interfering compounds elute from the GLC column in the area where MMC appears.

The EC detector system is linear in the range of 0 to 2.5 ng of MMC. The detector is sensitive to 5 picograms of MMC with a signal to noise ratio of 3/1.

In order to examine the efficacy of this method, two groups of rats were fed a diet containing 1.5 and 0.15 ppm of MMC for one and two months respectively, and the amount of MMC excreted in the urine was monitored. A plot was made of the amount of MMC found in the urine as a function of the number of days of exposure, Figure 2. The group which was fed 1.5 ppm showed

very little MMC excreted up to twelve days. After this time, the amount excreted increased rapidly up to approximately twenty-one days after which a leveling off effect was observed. No MMC could be found in the urine of rats which were fed 0.15 ppm until the 21st day of exposure. After this time, the amount excreted remained approximately constant for 30 days.



EXCRETION OF MMC IN THE URINE OF RATS

FIGURE 2

The data indicate that MMC is excreted in the urine after some build up of the compound in various tissue sites has taken place. This is illustrated by the fact that no MMC was found in the urine of rats fed a diet containing 0.15 ppm until after 21 days, whereas urinary excretion of MMC in the group of rats on the 1.5 ppm MMC diet began much earlier in the experiment. No attempt was made to quantitate the amount of MMC which could have been converted to inorganic mercury and excreted as such (GAGE 1964).

In summary, MMC is excreted in the urine of animals exposed to this material through dietary intake. Since urinary excretion is of special interest for monitoring exposure to toxic substances, the data produced in this investigation suggests the possibility of monitoring levels of MMC in the general population employing the described procedure. The method is capable of detecting ppb of MMC and combines high recoveries and rapid analysis time in a single procedure. Since binding of MMC to urine constituents is fast and complete, acidification of the urine to pH 1-2 is absolutely necessary prior to benzene extraction. The cleanup procedure using cysteine acetate solution is also essential for the elimination of interfering GLC peaks. This procedure has been used previously for determining MMC from blood and tissue, and the cleanup step was shown to be unnecessary for these samples (ROSS and GONZALEZ, 1971).

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