Bull. Environ. Contam. Toxicol. (1998) 60:511-518

Bulletin of Environmental Contamination and Toxicology

Aqueous Phase Ethylation Atomic Emission Spectroscopy for the Determination of Methylmercury in Fish Using Permeated Dimethylmercury Calibration

H. B. Swan

Australian Government Analytical Laboratories, Pymble, N.S.W. 2073, Australia

Received: 28 October 1997/Accepted: 17 February 1998

Mercury released to the ocean, whether from natural or anthropogenic sources, is transformed through biological processes to organic mercury. Methylmercury is found in trace amounts in nearly all fish, and for most fish this ranges from 0.01 ppm to 0.5 ppm with an average of less than 0.3 ppm for commercially important marine species (Foulke 1994). This is a more toxic form of mercury, due in part to higher liposolubility (Baeyens 1992). Fish of the highest order on the food chain concentrate the most methylmercury which binds strongly to proteins in tissue.

In 1979 the US Food and Drug Administration (FDA) set a limit for methylmercury of 1 ppm (mg/kg) for all seafood (Foulke 1994). In Australia the National Food Authority has recommended a mean national maximum permitted concentration of 0.5 mg/kg of total mercury in fish and fish products with an exception of 1.0 mg/kg for products from all shark, ray species and some specified fish species (NFA 1994). These guidelines are based on limited data industrial pollution events which incorporate high margins for safety and uncertainty. Presently the US FDA is searching for more extensive information on mercury toxicity by gathering data from a long term study of people from the Seychelles and Faeroe Islands where fish is the main source of protein. This study was begun 10 years ago but the results have been delayed for release because of a perceived serious public misunderstanding about the safety of seafood (FCN 1996a). There is concern that exaggerated estimates of the exposure and risk to seafood consumers than actually exist may be perceived by comparison with existing allowable daily intakes set for methylmercury (FCN 1996b). Controversy regarding this study has now led to an extensive review of mercury regulations (Johnson 1997).

A method for the determination of methylmercury present in fish tissue is described which uses aqueous-phase ethylation with sodium tetraethylborate (STEB) for the ethylation of labile CH_3HgX species in a buffered KOH/methanol digested fish sample. The derivatised methylethylmercury (MEHg) was purged

from solution onto a graphitic carbon trap, thermally desorbed and collected in a cryogenic trap prior to analysis by gas chromatographic atomic emission detection (GC-AED). The method presented provides for increased sample turnover by the use of permeated dimethylmercury (DMHg) for MEHg response calibration. This is possible because the AED (Hewlett-Packard 5921A) exhibits an essentially compound independent response for Hg from short-chain alkylated mercury compounds (Janak et al 1995). Typically to provide a calibration standard it is necessary to follow through all the steps used for sample analysis with a compound such as methylmercuric chloride. Generation of a Hg concentration range response with permeated DMHg takes a fraction of the time required to produce one with STEB reacted methylmercuric chloride.

MATERIALS AND METHODS

A selection of locally caught marine fish were purchased from a commercial supplier for analysis. Reagents were prepared according to the method of Bloom (1989) with adapted methodology. Fish tissue (1.00 ± 0.02 g wet wt) was weighed into a 50 mL plastic centrifuge tube and 10 mL of 25% KOH/methanol digestion solution was added. A cap was fitted to the tube with a hole just big enough pass the shaft of an homogeniser through. The homogeniser blade and shaft were thoroughly washed by immersion in distilled water followed by methanol while it was in operation. The sample was then homogenised for a few minutes till well dispersed. The sample was left overnight or alternatively placed in an ultrasonic bath for 1 hour. The digest was then diluted to 20.0 mL with methanol. An aliquot (2 to 50 µL) of the dilute digest was added to 100 mL of distilled water in a Dreschel bottle and the sample was neutralised with an equal volume of 2*M* acetic acid solution. Acetate buffer (200 µL) was added (pH 4.9 ± 0.1) followed by 50 µL of 1% STEB (Strem, USA) solution, and swirled to mix. The head was fitted, secured down against backpressure and the mixture was left to react for 15 minutes.

After derivatisation the Dreschel bottle was connected with a length of Teflon tubing to a Carbotrap 100 column (Supelco, USA) fitted with 1/8 in. Teflon Swagelok reducing unions. A tube from a cylinder of ultra high purity helium was connected to the inlet of the Drescheler head and the solution was purged at approximately 200 mL/min for 20 minutes. The helium line was then removed from the head inlet and connected directly to the Carbotrap column for 5 minutes to remove residual water from the adsorbent. The end of the Carbotrap column that faced the bubbler outlet of the Dreschel bottle was marked for identification, then both ends were capped with 1/8 in. blanking nuts.

A valving and cryotrap system was arranged as shown in Fig. 1. The cryogenic trap was constructed by passing 1/16 in. Teflon tubing through 1/8 in. copper tubing bent into a loop. A certified DMHg permeation tube (Dynacal, Vici Metronics, USA) was inserted into a glass tube with 1/2 in. to 1/8 in. Swagelok reducing unions and equilibrated in a water bath at 30°C. Actuation of the 8-port valve (Valco, USA) was used to switch the gas flow to load either of two stainless steel calibration loops of 0.4 mL and 0.8 mL capacity, thereby providing a standard volume of DMHg to be sent to the cryotrap. A flow-meter connected to the exit vent of the 8-port valve was used to calculate the amount of DMHg contained in a calibration loop. This amount was the time taken to fill the loop volume multiplied by the permeation rate of the calibration device. The amount could be regulated by adjusting the flow of UHP He across the permeation tube. The temperature of the water bath and the number of calibration loops switched also determined the amount of standard down-loaded to the cryotrap.

For sample introduction and analysis the cryotrap was cooled in liquid nitrogen for 1 min and the 6-port valve was switched to the “load” position. The Carbotrap column was then inserted into the Teflon line joining the two valves so that the end that faced the bubbler outlet was inserted facing the cryotrap intake. The Carbotrap was left for a minute to flush it with UHP helium, and a gas leak check was made with a flow-meter attached to the vent on the 6-port valve. The Carbotrap was then placed into a thermal desorption unit set at 300°C for 10 min. After the desorption period a check was again made to ensure there was no gas leak using the flow-meter, the 6-port valve was switched to the “inject” position, the cryotrap was placed in warm water at 35°C and the GC-AED was started via a remote switch. The Carbotrap was then taken out of the thermal desorption unit to cool.

The capillary column used was a BP1, 50 m x 0.32 mm x 1 µm film (SGE Scientific, Australia). The flow rate was 2 mL/min and the GC oven program was -50°C for 5 min, 30°C/min to 160°C for 1 min. The GC oven was taken down to -50°C just prior to sample injection with liquid carbon dioxide. The GC transfer line and the AED cavity were 250°C. The AED solvent vent and filter were off for the duration of the analysis. The emission wavelengths used were Hg 253.652 nm and C 247.856 nm, and the oxygen and hydrogen reagent gases were used without high He makeup flow. The spectrometer purge flow was set at 2L/min. The gas supply pressures were set at He 30psi, O_2 22psi, H_2 35psi, and the He flow measured at the cavity vent was 80mL/min with both H_2 and O_2 reagent gases on.

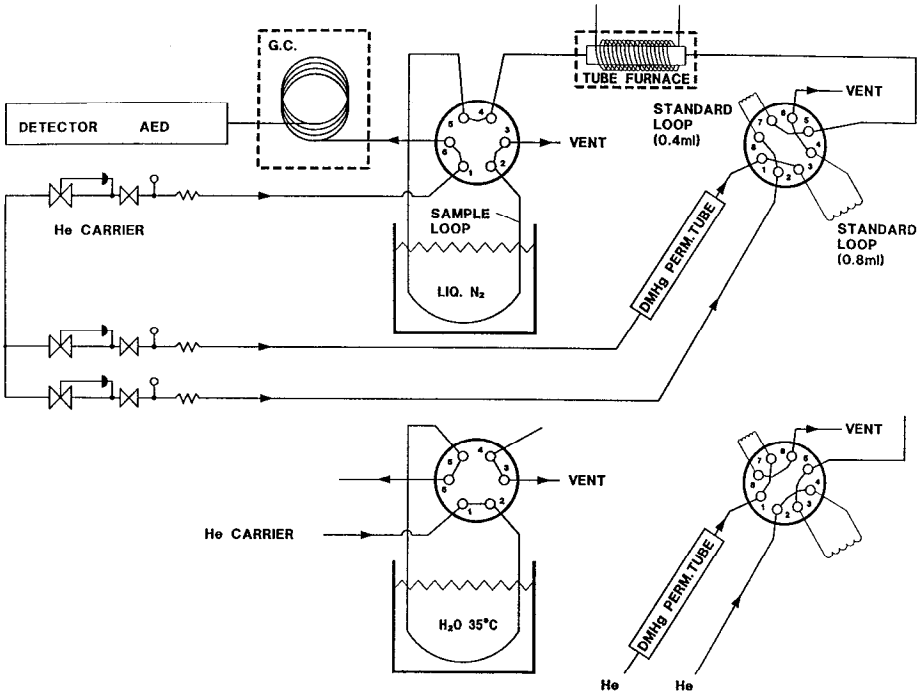

Figure 1. Configuration of the valving and cryotrap system for the analysis of organic mercury compounds using permeated DMHg calibration.

RESULTS AND DISCUSSION

The derivatisation of labile CH_3HgX species with STEB has been described as a rapid, precise and accurate determination of CH_3Hg^+ in aqueous solution (Rapsomanikis and Craig 1991) in comparison to previous methods (Collett et al 1980). Derivatisation with STEB is a convenient technique for alkylmercury determination and it has been used in conjunction with solid-phase microextraction for analysis by GC-MS (Yong and Bayona 1995).

The speciation of $Hg^{(II)}$ by reaction with STEB in aqueous solution allows ionic mercury species to be converted to their volatile ethyl derivatives. Labile CH_3HgX species react to form MEHg, and labile HgX_2 species form diethylmercury (DEHg). Nonionic forms such as. DMHg and elemental mercury (Hg°) do not react with the tetraethylborate anion, hence it is possible to

determine the various forms of mercury by GC separation (Fig. 2). The determination of alkylmercury by microwave induced plasma atomic emission spectroscopy has been previously reported (Koichi et al 1983; Carro-Diaz et al 1994). The 253.652 nm emission line was superior to the 184.950 nm line for Hg sensitivity on the AED, and a detection limit for Hg of 1 pg/sec was achievable using the former emission line. With this sensitivity it was possible to determine at least 5 pg methylmercury in wet fish tissue equivalent to 1 ng/g, by using only 100 µL sample digest, which is below naturally occurring concentrations found in most marine fish. Confirmation of an organomercurial was possible by observation of the emission spectra obtained over the operating wavelength range of the AED's diode array, a "snapshot" (Fig. 3).

The selected fish species were analysed for total mercury against a freeze-dried shark tissue standard reference material (Hg 11.82 ± 1.50 mg/kg) by cold vapour atomic absorption spectroscopy (CVAAS). This is a standard method where stannous chloride reduction is used for the liberation of mercury vapour from an acid digested fish sample. These same fish were analysed for methylmercury using the DMHg calibration technique presented here (Table 1). The DMHg was used as an external standard for the analysis but can be included with the sample because it is seldom present in fish (Cossa et al 1994). Comparison of the results from the two methods was used to assist in analytical validation (Green 1996) given that virtually all mercury present in fish tissue (>95%) is present as methylmercury (Puk and Weber 1994). The 9 fish species tested had total Hg concentrations ranging from 0.04 to 1.45 mg/kg wet wt. The mean methyl-mercury recovery as Hg was 91.2% (CV= 19.7%) for the 9 species. Repetitive analysis (n= 7) for methylmercury from the lowest concentration sample ('Blue eye') at 0.03 mg/kg Hg wet wt gave a relative standard deviation of 6.9%.

Table 1. Comparison of methylmercury by GC-AED with total mercury by CVAAS for various marine fish species (as mg/kg Hg wet weight)

Fish Species	Hg (methyl)	Hg (total)
Blue eye	0.029	0.04
Coral perch	0.107	0.14
Gemfish	0.356	0.37
Hapuka	0.096	0.12
Ling	0.812	0.72
Orange roughy	0.246	0.25
Silver flake	0.055	0.09
Skate	0.751	0.63
Swordfish	1.514	1.45

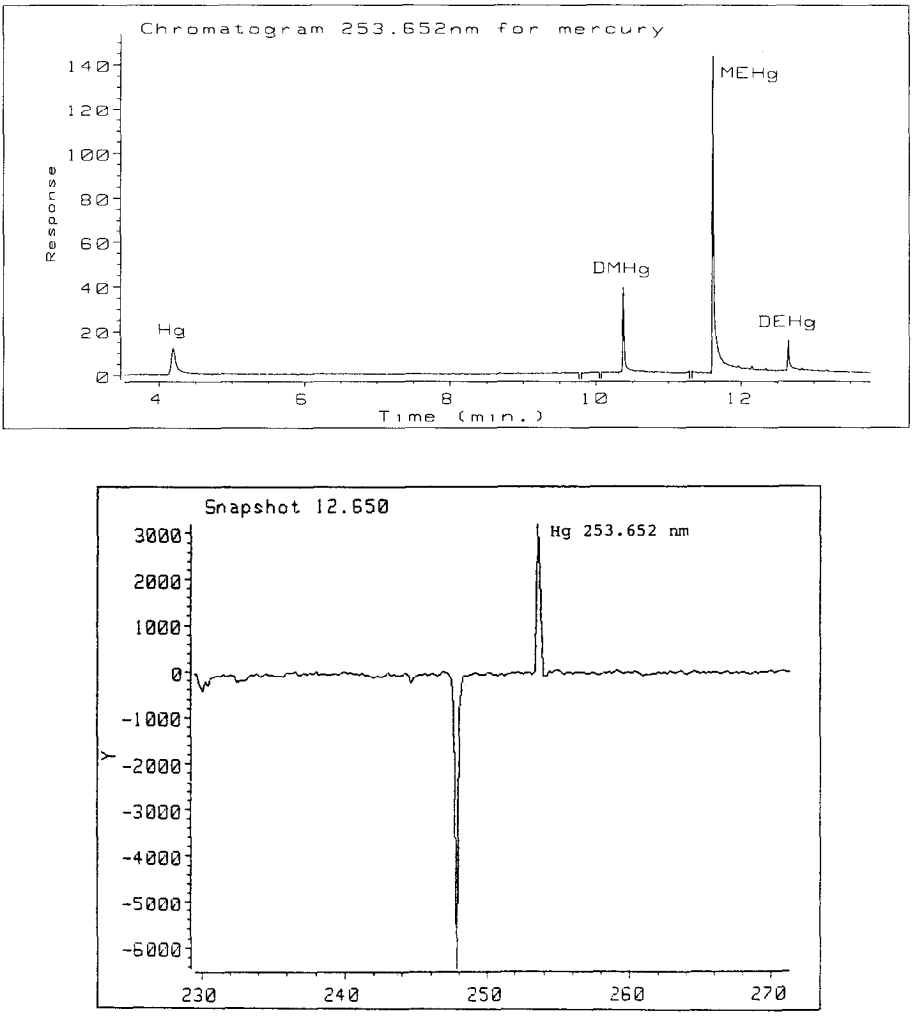

Figure 2. (top) GC-AED chromatogram of the 'Blue eye' fish sample with DMHg standard addition, using the cold oven temperature program -50°C (5mins) 30°C/min to 160°C. MEHg = 80 pg Hg. Figure 3. (bottom) A "snapshot" of DEHg (7 pg Hg at 12.65 mins). Matrix carbon response at 247.856 nm has been background subtracted from the Hg 253.652 nm emission.

The DMHg permeation tube was certified to have a permeation rate of 41.7 ng/min ± 5% @ 30°C. Using this device the AED displayed a linear dynamic range from 0.01 ng Hg of at least two orders of magnitude. The DMHg permeation tube was maintained at 30°C and periodic gravimetric determinations were made over a two year period. Linear regression analysis by least squares gave a compound loss of Y = -40.0 ng/min.

By derivatising and purging determined amounts of methylmercuric chloride (Strem, USA) onto the Carbotrap column it was found that the 450 mg of adsorbent used had an absolute maximum holding capacity of 0.25 ng Hg as alkylmercury. Prior analysis of the samples for total mercury by CVAAS was used to determine the appropriate aliquot of the diluted digest required for the determination of methylmercury so that breakthrough did not occur. Besides providing a rapid means of calibration, the use of DMHg avoided possible analyte contamination caused by methylmercuric chloride when used as a standard. A STEB reacted, purged and thermally desorbed standard or tissue sample will require 50 to 60 mins preparation time prior to injection, whereas a DMHg standard requires only a few minutes to down-load to the cryotrap prior to injection.

When non-cryogenic oven GC conditions were used (35°C for 1minute, 25°C/min to 160°, Swan 1996) peak tailing was experienced mainly due to the ambient transfer capillary column section from the 6-port valve to the GC column. To achieve sharper peaks it was necessary to cryogenically cool the oven with carbon dioxide. This allowed the alkylmercury compounds to be heldup at the head of the column after being swept from the 1st stage cryotrap prior to the temperature ramp.

The end of the Carbotrap that faced the bubbler outlet was placed towards the inlet of the cryogenic trap to avoid passing the organomercurials down the entire length of the heated Carbotrap column during desorption. This greatly minimised possible on-column breakdown of the organomercurials to form residual Hg° and monoethylmercury (Bloom 1989). To maintain a clean valving system it was necessary to remove the permeation tube periodically so that the Teflon tubing did not adsorb and subsequently bleed detectable residual DMHg. A small amount of Hg° and DEHg were observed in all samples analysed, but were also found present at the same concentrations in STEB reacted and purged distilled water reagent blanks, indicating a small source of labile inorganic mercury from the reagents and/or dilution water.

Acknowledgments. I thank Ron Plaschke at CSIRO Division of Oceanography, Hobart Tasmania for helpful comments, and John Cox at the AAD for drafting Figure 1. The Australian Government Analyst is acknowledged for permission to publish this work which was conducted via Public Interest Project funding.

REFERENCES

Baeyens W (1992) Speciation of mercury in different compartments of the environment. Trends Analyt Chem 11: 245-254

Bloom N (1989) Detection of picogram levels of methylmercury by aqueous phase ethylation followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Canadian J Fish Aquatic Sci 46: 1131-1140

Carro-Diaz AM, Lorenzo-Ferreira RA, Cela-Torrijos (1994) Speciation of organomercurials in biological and environmental samples by gas chromatography with microwave-induced plasma atomic emission detection. J Chromatog 683: 245-252

Collett DL, Fleming DE, Taylor GA (1980) Determination of alkylmercury in fish by steam distillation and cold-vapour atomic-absorption spectrophotometry. Analyst 105: 897-901

Cossa D, Martin J, Sanjuan J (1994) Dimethylmercury formation in the Alboran Sea. Marine Pollut Bulletin 28: 381-384

FCN (1996a) EPA delays methylmercury report, submits draft to science panel for review. Food Chem News, July 38: 25-26

FCN (1996b) Serious public misunderstanding about seafood safety could result from current EPA mercury study report draft, FDA says. Food Chem News, March 38: 3-5

Foulke JE (1994) Mercury in fish, cause for concern? FDA Consumer 28: 5-8

Green MJ (1996) A practical guide to analytical method validation. Analyt Chem News and Features, May: 305-309A

Janak K, Anders C, Ostman C (1995) Quantitative analysis using gas chromatography with atomic emission detection. J Chromatog Sci 33: 611-621

Johnson J (1997) Controversial EPA mercury study endorsed by science panel. Environ Sci Technol 31: 218A-219A

Koichi C, Kazuo Y, Kiyoshi T, Hiroki H, Keiichiro F (1983) Determination of alkylmercury in seawater at the nanogram per liter level by gas chromatography/ atmospheric pressure helium microwave-induced plasma emission spectrometry. Analytic Chem 55: 450-453

NFA (1994) Mercury in fish. Food Standard, (National Food Authority, Canberra, Australia) 13, Nov: 15

Puk R, Weber JH (1994) Determination of mercury (II), monomethylmercury cation, dimethylmercury and diethylmercury by hydride generation, cryogenic trapping and atomic absorption spectrometric detection. Analyt Chim Acta 292: 175-183.

Rapsomanikis S, Craig PJ (1991) Speciation of mercury and methylmercury compounds in aqueous samples by chromatography-atomic absorption spectrometry after ethylation with sodium tetraethylborate. Analyt Chim Acta 248: 563-567

Swan HB (1996) Detecting mercury in fish tissue using aqueous-phase ethylation with GC/AED. Peak (Hewlett Packard) No.2: 1-4.

Yong C, Bayona JM (1995) Determination of methylmercury in fish and river water samples using in situ sodium tetraethylborate derivatization followed by solid-phase microextraction and gas chromatography-mass spectrometry. J. Chromatog 696: 113-122