Preliminary Study of the Effects of Some Physical Parameters on the Stability of Methylmercury in Biological Samples*

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The effects of storage conditions (long-term storage of wet samples in a deep-freeze or thermal cycling), freeze-drying and gamma-irradiation at 1 and 5 Mrad on the stability of methylmercury in some biological samples were investigated. Methylmercury was determined by volatilization separation followed by gas chromatography and by ion-exchange separation of inorganic and organic species followed by measurement by cold vapour atomic absorption spectrometry (CVAAS). Total mercury was determined by CVAAS. Biological samples studied included fish and shellfish tissues, human hair and blood samples and appropriate reference materials. From the preliminary results obtained it can be concluded that fresh and dried fish muscle and fish certified reference materials show good stability with time and against temperature cycling. Shellfish and blood should not be repeatedly frozen and unfrozen otherwise possible losses of methylmercury can occur. Losses of methylmercury of up to 30% from wet mussels occurred on prolonged storage in a deep-freeze. Gamma-irradiation reduced the methylmercury content of the fish and shellfish only for hake (*Merluccius merluccius*). Further experiments should be carried out to confirm this and to investigate if this effect is species dependent. Apparent losses of methylmercury on freeze-drying of blood need to be reconfirmed on further samples.

Keywords: *Methylmercury; gamma-irradiation; freeze drying; long-term stability; cold vapour atomic absorption spectrometry*

In general, in many studies of analytical procedures for the determination of methylmercury, most attention has been paid towards the isolation and quantification stages. Relatively little is known about the effects of storage conditions on the stability of methylmercury in biological materials. Fresh samples are usually stored deep-frozen, or lyophilized and preferably stored in the dark in a refrigerator. Additionally, reference materials (RMs) are gamma-irradiated for sterilization.

In view of this, and as this laboratory is involved in many environmental and health-related mercury studies, it is important to know how storage conditions affect the stability of methylmercury in real samples. Therefore, the effects of long-term storage of wet samples in a deep-freeze, thermal cycling, freeze-drying and gamma-irradiation (1–5 Mrad) were investigated in preliminary experiments designed to provide information on the magnitude of such effects. Biological samples studied included fish and shellfish tissues, human hair and blood samples and appropriate RMs.

Experimental

The stability of methylmercury was investigated in both fresh and dried samples.

Fresh Samples

The following samples were used. (1) Muscle from three fish species: hake (*Merluccius merluccius*), sea bream (*Pagellus erytrinus*) and striped mullet (*Mullus barbatus*); (2) shellfish soft tissue (*Mytilus galloprovincialis*); (3) cuttlefish (*Sepia officinalis*); (4) whole blood (from a fish eater); and (5) whole blood from a chloralkali worker (occupationally exposed to mercury vapour).

Fish samples were caught in June 1988 in the central Adriatic Sea. Fish were filleted and the flesh tissue was homogenized. Soft parts from six shellfish were isolated, combined and homogenized. One cuttlefish sample was taken, intestinal organs were discarded and the remainder was homogenized. Samples were homogenized by chopping and mixing, and were then stored in polyethylene containers (Kartell, Italy). Total mercury and methylmercury were determined immediately after preparation, and the remainder of the samples stored deep-frozen until used for the stability experiments. Blood samples were heparinized and stored in a deep freeze until used for further experiments.

Dry Samples

(1) The following samples were used. Human hair with a low mercury content, which is used as a laboratory quality control standard; (2) Commission of the European Communities (CEC), Ispra, Research Sample Human Hair, intercomparison sample with a relatively high mercury content; (3) International Atomic Energy Agency (IAEA) Tuna-350,

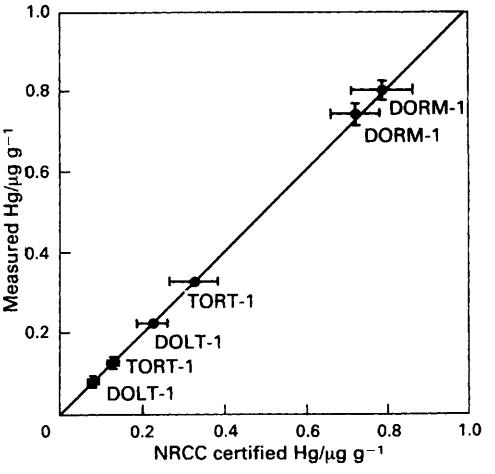

Fig. 1 Comparison of the results for CRMs from NRCC. ●, Total Hg (CVAAS); and ◆, methylmercury (mean of methods ± standard deviation)

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intercomparison sample with a high mercury content; (4) IAEA MA-B-3/TM, Fish Homogenate, Garkpike fish, CRM for total mercury concentration; and (5) National Research Council, Canada (NRCC) DORM-1, Dogfish Muscle, CRM for total mercury and methylmercury.

A freeze-drying apparatus from Christ, Alpha 1–4, LOC-1, was used. Gamma-irradiation was performed at two doses of 1 and 5 Mrad, using ^{60}Co gamma-rays. During irradiation, samples were deep frozen. Thermal cycling experiments were performed by allowing deep-frozen samples to reach room temperature, leaving for 4–5 h and then refreezing. This cycle was repeated on several days (up to six cycles).

Total mercury was determined by gold amalgamation cold vapour atomic absorption spectrometry (CVAAS).[1] Methylmercury was determined by volatilization separation followed by gas chromatography, and by ion-exchange separation of inorganic and organic species followed by CVAAS measurement.[2,3]

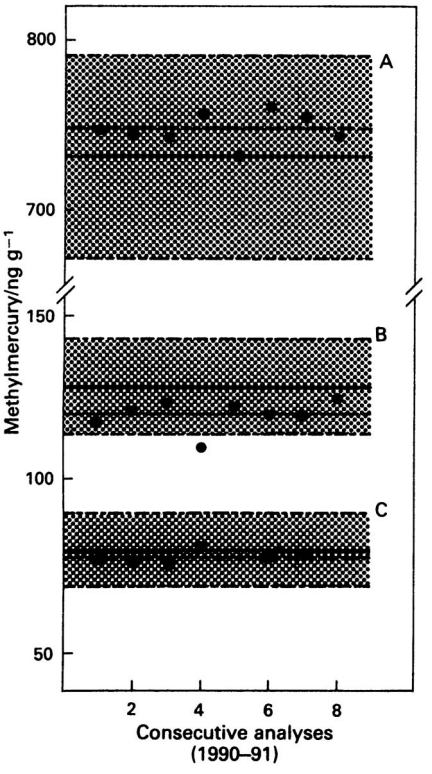

Fig. 2 Consecutive analyses of methylmercury in NRCC CRMs in the period 1990–91. A, DORM-1 Dogfish Muscle, certified value: 731 ± 60 ng g^{-1}; $\bar{x}$ = 748 ± 10 ng g^{-1} (n = 8); B, TORT-1 Lobster Hepatopancreas, certified value: 128 ± 14 ng g^{-1}; $\bar{x}$ = 120 ± 3 ng g^{-1} (n = 8); and C, DOLT-1 Dogfish Liver, certified value: 80 ± 11 ng g^{-1}; $\bar{x}$ = 78 ± 1 ng g^{-1} (n = 6)

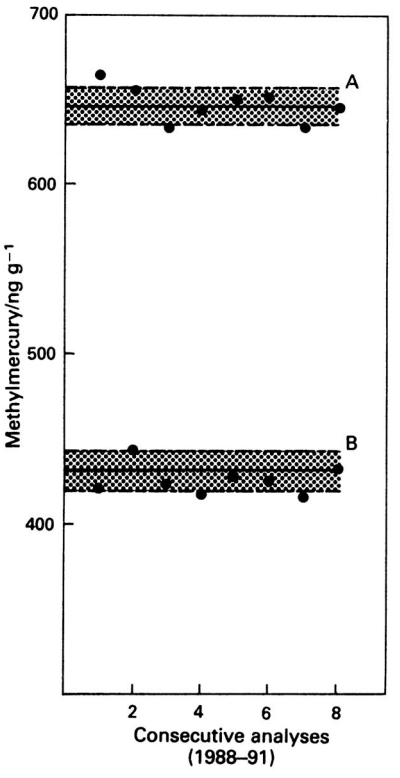

Fig. 3 Stability of methylmercury within the period 1988–91 in two laboratory standards. A, CEC Research Sample Human Hair, laboratory standard: 646 ± 11 ng g^{-1}; and B, IAEA MA-B-3/TM Fish Tissue Lyophilized, laboratory standard: 431 ± 12 ng g^{-1}. Laboratory standard: mean of five methods ± standard deviation

Table 1 Effect of lyophilization. Results for total mercury and methylmercury in fresh and lyophilized samples. Results are expressed on a dry mass basis as the mean ± standard deviation (1σ)

Sample	Total mercury/ng g^{-1} Hg		Methylmercury/ng g^{-1} Hg		c (MeHg) : c (total Hg) ratio	
	Fresh sample	Lyophilized sample	Fresh sample	Lyophilized sample	Fresh sample	Lyophilized sample
Whole blood (worker from chloralkali plant)	105 ± 3 (2)*	100 ± 2 (2)	7.00 ± 0.50 (4)	6.36 ± 0.06 (2)	0.066	0.063
Whole blood (fish eater)	39.6 ± 1.0 (2)	41.0 ± 0.7 (2)	21.4 ± 1.0 (4)	14.4 ± 0.1 (2)	0.54	0.35
Shellfish	116 ± 5 (2)	116 ± 4 (2)	64.5 ± 1.0 (4)	63.7 ± 0.6 (2)	0.56	0.55
Cuttlefish	162 ± 11 (2)	171 ± 4 (2)	133 ± 7 (4)	136 ± 6 (2)	0.82	0.80
	Total mercury/μg g^{-1} Hg		Methylmercury/μg g^{-1} Hg			
Hake	1.21 ± 0.10 (2)	1.14 ± 0.08 (2)	1.01 ± 0.02 (4)	0.85 ± 0.05 (2)	0.88	0.75
Striped mullet	2.15 ± 0.15 (2)	1.81 ± 0.09 (2)	1.30 ± 0.08 (4)	1.38 ± 0.04 (2)	0.61	0.76
Sea bream	2.27 ± 0.23 (2)	2.40 ± 0.02 (2)	1.52 ± 0.12 (4)	1.61 ± 0.10 (2)	0.67	0.67

* Values in parentheses are the number of independent determinations.

Results and Discussion

Comparison of the results for total mercury and methylmercury obtained in NRCC CRMs is shown in Fig. 1. The good agreement with certified values confirms the accuracy of the analytical methods.

Results of consecutive analyses for methylmercury in NRCC CRMs in the period 1990–91 are shown in Fig. 2. In Fig. 3 the results of consecutive analyses of CEC Research Sample Human Hair and IAEA MA-B-3/TM in the period from 1988 to 1991 are shown. From Figs. 2 and 3 it is evident that no changes in methylmercury occurred, which indicates the stability of methylmercury in these CRMs over a period of time.

Results for total mercury and/or methylmercury in fresh and lyophilized samples shown in Table 1 indicate no marked losses on lyophilization either for total mercury methylmercury, except for the blood sample from a fish eater. An approximately 20% lower value of methylmercury was found after lyophilization, but total mercury remained constant. More experiments on other blood samples need to be carried out to check if this single result is typical. A maximum loss of 5% of methylmercury from radiolabelled rat blood during lyophilization was observed by LaFleur.[4]

The larger variability of the results for fresh fish muscle is probably due to the inadequate homogenization technique (samples were chopped and mixed but not blended).

Table 2 Effect of gamma-irradiation. Concentrations of methylmercury in dry samples before and after gamma-irradiation at 1 and 5 Mrad doses. Results are expressed as the mean ± standard deviation (1σ) in ng g^{-1} of Hg on a dry mass basis

Sample	Before gamma-irradiation	1 Mrad	5 Mrad
Human hair	204 ± 8 (3)*	206 ± 4 (2)	203 ± 3 (2)
CEC Research Sample Human Hair	620 ± 21 (3)	631 ± 4 (2)	630 ± 7 (2)
IAEA MA-B-3/TM	431 ± 12 (3)	478 ± 8 (2)	450 ± 32 (2)
NRCC DORM-1	749 ± 14 (3)	738 ± 8 (2)	752 ± 9 (2)
IAEA Tuna-350	4066 ± 51 (3)	4032 ± 70 (2)	4284 ± 15 (2)

* Values in parentheses are the number of independent determinations.

The effect of gamma-irradiation on the stability of methylmercury in fresh and lyophilized samples is presented in Tables 2 and 3. Only for hake muscle was a significantly lower concentration of methylmercury found in fresh and lyophilized samples after gamma-irradiation. At higher doses even lower methylmercury concentrations were observed. On the other hand, no effect was observed in other fish or seafood.

In Table 4 results for methylmercury in four fresh marine samples stored for a 32 month period in a deep-freeze are presented. It is evident that long periods of deep-frozen storage do not affect the methylmercury concentration in fish muscle, but losses of up to 30% were observed in shellfish. This effect has also been observed previously for shellfish in this laboratory. The effect of thermal cycling on the stability of methylmercury in all the fresh samples included in this study showed that lower results were found in shellfish tissue and blood samples (Fig. 4). In all other samples it remained constant during many thermal cycles.

Conclusions

From the preliminary results described, it can be concluded that: (1) fresh and dried fish muscle and fish CRMs show good stability with time and against temperature cycling. However, on prolonged storage of frozen wet shellfish, losses of methylmercury occur; (2) shellfish and blood should not be repeatedly frozen and unfrozen, otherwise possible losses of methylmercury can occur; (3) gamma-irradiation reduced the methylmercury content of the fish and shellfish only for hake (*Merluccius merluccius*). More experiments should be carried out to confirm this and investigate if this effect is species dependent; (4) apparent losses of methylmercury on freeze-drying blood need to be reconfirmed on further samples; and

Table 4 Stability of methylmercury in some marine organisms stored deep-frozen for 32 months. Results are expressed as the mean of three determinations ± standard deviation in ng g^{-1} wet mass.

Sample	Date of analysis: July 1988	March 1991
Sea bream	430 ± 14	417 ± 20
Striped mullet	330 ± 20	311 ± 8
Hake	220 ± 5	213 ± 8
Shellfish (*M. galloprovincialis*)	24.3 ± 2.4	17.3 ± 1.8

Table 3 Effect of gamma-irradiation. Concentrations of methylmercury in fresh and lyophilized samples before and after gamma-irradiation. Results are expressed as the mean ± standard deviation (1σ) on a dry mass basis

	Fresh samples			Lyophilized samples		
Sample	Before gamma-irradiation	1 Mrad	5 Mrad	Before gamma-irradiation	1 Mrad	5 Mrad
Whole blood (worker from chloralkali plant)	6.36 ± 0.06 (4)*	6.71 (1)	7.52 (1)	6.36 ± 0.06 (2)	5.84 (1)	6.12 (1)
Whole blood (fish eater)	21.4 ± 1.0 (4)	20.9 (1)	16.2 (1)	14.4 ± 0.1 (2)	14.5 (1)	15.4 (1)
Shellfish	64.5 ± 1.0 (4)	62.5 (1)	62.5 (1)	63.7 ± 0.60 (2)	61.2 (1)	62.4 (1)
Cuttlefish	133 ± 7 (4)	130 (1)	133 (1)	136 ± 6 (2)	133 ± 8 (2)	134 ± 2 (2)
Hake	1.01 ± 0.02 (4)	0.55 ± 0.01 (2)	0.32 ± 0.01 (2)	0.85 ± 0.05 (2)	0.42 ± 0.02 (2)	0.25 ± 0.01 (2)
Striped mullet	1.30 ± 0.08 (4)	1.31 ± 0.02 (2)	1.37 ± 0.02 (2)	1.38 ± 0.04 (2)	1.28 ± 0.10 (2)	1.47 ± 0.21 (2)
Sea bream	1.52 ± 0.12 (4)	1.33 ± 0.04 (2)	1.52 ± 0.02 (2)	1.61 ± 0.10 (2)	1.63 ± 0.15 (2)	1.52 ± 0.14 (2)

* Values in parentheses are the number of independent determinations.

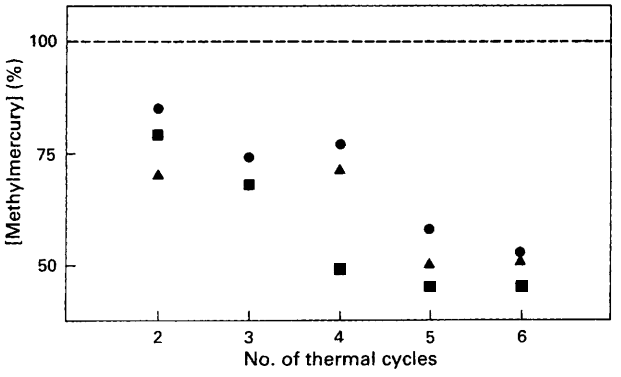

Fig. 4 Effect of thermal cycling on methylmercury concentration in blood and shellfish samples. ■, *M. galloprovincialis*; ●, blood (fish eater); and ▲, blood (worker in chloralkali plant)

(5) the effect of light and ultraviolet (UV) irradiation needs to be studied.

If it were to be confirmed that gamma-irradiation of certain seafoods substantially reduces methylmercury concentrations, this could form the basis for a detoxification process of commercial importance. Similarly if UV irradiation of a thin, semi-transparent slurry of fish were to convert methylmercury into the inorganic form efficiently, this might be explored in a continuous, industrial-scale demethylation process.

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