MERCURY AND SELENIUM IN MARINE- AND FRESHWATER FISH

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ABSTRACT

Mercury, methylmercury and selenium concentrations were determined in fillets and livers of various marine- and freshwater fish species. About 90% of the mercury in the fillets was found to be present as methylmercury. The concentration of mercury in some freshwater fish samples exceeded the FDA guideline of 1.0 mg/kg, whilst the mercury concentration in marine fish was below this level. In nearly all marine fish samples the stoichiometric Hg:Se ratio was smaller than one. Accumulation of mercury in freshwater fish took place in such a way that the stoichiometric Hg:Se ratio was larger than one. A linear relationship was observed between the methylmercury and selenium concentration in the livers of eight perch samples.

INTRODUCTION

IN RECENT YEARS much attention has been given to the possible danger of mercury present in fish.

The toxicity of methylmercury, the predominant chemical form of mercury in fish, gave rise to a provisional maximum permissible level of 1.0 mg/kg established by the Food and Drug Administration (1979) in the United States. As a result fish species like swordfish, shark, and tuna, which often exceed this tolerance level, will be sometimes considered unsuitable for human consumption. Many investigators, however, have pointed out that the toxicity of methylmercury can be lowered by selenium as referred to below.

Mercury-selenium antagonism

Parizek and Ošťádalová (1967) showed that sodium selenite has a protective effect against the mortality of rats caused by mercuric chloride when administered simultaneously. The protective effect of sodium selenite against methylmercury poisoning of rats was demonstrated by Iwata et al. (1973). Ganther and Sunde (1974) reported that the toxicity of methylmercury was reduced in Japanese quail by a diet containing tuna and found that tuna had a relatively high selenium content. Selenium might therefore be responsible for the decreased toxicity. Stoewsand et al. (1974) showed that the addition of sodium selenite (5 mg/kg) to diets of Japanese quail protected against methylmercury (20 mg Hg/kg) intoxication for 9 wk. Dietary interaction between methylmercury and sodium selenite were studied in Japanese quail by El-Begearmi et al. (1977). They showed that the addition of 0.35–6 mg Se/kg to diets containing toxic levels of Hg (5–30 mg/kg) reduced the toxicity of methylmercury and increased the survival of Japanese quail.

Experiments with rats fed diets containing methylmercury and mercuric chloride with and without sodium selenite have been performed by Potter and Matrone (1974).

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Table 1—Fishing grounds of the various fish species

Marine fish	Fishing ground
ling (Molva molva)	Northern North Sea
redfish (Sebastes spp)	South of Greenland
plaice (Pleuronectus platessa)	Central North Sea
sole (Solea solea)	Central North Sea
mackerel (Scomber scombrus)	Dutch coast/the Channel
blue whiting (Gadus poutassou)	South of Får Öer
cod (Gadus morhua)	North Sea
shrimp (Crangon crangon)	Texel
mussel (Mytilus edulis)	Hammen
Freshwater fish	
eel (Anguilla anguilla)	Maas-Waal channel
pike (Esox lucius)	Loenerveense plas[a]
perch (Perca fluviatilis)	Lauwersmeer/Loenerveense plas[a]
pike-perch (Stizostedion lucioperca)	IJsselmeer[a]

[a] Inland lakes

They showed that selenite protected against mortality and neurotoxicity due to dietary methylmercury.

Recently Nobunaga et al. (1979) investigated the effects of selenium on the embryotoxicity and teratogenicity of methylmercury. A low dose of sodium selenite in drinking water (3 mg Se/ml) in combination with a high dose of methylmercury in food (8 mg/kg) increased the number of placenta sites without embryos and the number of remnants of implantation without placenta in female mice. Embryolethality was decreased and the incidence of cleft palate was increased in the group receiving a high dose of selenite (6 mg/kg) combined with the high dose of methylmercury.

It is obvious that the antagonistic action of selenium against mercury is rather complex and up till now its mechanism is only partially understood.

It has been suggested that mercury and selenium occur together in animal tissues and are associated to proteins by means of sulphur linkages.

Sumino et al. (1977) showed that methylmercury bound to proteins of various organs of mice could be partially released by an intravenous injection of a sodium selenite solution.

Mercury-selenium correlation

Koeman et al. (1973) observed a linear relationship between the concentration of mercury and selenium in livers of marine mammals. A molar 1:1 mercury-selenium increment was reported. A similar molar increment has been reported by Ganther and Sunde (1974).

A strong linear correlation between mercury and selenium in the organs (thyroid, pituitary gland, kidney, and brain) of miners have been observed by Kosta et al. (1975). A Hg-Se increment of approximately 1:1 has been established as well.

Recently Freeman et al. (1978) determined the concentration of both mercury and selenium in 47 samples of swordfish. Most of the samples had mercury levels exceeding 0.5 mg/kg. When the data pairs of the mercury and selenium concentration were analyzed for correlation, it

Table 2—Concentration of mercury, methylmercury, and selenium in fillets of various fish species

	No. of samples	Concentration					
		Mercury (mg/kg)		Methylmercury (mg Hg/kg)		Selenium (mg/kg)	
		Avg	Range	Avg	Range	Avg	Range
ling	4	0.37	0.20–0.83	0.34	0.19–0.73	0.34	0.31–0.36
redfish	4	0.15	0.10–0.17	0.15	0.10–0.19	0.44	0.26–0.53
plaice	4	0.16	0.04–0.31	0.14	0.03–0.29	0.65	0.26–1.04
sole	5	0.13	0.02–0.29	0.11	0.01–0.28	0.24	0.15–0.29
mackerel	6	0.33	0.05–0.70	0.27	0.04–0.64	0.35	0.22–0.44
blue whiting	15	0.15	0.03–0.29	0.14	0.02–0.26	0.31	0.25–0.43
cod	53	0.16	0.03–0.49	0.16	0.03–0.48	0.29	0.17–0.43
shrimp	4	0.14	0.12–0.16	0.13	0.12–0.15	0.31	0.28–0.36
mussel	5	0.07	0.05–0.12	0.03	0.02–0.05	0.48	0.46–0.51
eel	4	0.26	0.21–0.31	0.26	0.26–0.31	0.40	0.35–0.46
pike	14	0.60	0.11–2.42	0.58	0.11–2.31	0.13	0.08–0.24
perch	15	0.82	0.10–1.74	0.74	0.08–1.63	0.24	0.12–0.66
pike-perch	11	0.85	0.53–1.14	0.76	0.50–0.93	0.26	0.21–0.31

was found that a power curve with an equation form $y = ax^b$ fits best.

It is obvious that the observed mercury-selenium interaction and relationship should be taken into account with the establishment of a maximum permissible level of mercury in fish. Therefore it is preferable to determine not only the mercury concentration in fish but also the selenium concentration.

In such a way a possible relationship between mercury and selenium in fish can be observed as well. In this study the concentration of mercury and selenium in various fish species is determined in order to give a contribution to the discussion about a tolerance level of mercury in fish.

EXPERIMENTAL

MARINE FISH SAMPLES were obtained from the Netherlands Institute for Fishery Investigations, Ijmuiden, the Netherlands. Samples of freshwater fish species were taken by the Institute for Fishery Products TNO. All fish samples were collected ungutted and preserved (deep frozen) as soon as practicable after collection. Next the edible part and the livers were homogenized separately in a Retsch centrifugal mill (speed 10.000 r.p.m.; Φ of the rotor 10 cm; Φ of the holes in the surrounding sieve 5 mm). A mussel sample consisted of at least 50 individuals. The individual animals were carefully freed from their shells by cutting the adductor muscle. The shell cavity liquor was discarded and the entire remaining shell contents were collected and homogenized. A sample of shrimps, consisting of at least 100 individuals, was peeled and homogenized. All samples were stored at −20°C. The analyses were performed in duplicate. The relative standard deviation of the elements concerned was ⩽ 5%. Table 1 presents a survey of the investigated fish species and the fishing grounds.

Methods of analysis

Mercury. One gram of wet material was provided with 3.4 ml of 90% nitric acid and 0.3 ml of water. The mixture was heated in a Teflon-lined stainless steel decomposition vessel for at least 3 hr at 150°C.

After blowing off most of the nitrous fumes and subsequent treatment with hydroxylamine · HCl, Hg^{2+} was reduced to elemental mercury by means of $SnCl_2$. The volatile elemental mercury was preconcentrated on a wrapped gold foil (∿ 4g) by purging hydrogen

Table 3—CH_3 Hg/Hg and Hg/Se ratio in fillets of various fish species

	No. of samples	CH_3 Hg/total Hg (stoichiometric)		Hg/Se (stoichiometric)	
		Avg	Range	Avg	Range
ling	4	0.95	0.88–1.00	0.45	0.22–1.02
redfish	4	0.98	0.88–1.00	0.15	0.08–0.25
plaice	4	0.86	0.75–0.93	0.10	0.05–0.17
sole	5	0.76	0.20–1.00	0.19	0.04–0.40
mackerel	6	0.74	0.58–0.93	0.32	0.07–0.63
blue whiting	15	0.92	0.66–1.00	0.21	0.03–0.46
cod	53	0.97	0.83–1.00	0.22	0.07–0.63
shrimp	4	0.93	0.92–1.00	0.18	0.13–0.21
mussel	5	0.39	0.33–0.42	0.05	0.04–0.09
eel	4	0.99	0.96–1.00	0.26	0.22–0.34
pike	14	0.96	0.83–1.00	1.97	0.36–9.5
perch	15	0.89	0.80–1.00	1.60	0.24–4.0
pike-perch	11	0.90	0.79–1.00	1.31	0.80–1.85

Table 4—Concentration of mercury, methylmercury, and selenium in livers of various fish species

	No. of samples	Concentration					
		Mercury (mg/kg)		Methylmercury (mg Hg/kg)		Selenium (mg/kg)	
		Avg	Range	Avg	Range	Avg	Range
ling	1	1.26	–	0.74	–	2.84	–
redfish	1	0.07	–	0.02	–	1.76	–
plaice	1	0.28	–	0.10	–	1.81	–
sole	2	0.11[5]	0.05–0.18	0.06[5]	0.03–0.10	2.01	1.95–2.08
mackerel	7	1.41	0.12–4.6	0.48	0.04–1.42	3.87	2.76–4.60
blue whiting	3	0.07	0.03–0.11	0.02	0.01–0.03	0.76	0.45–1.07
cod	4	0.07	0.02–0.18	0.04[5]	0.01–0.10	1.32	0.86–1.95
eel	1	0.37	–	0.34	–	5.00	–
pike	8	0.63	0.06–4.50	0.41	0.04–2.92	0.88	0.56–2.05
perch	10	0.73	0.08–1.76	0.54	0.01–1.00	0.78	0.63–1.00
pike-perch	2	0.59	0.48–0.74	0.47	0.40–0.60	1.01	0.81–1.18

(300 ml/min) through the reduction vessel during 10 min. Next the gold foil was heated (∿ 320°C) within 30 sec. A stream of hydrogen transferred the evaporated mercury to a HGM 2300 mercurymeter (Incentive Research & Development AB, Stockholm, Sweden). Recovery experiments have shown that all the present mercury was recovered quantitatively.

Methylmercury. A modified version of the method of Talmi (1975) was used for the determination of methylmercury. To 5.0g of wet material 5 ml of water and 5 ml of concentrated hydrochloric acid (36%) were added. The mixture was shaken for 10 min and 4 ml of toluene/carbontetrachloride 9:1 was added subsequently. The mixture was shaken again for 10 min, centrifuged for 5 min at 3000 rpm and the organic layer, after drying over anhydrous sodium sulphate, was submitted to gas chromatography. Recovery experiments showed that 75% of the present methylmercury was extracted. Detection was performed with a microwave emission spectrometric detector. The detector was designed and built by the Central Laboratory TNO, Delft, the Netherlands, and modified at our own Institute.

The gas chromatographic conditions and those of the atomic emission detector were:

Glass column	: 1.40 × 2.0 mm i.d. packed with 4% FFAP on Gas Chrom Q 80–100 mesh
Column temperature	: 185°C (isothermal)
Injection port temperature	: 200°C
Wavelength	: 253.65 nm
Split width	: 0.2 nm
Microwave generator output	: 75 Watt
Carrier gas	: 30 ml He/min
Pressure in the capillary	: 20 Torr

Selenium. One gram of wet material was decomposed as described under mercury and post-treated with perchloric acid and hydrochloric acid in order to complete the destruction and to bring all Se into the quadrivalent state, respectively. After coupling to 2,3-diaminonaphthalene (DAN), the resulting benzopiazselenol was extracted into hexane and measured fluormetrically (excitation at 378 nm, emission at 520 nm). Experiments have shown that selenium present in the samples were recovered quantitatively by the proposed analytical method.

RESULTS & DISCUSSION

THE AVERAGE concentrations and concentration ranges of mercury, methylmercury and selenium in fillets and livers of marine fish samples (ling, redfish, plaice, sole, mackerel, blue whiting, and cod) and freshwater fish samples (pike, perch, and pike-perch) are given in Table 2 and 3. Results of mercury and selenium in the edible part of some samples of mussels and shrimps are tabulated as well.

Table 4 and 5 show the average and range of the methylmercury:mercury ratios and mercury:selenium ratios present in the fillets and livers.

Fillets

In the case of marine fish the mercury concentration in all the investigated samples was below the FDA guideline of 1.0 mg/kg.

Samples of pike, perch and pike-perch often possessed mercury concentration levels which are beyond the tolerance level of 1.0 mg/kg. However the average mercury concentration in the freshwater species concerned was below 1.0 mg/kg.

About 90% of the mercury in the edible part, except in mussles, was present as methylmercury.

In nearly all marine fish samples the stoichiometric Hg:Se ratio was smaller than 1. Stoichiometrically, selenium was always in excess of mercury.

No relation could be found between the mercury and selenium concentration in the fillets of 53 cod samples. The mercury concentration in freshwater fish samples was relatively high whilst the variability of the selenium concentration in the samples was small. Therefore the stoichiometric Hg:Se ratio became larger than one. Obviously the accumulation of selenium in the fillets of these samples did not keep step with the accumulation of mercury.

Table 5—CH_3Hg/Hg and Hg/Se ratio in livers of various fish species

	No. of samples	CH_3Hg/total Hg (stoichiometric)		Hg/Se (stoichiometric)	
		Avg	Range	Avg	Range
ling	1	0.59	–	0.17	–
redfish	1	0.29	–	0.02	–
plaice	1	0.36	–	0.06	–
sole	2	0.57	0.56–0.58	0.02	0.01–0.04
mackerel	7	0.37	0.22–0.62	0.15	0.01–0.45
blue whiting	3	0.34	0.27–0.43	0.04	0.02–0.06
cod	4	0.65	0.50–0.80	0.013	0.01–0.02
eel	1	0.92	–	0.03	–
pike	8	0.66	0.47–0.83	0.14	0.03–0.85
perch	10	0.64	0.55–0.83	0.42	0.06–0.74
pike-perch	3	0.81	0.78–0.84	0.23	0.18–0.28

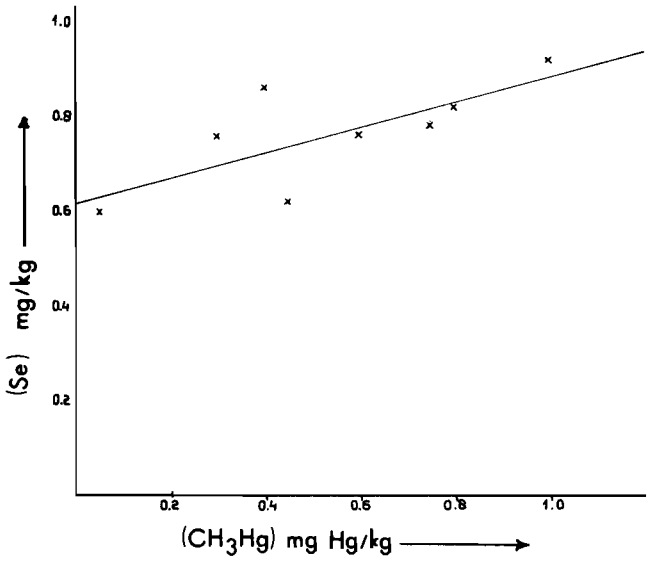

Fig. 1—Relation between methylmercury and selenium in livers of eight perch samples.

Livers

In comparison with the fillets the selenium concentration in all livers was relatively high, whilst a smaller part of the mercury was present as methylmercury. High mercury concentrations were present in the livers of mackerel, pike, perch, and pike-perch samples. However, the stoichiometric Hg:Se ratio never exceeded one.

In the case of perch a significant correlation existed between the methylmercury concentration and the selenium concentration (Fig. 1).

Application of the least squares method leads to the following regression line with a correlation coefficient of 0.74:

$$[Se] = 0.26\,[Hg]_{CH_3Hg} + 0.62$$

Although the number of investigated samples was limited a tentative conclusion can be drawn. If at least a molar equivalent selenium concentration is necessary for protection, it is to be expected that the selenium concentration in marine fish will be sufficient to give a protective effect against (methyl)mercury toxicity.

Probably the protection of selenium present in the freshwater fish samples will be less efficient because a molar excess of mercury often occurs. However, Ganther and

Sunde (1974) and El-Begearmi et al. (1977) showed that a small selenium concentration gives already protection against the toxicity of relatively high mercury concentrations.

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yield reached in the PFR. The CSTR system is valuable for systems undergoing substrate inhibition (Vieth and Venkatasubramanian, 1974) and this is a problem with laminarinase (Jacober et al., 1980). Also, this reactor may minimize diffusion limitation problems caused by the crosslinking procedure. This may explain, in part, why the CSTR approach yielded higher product concentrations.

The surf clam crystalline style was stabilized against disintergration with glutaraldehyde to yield a catalytically active, immobilized enzyme system which could be introduced into a reactor. The utilization of a biological organ as a natural carrier matrix for the immobilization of endogenous hydrolytic enzymes was a significant finding of this research.

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