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Total and Organic Mercury in Barents Sea Pelagic Fish

C. R. Joiris, I. B. Ali, L. Holsbeek, M. Bossicart, G. Tapia

Laboratory for Ecotoxicology and Polar Ecology, Free University of Brussels (VUB), Pleinlaan, 2, B-1050 Brussels, Belgium

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One of the main questions, when studying mercury levels in natural samples, is to define how far the measured concentrations correspond to natural -or background- levels or to actual contamination due to human activities. To establish background pristine levels of Hg in the marine environment, areas of very low human activities are often proposed. Arctic and Antarctic waters, together with deep oceans waters, are best suited and provide themselves for such studies. Barents Sea areas were used in this study, even if the existence of an important atmospheric transport of Hg probably caused an increase of Hg levels at a global scale.

Instead of analyzing mercury from the very low concentrations in sea water, it is much easier to identify it from the higher concentrations which organisms, used as bioindicators, have built up in their tissues. By using these bioindicators to study the bioavailable fraction of the stable residues, one also integrates small scale temporal and spatial variations.

Pelagic fish were used in this work to study the ecotoxicology of Hg in the Barents Sea. This study has been made possible due to recent access of the Barents Sea to western scientists and it is to serve as a complement to existing studies by the same team in the Greenland and Norwegian seas, and the southwestern part of the Barents Sea.

MATERIALS AND METHODS

Samples were collected during three different cruises: capelin *Mallotus villosus* and redfish *Sebastes sp (marinus* and/or *mentella)* were sampled during the EPOS II cruise of RV Polarstern, in June-July 1991 with Otter trawl. MMBI/VUB ARCTIC cruises of RV Dalnie Zelentsy, provided haddock *Melanogrammus aeglefinus* and capelin in July-August 1991, and redfish in July-August 1992. Fish were immediately deep frozen, and analyzed for their total and organic Hg concentration in muscle a few months later. Figure 1 shows the position of the sampling stations and the approximate limits of the main water masses (polar front), based on water temperature determinations.

Total mercury (Hg) was determined with atomic absorption spectrometry (MAS-50 Mercury analyzer, Perkin-Elmer) after mineralization of fresh samples with sulfuric acid (10 ml of 97% acid per g fresh weight) and oxidizing the mercury to Hg^{2+}.

Correspondence to: C. R. Joiris

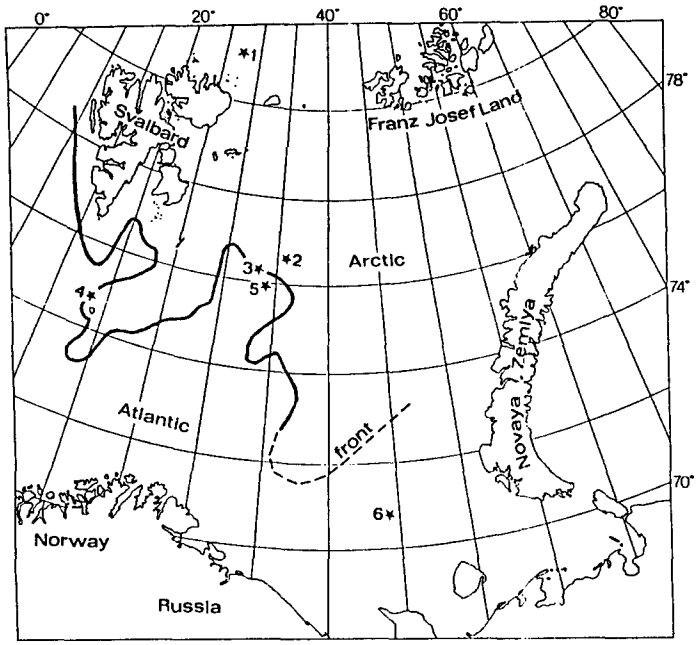

Figure 1. Map of the Barents Sea, showing the main water masses and the sampling stations for pelagic fish, on board RV Polarstern (PS) and RV Dalnie Zelentsy (DZ). Station 1: PS 1991, Arctic water, redfish *(n = 24);* 2: DZ 1991, Arctic water, capelin *(12);* 3: PS 1991, Polar front zone, capelin *(11);* 4: DZ 1991, Polar front, redfish *(5)* and haddock *(26);* 5: PS 1991, Atlantic water, redfish *(15)* and capelin *(11)* ; 6: DZ 1992, Atlantic water, redfish *(6)*

After reducing the Hg^{2+} to Hg° with stannous chloride, the volatile Hg° is bubbled into the closed system of the analyzer and the absorption is measured (wavelength: 253.7 nm). The method used (Hatch and Ott 1968, modified by Bouquegneau 1975), was already described with more detail, including intercalibration (Joiris *et al.* 1991). Mercury content was calculated using an external standard curve. The values were expressed in µg Hg/g dry weight. To test the reproducibility of the method, a homogenized sample of redfish was divided into ten identical parts. Each was mineralized separately and analyzed for total mercury.The mean and median were equal (0.14 µg/ g dw), the standard deviation was 0.01: all measurements fell within 1.96 standard deviations of the mean, and the coefficient of variation (c.v. = standard deviation/mean) was very low (0.07). This is why a normal distribution is assumed and the test of reproducibility considered as satisfactory. Another means of testing for reproducibility is by comparing the standard deviation of the measurements with the theoretical error calculated from the error on the measuring instruments used (in this case a weighing scale, a 100-ml graduated cylinder and the spectrophotometer). Since the mean calculated theoretical error of 0.01 µg/g dw exactly corresponds with the standard deviation of the measurements, it further confirms the high reproducibility of measurements for total mercury. In order to test the representativity of a determination for the whole fish muscle, a test of homogeneity of the tissue was carried out as well: total Hg was measured in 10 different samples of the same fish. The results showed a good homogeneity, since

the same level of total Hg (0.14 as mean and as median) and standard deviation (0.01) were found as for the test of reproducibility. It can be concluded that the variability in the measurements is entirely explained by the reproducibility of the method, and that a possible heterogeneity of the fish tissue does not seem to play any significant role. A test was conducted to check for the possibility of any interaction between mercury and the biological material in which it was analyzed (matrix effect). One muscle sample (haddock) was divided into seven identical parts. Sample 1 was prepared for analysis as usual. Known quantities of inorganic mercury were added to 7 samples before mineralization (internal standard: 0.05, 0.10, 0.15 and 0.20 µg). Measured concentrations increase linearly with quantity of mercury added, with a slope of 1 ($y = 0.973x + 0.062$, $r = 0.99$), so that no matrix effect had to be considered. Intercalibration was performed, using a certified dogfish muscle (DORM-1, Marine Analytical Chemistry Standards Program, Ottawa, Canada). The certified value was 0.789 µg/ g dw +/- 0.074. Our measurements provided a mean value of 0.733 µg/ g dw, with a standard deviation of 0.03, i.e, 93% of the target value ($n = 15$). The detection limit, for samples of 1 to 2 g fw as used in this study, corresponds to about 0.01 µg/ g dw.

Organic Hg was determined by gas chromatography with electron capture detector (Westöö 1966)(Shimazu CR-14). In order to extract all the organic Hg, liophilized samples were treated with NaBr and $CuSO_4$ in sulphuric medium. This facilitates the liberation of methyl bound to thiol groups and the bromine forms a stable compound in a sulphuric medium with methyl. This compound was extracted with toluene. The organic Hg was extracted with thiosulfate hydroalcoholic solution which makes up a specific thio-organic mercury compound. The compound is reconverted into bromide and re-extracted with toluene. The organic Hg detected is mainly methylmercury CH_3Hg^+. One muscle sample (haddock) was divided into five identical parts to test for matrix effect. Sample 1 was prepared for analysis as usual. Known quantities of methylmercury were added to samples 2 through 5 before they were prepared for analysis. The high correlation between added and measured MeHg ($y = 1.08x + 0.065$; $r = 1$) showed a slope sufficiently close to 1 to consider the matrix effect as negligible. Intercalibration was performed, using a certified dogfish muscle (see above). The certified value was 0.731 µg/ g dw +/- 0.06. Our measuremets provided a mean value of 0.62 µg/ g dw, with a standard deviation of 0.07 ($n = 12$), or 85 % of the target value. Considering 10 - 15% variation as acceptable, we decided not to introduce any correction factor on the MeHg measurements. The detection limit, for samples of 1 to 2 g fw, corresponds to about 0.005 µg/ g dw.

"Total" extractable lipids were extracted with a 135 ml hexane and 15 ml acetone mixture for 10 hr at 75° C, using Soxhlet apparatus; the hexane-acetone mixture was then evaporated and the extracted lipids weighed.

The results discussed here did not show a normal distribution; thus, median values are presented rather than the mean, and the significance of differences was tested with a non-parametric Kruskal-Wallis test.

RESULTS AND DISCUSSION

Redfish from the different water masses showed significant differences in median total Hg concentration among the three different water masses, i.e., Arctic water, Atlantic water and mixed Polar front water ($P < 0.01$; Table 1). There was also a significant difference ($P < 0.05$) in distribution of total body length in the different zones, reflecting a different age structure in the fish populations. A positive

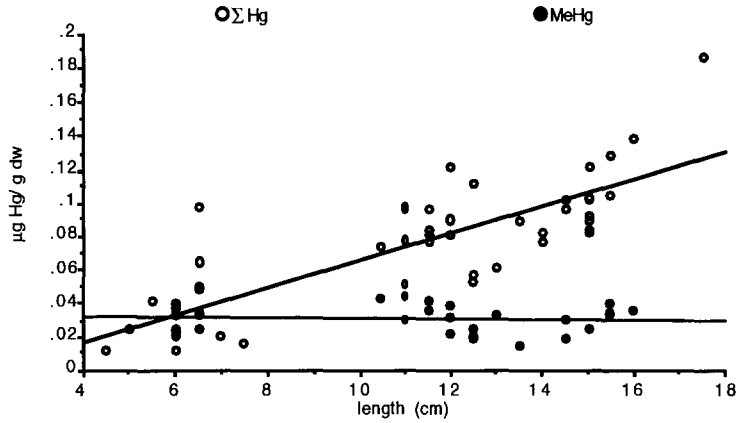

Figure 2. Total and methylHg concentrations (µg/g dw) in redfish, *Sebastes sp.*, from the Barents Sea as a function of body length (cm)

correlation between length and total Hg concentration was found (Fig. 2, upper curve, r = 0.83), reflecting an accumulation of Hg with time, considering body length as directly dependent on age. This result could not be interpreted using a metabolic model by which mercury is fixed and released so that an equilibrium is reached between the quantity fixed and the quantity eliminated (WHO 1976): it implies that mercury concentration for a given species in an environment should be fairly constant with time. The above observation suggests rather a genuine, almost irreversible, association of Hg with biological material, like lipids for organic Hg, selenium or metallothioneins for inorganic Hg. For the capelin, total Hg concentrations were significantly different in the three zones ($P < 0.05$; Table 1). A difference in length distribution in the different zones was also detected ($P < 0.01$). Again, the total Hg concentration was directly dependent on length (Fig. 3, upper curve, r = 0.84). All samples of haddock were taken in the mixed frontal zone and showed a total Hg concentration of 0.07 (Table 1). A similar correlation was noted between total Hg concentration and length (Fig. 4, upper curve, r = 0.82). The apparent geographical differences observed for a given fish species (i.e., redfish, capelin) were due to differences in age structure among the populations, reflected by differences in length. Redfish consisted of different age groups and capelin was represented by an adult summer population (Hopkins and Nilssen 1991).

Table 1. Median total Hg concentration in muscle, and total body length of pelagic fish from the Barents Sea (*n:* number of samples)

species	water mass	*n*	total Hg µg/ g dw	length cm
redfish	Arctic	*24*	0.083	11.5
Sebastes sp.	Atlantic	*21*	0.035	6.5
	polar front	*5*	0.061	13.0
	all	*50*	0.077	11.5
capelin	Arctic	*12*	0.049	11.8
Mallotus villosus	Atlantic	*11*	0.081	14.0
	polar front	*11*	0.070	13.0
	all	*34*	0.073	13.0
haddock				
Melanogrammus aeglefinus	polar front	*26*	0.067	22.3

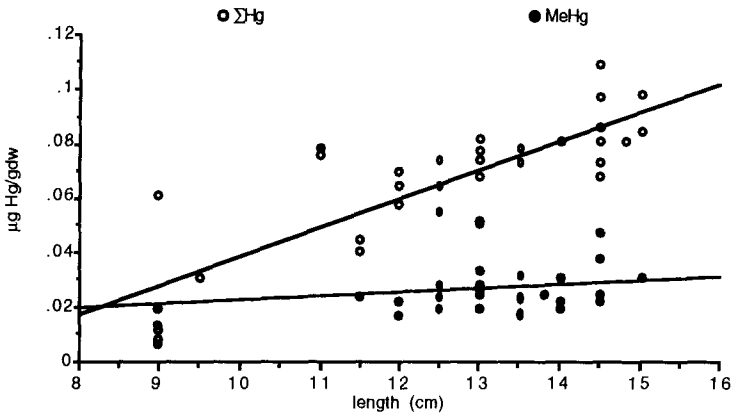

Figure 3. Total and methylHg concentrations (µg/g dw) in capelin, *Mallotus villosus,* from the Barents Sea as a function of body length (cm)

Hence, for such types of study, different stages, i.e., different age groups, should be represented in the sample in order to allow normalization of the data as a function of length. The increase in Hg concentration against length or weight has often been reported in the literature on a wide range of marine fish (see synthesis in Thompson 1990). From the significant correlation observed in all the samples it is clear that differences in length distribution were the major factor explaining the observed differences in total Hg concentration. Since Hg concentrations found in fish muscle are directly dependent on the bioavailable Hg fraction in the ecosystem, it can be concluded that no clear differences could be detected in the Hg levels of the different water masses of the Barents Sea.

The relative concentration of organic mercury (mainly methylmercury MeHg) is often expected to be high (around 80%: see synthesis in Thompson 1990). In our Barents Sea samples, however, it varied between 11 and >100 % (Table 2). The meaning of such median values is, however, very limited: the MeHg concentration (in absolute value) was very constant with length (Figs. 2, 3, and 4, lower curves, for redfish, capelin and haddock, respectively. This resulted, of course, in a decreasing relative MeHg concentration with length.

Table 2. Median organic Hg (MeHg) concentration, relative MeHg concentration (% of total Hg) in muscle, and median total body length of pelagic fish from the Barents Sea (*n:* number of samples).

species	water mass	*n*	MeHg µg/ g dw	% MeHg min.-max.	length cm	lipid g/ g dw
redfish	Arctic	*17*	0.042		12.0	0.04
Sebastes sp.	polar front	*5*	0.024		13.0	0.10
	all	*22*	0.041	22-80	12.0	0.09
capelin	Arctic	*7*	0.019		13.0	0.32
Mallotus villosus	Atlantic	*6*	0.026		14.0	0.23
	polar front	*19*	0.025		11.5	0.23
	all	*32*	0.027	22->100	13.0	0.23
haddock						
Melanogrammus aeglefinus	polar front	*15*	0.041	11->100	22.3	0.03

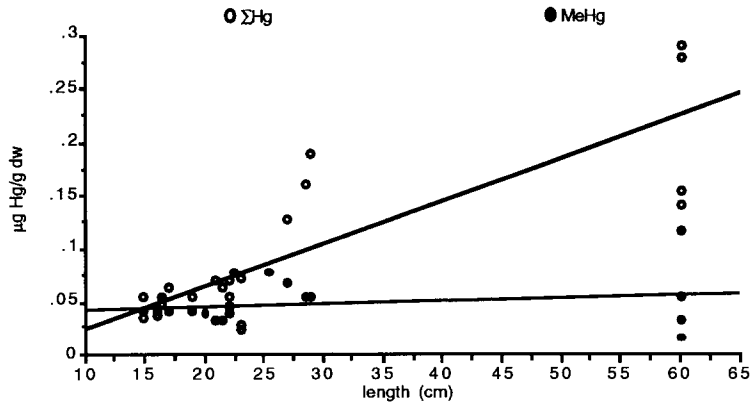

Figure 4. Total and methylHg concentrations (µg/g dw) in haddock, *Melanogrammus aeglefinus,* from the Barents Sea as a function of body length

Such data suggest the existence of a slow demethylation (mineralization) process in the pelagic fish from the Barents Sea. Biodemethylation has been reported in the literature for the white marlin, *Tetrapturus albidus* (Thibaud 1986); it has also been demonstrated in the laboratory in the bluegill, *Lepomis macrochirus* (Burrows and Krenkel 1973). Similar conclusions were drawn for toothed cetaceans (see Joiris *et al.* 1991). In case of contamination of organisms by liposoluble stable residues directly from the water, a correlation can be expected between residues concentrations and lipid content. No correlation between MeHg and extractable lipids (in fact mainly neutral lipids) was noted in our data suggesting that the main route of contamination is the indirect one, through ingested food. A similar fact has been recorded in the case of herring and cod (Perttila *et al.* 1982).

The contamination level of marine organisms presents spatial and temporal variations related not only to the concentration of pollutants in their environment, but also to the time of exposure and to biological characteristics such as age, sex, feeding habits and seasonal variations (Bouquegneau and Joiris 1988). Hence, it is pertinent for understanding the meaning of the data on stable residue concentrations and for the assessment of the pollution level of ecosystems to have adequate knowledge of the above factors. This also stresses the importance of adequate information on the mechanisms of contamination within an ecosystem before trying to establish and compare contamination levels. In this study, differences in length distribution (as a measurement for age) were clearly the main factor explaining the geographical differences in Hg content encountered. After normalization of the data as a function of length, no geographical difference could be detected any longer.

Even though high metal burdens in animals from a seemingly pristine area in the Canadian Arctic have been reported (Smith and Armstrong 1975; Muir *et al.* 1992), the concentration of mercury found in the Barents Sea is low despite the intense activities carried out there by the former USSR. Our sampling, however, did not include any coastal areas. Mercury concentration in Barents Sea pelagic fish (0.04 - 0.08 µg total Hg/ g dw) can be compared with other data obtained by the same team in the North Sea (0.7 - 2.6) and Antarctica (0.13 - 0.66)(Joiris *et al.* unpublished), even if such a comparison is very rough since the data were not corrected for length, and thus provide order of magnitudes only. Hence, the concentration of mercury found in the Barents Sea fish could be proposed as close to background levels, even if real background levels are improbable in any sea,

taking the importance of atmospheric transport at a global scale into account. Another difficulty in comparing data from different regions is the importance of ecological factors, making the direct comparison dangerous or even impossible. Such a situation was already discussed for PCB contamination of the Antarctic: the ecosystem is 7 times less contaminated than the North Sea (when data are expressed in PCB contamination of particulate matter per volume of seawater), but the low particulate matter concentration in the water causes a concentration of PCBs on a dry weight basis as high as in the North Sea (Joiris and Overloop 1991). If representative of real geographical differences, the lower Hg concentrations in the Arctic may be due to the fact that, unlike Antarctica, it forms a closed hydrological basin. The Antarctic is more open to the Atlantic and Pacific oceans, and is also subjected to intense pollution as a result of the agricultural and industrial activities of developing countries surrounding it. By comparison with older data from the Barents Sea, no obvious change of Hg concentration in fish muscle could be detected: in 1975, levels of 0.06 µg/ g dw were noted in capelin (ICES 1977), and 0.07 in this study. Such an observation confirms that the obtained values indeed could be close to background levels, since no clear change took place between 1975 and 1991/92. In Barents Sea pelagic fish, total Hg concentration increased with length (age), while MeHg level remained constant. As a consequence, the relative MeHg concentration strongly decreased with time: from about 100% in young fish (Figs. 2, 3, and 4), to 10-20% in older individuals. The proposed interpretation is that fish are indirectly contaminated by MeHg through their food, and than slowly mineralize these residues and accumulate inorganic Hg in time. This is why another essential aspect to be studied is the speciation of Hg residues. Such low relative concentrations of MeHg are unexpected in fish: ratios of 0.8 and higher are generally recorded, with variations apparently due more to a low reproducibility of organic Hg measurements than to actual low concentrations (Bloom 1992). In our case, however, reproducibility was good, and could not explain the low MeHg values registered. A possible explanation is that in (almost) pristine conditions like the ones of this study, the relative contribution of organic Hg could be limited, but much higher in polluted areas. In older fish, one should also determine if this inorganic Hg is detoxified, i.e., by an association with selenium (Martoja and Viale 1977) or metallothioneins (Roesijadi 1981; Bouquegneau *et al.* 1984).

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