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Bioconcentration and Biomagnification of Mercury and Methylmercury in North Sea and Scheldt Estuary Fish

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Abstract. Total Hg and MMHg concentrations were assessed in more than 350 fish and shellfish samples. Hg concentrations in Greater North Sea fish of prey range from 0.039 mg kg^{-1} wet weight (ww; for ray) to 0.61 mg kg^{-1} ww (for dogfish) and for all other fish species, from 0.045 mg kg^{-1} ww (for plaice) to 0.33 mg kg^{-1} ww (for sand sole), with 95 ± 2% of the Hg content in the MMHg form. In Belgian coastal zone, fish concentrations range from 0.063 mg kg^{-1} ww for plaice to 0.13 mg kg^{-1} ww for flounder, with 82–87% of the Hg content in the MMHg form. In fish of the Scheldt, which is a very polluted estuary, Hg levels, as well as the percent MMHg of the total Hg, were lower than in the two zones previously mentioned. The intraspecies variability is of the order of 50% in each of the three zones. In liver tissue, a much larger variability was observed than in muscle tissue, except for fish species of the Scheldt. In most cases, the MMHg fraction in a particular fish species is inversely related to the intraspecies variability. Bioconcentration and biomagnification factors (BCF and BMF, respectively) were assessed. MMHg-BMFs were a few orders of magnitude higher than Hg(inorganic)-BMFs, and for the same species were always highest in the Greater North Sea and lowest in the Scheldt. For each of the Belgian coastal zone four species, a weak positive correlation between Hg content and fish length was found; however, the larger the size-range, the better the correlation. Taking fish length into account, a statistically significant difference in contamination level was observed for species sampled from the different geographical zones.

The toxicity of mercury (Hg) compounds is well known and seems to be primarily governed by its high affinity for SH-groups. Monomethylmercury (MMHg), one of the most toxic of the various Hg species, is a systematic and delayed toxin that acts on various organ systems and functions, the nervous system being by far the major target (Clarckson *et al.* 1984; Scheuhammer, 1991). It is widely assumed that the principal pathway for mercury and MMHg exposure in humans is food consumption, particularly of fish and fish-derived products, although there are some exceptions, such as exposure in areas with high Hg levels in multiple environmental compartments (*e.g.*, Gustin *et al.* 1994; Baeyens *et al.* 2003). Many studies have also been dedicated to the origin of MMHg in fish and its derived products. As most of the anthropogenic Hg enters the ecosystem in its inorganic form via point discharges (chlor-alkali and nonferrous industries) or diffuse sources (dentistries; Andersen and Niilonen 1995), MMHg must be formed *in situ*. It is now known that methylation is an important process in the Hg-cycle, mainly occurring in sediments and anoxic aquatic systems (*e.g.*, Craig and Moreton 1986; Compeau and Bartha 1987). Sulphate reducing bacteria are often involved in the transformation of inorganic into organomercury compounds, but the methylcobalamine coenzyme seems to play a major role too. Factors favoring the methylation process are higher temperature, lower pH, anoxic conditions, higher organic matter content and appropriate sulphate (200–500 μM) concentrations (Bloom *et al.* 1991; Gilmour and Henry 1991; Muhaya *et al.* 1997), while sulphides appear to limit the production of methylmercury in saline sediments (Craig and Moreton 1986; Compeau and Bartha 1987). Besides methylation, demethylation also occurs, thus the net result observed in a natural system will be the difference between both processes.

High MMHg concentrations have been observed in fish from remote, often acidified lakes in the northern United States, Canada, and Sweden (Lindqvist *et al.* 1991; Watras *et al.* 1995). Due to its volatility, Hg (mainly in the elemental form) is transported over very long distances in the atmosphere. Reactions with small particles (mainly soot) and reduction of its volatility in colder areas, like the northern US, Canada, and Scandinavia, let it reenter the aquatic system. In addition, acidified lakes present favorable methylation conditions

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(Watras *et al.* 1995), so that mercury will accumulate in its most dangerous form in the fish.

Besides methylation, other important characteristics of the Hg cycle to be taken into account are bioconcentration (Hg enrichment in suspended matter and plankton versus water column), bioaccumulation (increase of the Hg levels in fish with age), and biomagnification (increase of the Hg levels and the percent MMHg through the trophic food chain).

In this paper, we will try to explain the impact of Hg and MMHg levels, observed in various compartments of the North Sea and Scheldt estuary ecosystems (Coquery and Cossa 1995; Baeyens and Leermakers 1996; Leermakers *et al.* 2001), on fish contamination (bioconcentration and biomagnification), including the effect of the length of exposure (bioaccumulation). This information will help us to assess the potential danger to human health of consuming North Sea fish contaminated with Hg species.

Material and Methods

Sampling

The fish samples discussed in this paper can be subdivided into three sets (Figure 1): species caught in (1) the Greater North Sea, including the Channel; (2) the Belgian coastal zone; (3) the Scheldt estuary.

Greater North Sea. By trawling in different areas of the North Sea and the English Channel, 180 fishes and 29 shellfishes were caught, representing 23 different fish and four shellfish species. The species of which only a limited number of samples were obtained (eight fish and two shellfish species) were not included in the statistical analysis. According to Belgian and European Union regulations (93/351/CEE) on trace metals and persistent organic substances in food, fish species are subdivided into two categories—A and B. Species classified in category A are fishes of prey and include angler ($n = 20$), dogfish ($n = 20$), ray ($n = 20$), conger ($n = 1$), and seabass ($n = 1$). The allowable Hg-concentration upper limit for these species is 1 $mg.kg^{-1}$ wet weight (ww). All other fish species belong to category B and Hg concentration should not exceed the 0.5 $mg.kg^{-1}$ (ww) limit. The allowed Hg concentration for shellfish species is the same as for category B fish.

After determination of length and weight of each fish, muscle tissue was sampled, in 25 fish, liver tissue was also sampled.

Belgian Coastal Zone. During routine surveys of the *MS Belgica*, the Belgian oceanographic vessel, 68 samples, representing four fish species (flounder, plaice, dab, and whiting), were collected from the coastal zone. Liver tissue was sampled in all of these fishes.

Scheldt Estuary. 84 fishes were caught in the Scheldt estuary by local fisherman as well as by our colleagues from the University of Leuven (KUL) at the nuclear power plant at Doel. They represent 21 different species, of which nine are commercially available. Muscle tissue of all samples was analyzed, liver tissue only of flounder and conger. In addition, 24 mussels of the downstream estuary were sampled.

Analytical Procedures

Prior to analysis, the fish and shellfish subsamples were weighed, deep-frozen, lyophilized. They were weighed again to determine their water content and then manually homogenized.

Determination of Total Mercury in Biological Samples. All reagents used were of low Hg A.R. grade: digestion acids were HNO_3 65% (Merck, p.a, for Hg analysis) and H_2O_2 30% (Merck, p.a). For the digestion of the samples a microwave digestion system (CEM 2000, CEM Corporation) was used. Hg analysis was done by cold vapor atomic absorption spectrometry (CVAAS; Hg Analyzer II, Thermo Separation Products).

The sample digestion procedure should be suitable for the determination of Hg with CVAAS and of other trace metals with ICP-MS, in the same sample solution. Since it is best to avoid the use of H_2SO_4 in ICP-MS analysis, for reasons of interferences, as well as to avoid destruction of the Ni sampler and skimmer cones, a digestion procedure using only HNO_3 and H_2O_2 was tested. First, the acidified sample (6 mL of HNO_3 were added to about 0.2 g of sample) was placed in a microwave oven for 1 h and a ramped pressure program was applied (up to 150 psi). After adding 1 mL of H_2O_2, the solution was again placed in the microwave oven for 30 min at 80 psi. After digestion, 50 mL Milli-Q water was added. Pressurized digestions have the advantage of increasing the boiling point of an acid substantially. The pressure-versus-temperature curve of HNO_3 shows that at 100 psi a temperature of 185°C is reached. At 150 psi, 195°C is reached and pressures above 150 psi do not lead to further increases in temperature. However, care must be taken not to overpressurize the vessel, as gases released through the valves can result in loss of analyte. In the tested digestion procedure, no rupture of the membranes was detected in any of the samples. The digestion resulted in entirely clear solutions. The results obtained for reference samples were in good agreement with the certified values (Table 1) and recoveries for samples spiked with inorganic and methylmercury (Table 2) were good. The detection limit, based on three times the standard deviation of the digestion blank, was 4 $ng \cdot g^{-1}$.

Determination of Methylmercury in Biological Samples. For alkaline digestion, approximately 0.1 g lyophilized sample was weighed into a 30-mL Teflon (FEP) bottle and 2 mL of 25% KOH in methanol was added. The bottles were capped and placed in an oven at 75°C for 3 h. After digestion, the solutions were diluted with 30 mL methanol.

For ethylation-isothermal GC-AFS 30 mL Milli-Q water and 50 μL of acetate buffer were mixed in a reaction flask. 10–100 μL of the alkaline-digested sample were pipetted into the flask. The pipet tip was rinsed with the solution to transfer all aliquot to the reaction vessel. The vessel was gently swirled to mix, then 100 μL of ethylating agent (0.07 M tetraethylborate) was added. Calibration standards between 20 and 400 pg MMHg were used.

Hg species were transformed to their volatile ethylderivates (methylethylmercury and diethylmercury), purged out of solution and collected on Carbotrap or Tenax columns. GC separation was performed isothermally (Liang *et al.* 1994). In this procedure, the GC column was held at a constant temperature of 75°C and the Tenax column is heated to 350°C for 20 s. The resolution between the GC peaks of the various Hg compounds was sufficient. Table 3 shows the certified values and results of six replicate analyses obtained using the procedure described above. The detection limit for a 100-mg solid sample and a 100-μL sample volume is 4 $ng \cdot g^{-1}$, based on three times the standard deviation of the blank.

Results

Average total Hg and MMHg concentrations, as well as corresponding standard deviations, were calculated for all fish species in each of the three zones (Table 4 and Tables 1A–3A in Appendix). When comparing contamination levels between species, it is necessary to take the variability within the species (intraspecies variability) into account. Especially in the Greater North Sea, which covers about 750,000 km^2, significant subregional differences can not be excluded, rendering the use of averages mean-

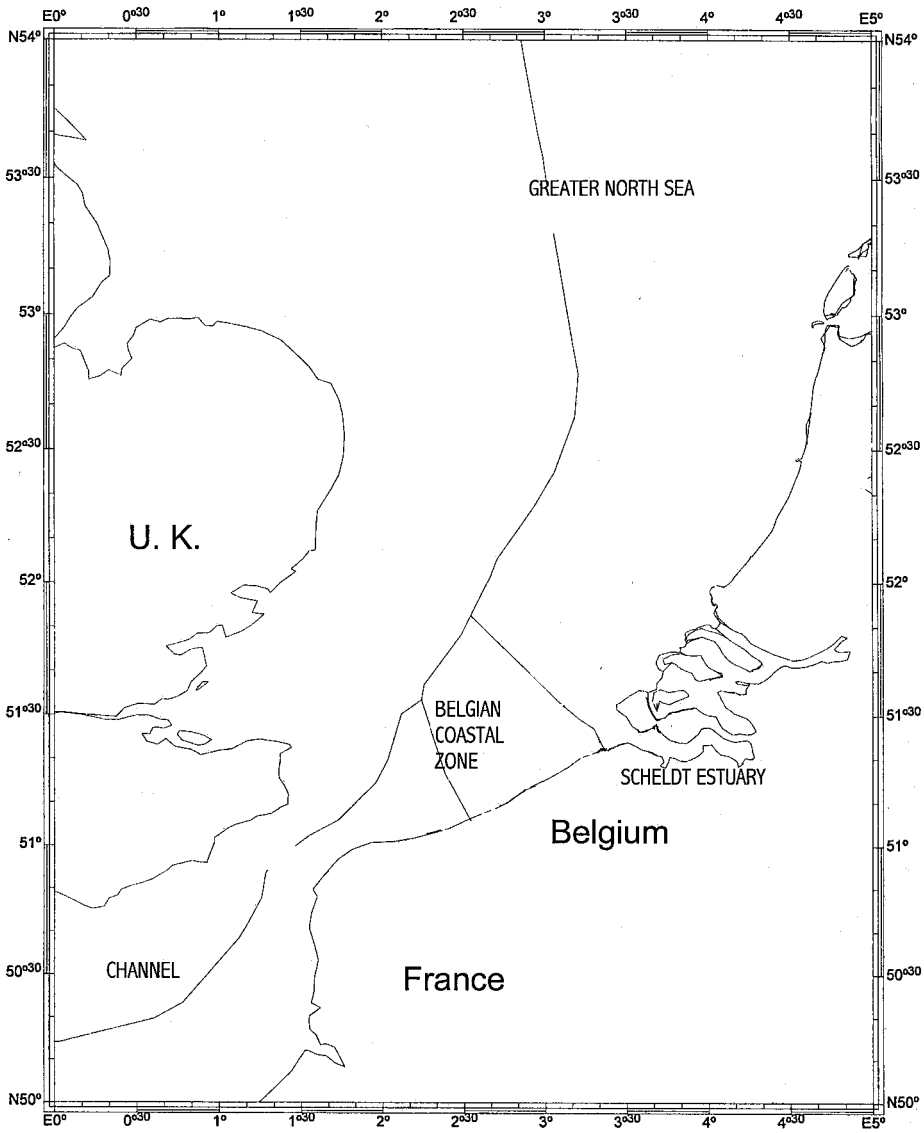

Fig. 1. Map of the Greater North Sea, the Belgian coastal zone, and the Scheldt estuary. The line in the middle of the sea separates the territorial waters of U.K. and continental Europe

Table 1. Observed Hg concentrations in Certified Reference Materials

Sample	CEM: Hg mg kg^{-1}	Certified: Hg mg kg^{-1}
DORM-2	4.61 ± 0.21	4.64 ± 0.26
DOLT-2	2.01 ± 0.20	1.81 ± 0.10
TORT-2	0.31 ± 0.02	0.27 ± 0.06

Table 2. Spike recoveries of Certified Reference Materials (spike of 0.5 μg Hg)

Sample	Hg^{2+}	MMHg
DORM-2	103 ± 2%	99 ± 1%
DOLT-2	105 ± 3%	102 ± 1%

ingless. Therefore, statistical procedures such as ANOVA were used to test for example whether the difference between species averages is large enough to be explained by random error.

Contamination Levels in Fish of the Greater North Sea and Channel

A one-way ANOVA analysis was carried out to compare the contamination levels in fish species from the Greater North Sea and Channel (GNS). This zone was chosen, because (1) most of the samples originated from that zone and (2) it is by far the largest one and thus more subject to subregional variability. Table 4 presents species averages and relative standard deviations (RSD) of total Hg and MMHg concentrations in muscle of the GNS fish. Only small differences between the results on total Hg and MMHg were observed, as MMHg constitutes about 95% (average value) of the total Hg content in fish, except for St. James'shells (MMHg is only 35%). Therefore, the statistical analysis was only carried out on MMHg levels and percent MMHg values.

An ANOVA analysis on all species, showed a statistically

Table 3. Observed MMHg concentrations in Certified Reference Materials

Certified Reference Material	MMHg mg kg^{-1} certified	MMHg mg kg^{-1} obtained ($n = 6$)
NRC DORM-2 Dog fish muscle tissue	4.47 ± 0.32	4.32 ± 0.40
NRC DOLT-2 Dog fish liver	0.693 ± 0.053	0.730 ± 0.060
NRC TORT-2 Lobster Hepatopancreas	0.152 ± 0.013	0.148 ± 0.015

significant difference between the mean MMHg concentrations ($F = 29.7$, $p[\leq \text{Fcrit}, 95\%] < 0.001$). This result confirms a factual analysis of the data (Table 4): when ranking them we observed that they are all quite different one from another. According to a pairwise multiple comparison procedure (Tukey test), only for a small subgroup of species including thornback ray, lemon sole, brill, plaice, and Atlantic cod, the sample means did not differ significantly ($F = 1.7$, $p[\leq \text{Fcrit}, 95\%] < 0.168$). The mean percentage of MMHg to Hg total also differed significantly among the species ($F = 73.2$, $p[\leq \text{Fcrit}, 95\%] < 0.001$), essentially because of the existence of two subgroups, one of them including lesser spotted dogfish, thornback ray, saithe, dab, plaice, megrim, and St. James' shells. For the remaining species (Table 4), differences in MMHg percentages were small enough to be explained by random sampling variability ($F = 1.4$, $p[\leq \text{Fcrit}, 95\%] < 0.159$).

In liver tissue of dogfish and ray (see Appendix, Table 1A), the variability in MMHg concentration and in MMHg fraction was much higher than in muscle tissue. In the liver of dogfish, the Hg concentrations were about 20 times higher than in the liver of ray, but for both species concentrations in liver were lower than in muscle. Additionally, in the liver about half of the Hg was in the form of MMHg.

Hg concentrations in dogfish, conger, and seabass exceeded 0.3 mg · kg^{-1} (ww), but these values were still far beneath the Belgian limit of 1 mg · kg^{-1} (ww). This was also the case for the Hg concentrations in sea fish of category B (Belgian limit of 0.5 mg · kg^{-1} ww).

Contamination Levels in Fish of the Belgian Coastal Zone and Scheldt Estuary

In the Belgian coastal zone, flounder, and whiting were the species with highest Hg content (see Appendix, Table 2A), exceeding 0.1 mg · kg^{-1} (ww), while plaice and dab, showing values below 0.07 mg · kg^{-1} (ww), were apparently less contaminated. The fraction of MMHg in muscle tissue of the four fish species from the Belgian coastal zone was substantially lower (82–87%) than in the same species of the Greater North Sea (91–98%).

In the liver of the four studied species, the total Hg and MMHg levels were fairly similar (0.1 and 0.035 mg · kg^{-1} ww). In addition, these latter values confirmed the observations for dogfish and ray in the Greater North Sea, indicating that the MMHg fraction in the liver (around 35%, except for whiting, 50%) was much lower than in muscle tissue.

The average concentrations in commercially available fish (see Appendix, Table 3A) from the Scheldt estuary were 0.080 mg · kg^{-1} (ww) for total Hg and 0.046 mg · kg^{-1} (ww) for MMHg. In noncommercially available fish species, these values were somewhat lower, respectively, 0.054 mg · kg^{-1} (ww) and 0.030 mg · kg^{-1} (ww). The MMHg fractions for commercial (average 58%) and noncommercial species (average 56%) were similar and the lowest of the three zones, while their variability (RSD) was the highest. Mussels showed a low MMHg fraction (average 24%).

The variability in the percentage of MMHg in liver was comparable to that in muscle tissue: 25–42%.

Comparison of Contamination Levels in Fish Between the Three Zones

A comparison of the contamination levels in fish between the three zones was useful since the pollution of the water column was very different. In particular, the Scheldt estuary was and still is a highly polluted and partially anoxic estuary (Baeyens 1998). The levels of MMHg and total Hg in the water column (dissolved as well as particulate) were much higher in the Scheldt than in the North Sea (Leermakers *et al.* 2001). However, age may influence the Hg content of the fish and it appeared that the sample sizes were not similar in each of the sampling zones. For example, the whiting species originating from the Greater North Sea had a larger size than those inhabiting the Belgian coastal zone (respectively 379 ± 19 mm versus 276 ± 42 mm). The same is observed for dab—294 ± 19 mm in the Greater North Sea versus 230 ± 43 mm in the Belgian coastal zone—but not for plaice.

ANOVA analysis was carried out on all data (species concentration, length, and geographical zone) available for flounder, dab, and whiting. Because there were no observations for all combinations of the three factor levels (species, length, and location), a one-way ANOVA was applied, each cell in the input table being treated as a different level of a single experimental factor. This approach is the most conservative because it requires no additional assumptions about the nature of the data or experimental design. Firstly, the Spearman rank order correlation indicated that except for flounder in the Belgian coast ($rs = 0.625$, $p = 0.001$), there were no significant relationships between length and MMHg concentration. According to the one-way ANOVA, the difference in mean MMHg concentrations between the sampling zone was greater than the one that could be expected by chance for the three species: flounder ($F = 10.5$, $p[\leq \text{Fcrit}, 95\%] = 0.003$), dab ($F = 3.6$, $p[\leq \text{Fcrit}, 95\%] < 0.043$), and whiting ($F = 89.1$, $p[= \text{Fcrit}, 95\%] < 0.001$). There is thus a statistically significant difference in contamination level for these three species between the geographical zones.

Discussion

Effect of Methylation

A long-term study of the biogeochemical behavior of Hg in the Scheldt estuary and the Belgian coastal zone (Baeyens and

Table 4. Average Hg and MMHg concentrations in muscle of Greater North Sea fish

Species	*n*	Hg ww (mg kg^{-1})	s.d.	MMHg ww (mg kg^{-1})	s.d.	MMHg (%)	s.d.
Angler	20	0.087	0.024	0.080	0.022	92.5	5.4
Lesser spotted dogfish	20	0.613	0.230	0.598	0.247	97.0	1.1
Thornback ray	19	0.039	0.021	0.037	0.019	97.8	6.4
Lemon sole	20	0.052	0.026	0.049	0.023	95.7	5.9
Pouting	5	0.172	0.052	0.160	0.053	92.4	4.5
Whiting	5	0.101	0.021	0.091	0.015	90.9	8.4
Atlantic cod	5	0.053	0.018	0.049	0.016	93.2	4.3
Brill	5	0.064	0.024	0.059	0.025	91.8	6.8
Ling	5	0.117	0.026	0.106	0.020	91.0	3.9
Saithe	5	0.091	0.058	0.088	0.056	97.4	3.5
Dab	13	0.101	0.050	0.098	0.051	97.2	6.2
Sand sole	9	0.327	0.309	0.308	0.290	94.4	6.0
Plaice	17	0.045	0.023	0.043	0.023	97.0	6.2
Common sole	16	0.088	0.067	0.086	0.071	96.2	5.8
Megrim	6	0.083	0.046	0.080	0.046	96.7	4.3
St. James's shell	24	0.022	0.009	0.007	0.002	35.4	17.0
Common whelk	3	0.101	0.064	0.094	0.063	89.8	7.3

Table 5. Typical total Hg and MMHg concentrations in each of the three studied areas

Hg-tot diss. (ng L^{-1})	MMHg-diss (pg L^{-1})	Hg-tot part. ($\mu g\ g^{-1}$)	MMHg-part. (ng g^{-1})
Greater North Sea			
0.3	15	0.044	1.2
Belgian coastal zone			
0.7	30	0.16	2.4
Scheldt estuary			
1.5	120	0.9	3.4

Table 6. BCF and BMF of inorganic mercury and methylmercury

BCF values	Hg-inor.	MMHg
Greater North Sea	5.18	4.90
Belgian coastal zone	5.37	4.90
Idem (high Chl-a levels)	5.47	5.07
Scheldt estuary	5.81	4.45

	Greater North Sea		Coastal zone		Scheldt estuary	
BMF values	Hg-inor.	MMHg	Hg-inor.	MMHg	Hg-inor.	MMHg
Dogfish	0.21	3.37				
Plaice	−0.63	2.24	−0.50	2.02	−1.21	1.45
Whiting	0.07	2.59	−0.42	2.26		
Dab	−0.63	2.59	−0.42	2.05		
Common sole	−0.63	2.49			−0.58	1.49
Flounder			−0.16	2.34	−0.84	1.90
Eel					−0.65	2.12
Mussel					−1.60	0.33

Leermakers 1998; Leermakers *et al.* 2001) allowed us to identify two areas of methylation, the anoxic upstream area of the Scheldt estuary and the coastal-estuarine mixing zone. Both zones were characterized by muddy, organic-rich sediments and—as a result of reducing conditions, sufficiently high sulphate, and high organic matter content—were favorable for methylation. MMHg concentrations up to 0.35 ng · L^{-1} in the dissolved phase and 10 ng · g^{-1} in the particulate phase could be observed in these zones. Average values of dissolved and particulate total Hg and MMHg are presented in Table 5 and allow estimates of bioconcentration (BCF) and biomagnification (BMF) factors (Table 6).

Bioconcentration and Biomagnification

Especially in estuaries, and to a lesser extent in coastal seas, bioconcentration factors (BCF) represent the distribution between the particulate and the dissolved phases. The particulate suspended matter pool (SPM) includes, however, a biogenic fraction of living organisms and detritus, and a nonbiogenic fraction. Especially in estuaries and coastal areas, this latter fraction may become very important. We calculated the log-BCF values for inorganic Hg as (Hg total − MMHg) and MMHg, with BCF = [particulate Hg (ng · g^{-1}, dry weight)]÷[dissolved Hg (ng · mL^{-1})] in the Scheldt estuary, Belgian coastal zone, and the Greater North Sea. For inorganic Hg, a slight increase in log-BCF from the open sea (5.18) to the Scheldt estuary (5.81) was observed. The suspended matter of the estuary was relative to the dissolved phase, more enriched in inorganic Hg than that of the open sea. This was not the case for MMHg, since in the two marine zones SPM was slightly more enriched in MMHg, relative to the dissolved phase, than that in the Scheldt estuary. In the Belgian coastal zone, we observed high chlorophyll-a and POC concentrations in the summer. BCF values corresponding to this chlorophyll-a enriched SPM better approach phytoplankton BCFs. They turned out to be slightly higher than in case average SPM is considered, for inorganic Hg as well as MMHg (see Table 6). Our BCF values are in the same range as those observed by other authors, for example Back and Watras (1995). They reported BCF values between water column and SPM ranging from 4.24 to 6.07 for Hg-total (inorganic Hg BCFs in the water column are almost equal to those of total Hg), and from 4.58 to 6.78 for

MMHg, in 12 northern Wisconsin (USA) lakes. However, they also observed an inverse relationship between both Hg BCF and MMHg BCF for SPM and the lake's DOC. DOC concentrations in our three zones increased from the Greater North Sea (ca 60 μM), over the Belgian coastal area (ca 150 μM), and to the Scheldt estuary (ca 400 μM), but no inverse relationship with the BCF values was seen. The range of DOC concentrations in the study of Back and Watras (1995) was, however, five times larger (up to 2 mM) than in our study, and the effect of DOC was only seen at concentrations above 1 mM. These results suggest that the bioavailability of Hg in the North Sea was not influenced by the presence of organic ligands.

The high concentrations of Hg in fish led to an increasing interest in the biomagnification features of this element in the lower food web. We do not have recent results for Hg concentrations in North Sea zooplankton, but Back and Watras (1995) found that bioconcentration of MMHg was higher than for total Hg in the 12 northern Wisconsin lakes. This supports the hypothesis that MMHg progressively accumulates in higher trophic levels of food webs, while nonmethyl Hg declines. However, in contrast to these findings, they also found lower BCF and BMF in an invertebrate predator, than in the presumed prey, concluding that the transport of Hg species in the lower levels of aquatic foodwebs may be very complex. Biomagnification for MMHg was always highest in the Greater North Sea area and lowest in the Scheldt estuary for the same species (Table 6). Dogfish, a fish of prey, had by far the highest BMF values of all species studied, while mussels, which are filter feeders, showed the lowest BMFs. In addition, MMHg BMFs were always positive and much higher (up to three orders of magnitude) than inorganic Hg BMFs, which were mostly negative. The reason for this preferential accumulation of MMHg in fish has its source at the phytoplankton level. Uptake of Hg by phytoplankton takes place via passive diffusion: neutral complexes such as MMHgCl or $HgCl_2$ are favored, compared to ionic species such as $HgCl_3^-$ or $MMHgCl_2^-$ (Mason *et al.* 1995). The latter authors demonstrated that there is, however, a differentiation between inorganic Hg and MMHg assimilation. MMHg enters the cytoplasm, while inorganic Hg is mainly bound to the cell membrane. Planktonic predators such as zoopankton and planktivorous fish, digest the dissolved cytoplasmic but defecate the membrane material, thus they poorly assimilate inorganic Hg. When uptake exceeds excretion, the net result is accumulation. Further discrimination up the food chain can result from the functioning of the metabolic system, in particular the liver of the organisms. Moreover, the functioning of this metabolic system is influenced by various parameters such as the age of the organism, but also by the intensity and the period of Hg exposure.

In the North Sea, we observed MMHg percentages ranging from 2.9 to 4.8% in the dissolved phase and from 0.6 to 2.5% in the particulate phase, while in fish, for both categories A and B, about 95 ± 2.5% of the Hg content was MMHg. However, the MMHg in liver tissue was significantly lower (46 ± 18%). In addition, a peculiar relationship between the MMHg fraction, the contamination level (total Hg content of the fish) and the ratio of total liver Hg to total muscle Hg was observed. The lower the contamination level of the fish, the lower the MMHg fraction in muscle and liver, and the higher the ratio of total liver Hg to total muscle liver. A plausible mechanism is that, at low exposure, the MMHg transport from muscle to liver is efficient. An efficient transport means a decrease of MMHg in muscle and thus also a decrease of total Hg (on average about 95% of total muscle Hg is MMHg). When total muscle Hg decreases, the ratio of total liver Hg to total muscle Hg increases.

Bioaccumulation

Bioaccumulation was assessed for flounder, dab, whiting, and plaice. All species showed a positive correlation between Hg content and length, but while this correlation was relatively strong for flounder ($r = 0.71$), it was very weak for dab ($r = 0.20$). The size range for flounder (about 280 mm) was, however, much larger than for dab (about 140 mm). Bioaccumulation was also observed for cod ten years ago (Lansens *et al.* 1991), but again the size range was sufficiently large (about 250 mm). Figure 2 represents the results for flounder, dab, plaice, and cod. Thus, care must be taken when drawing conclusions about bioaccumulation: the size range of fish samples should be sufficiently large (*e.g.*, Cossa *et al.* 1992) and as large as possible, which was often not the case in our study.

In the liver of the four species, different trends between Hg content and fish length were found, even decreasing ones (for plaice and dab, negative correlation coefficients of 0.75 and 0.69, respectively, were obtained).

Despite the fact that Hg levels in the Scheldt estuary were much higher than in the North Sea, it appeared that Hg concentrations in fish were slightly lower in the Scheldt. A plausible explanation is the much smaller size (younger fish) of the Scheldt species. A more striking observation is the very low MMHg fraction in Scheldt fish (average 57%), compared to that in North Sea fish (average 95%). Here, no explanation can be forwarded, but it will be worthwhile to investigate in future following processes: (1) the MMHg breakdown efficiency in younger and older species; (2) the feeding habits of these Scheldt species. They are not only feeding on phytoplankton, but also on the large amounts of detritus from untreated domestic sewage directly discharged into the water column. As mentioned above, phytoplankton plays a vital role in the discrimination of inorganic Hg and MMHg towards higher trophic levels, while that of detritus is less clear.

Comparison with Similar Marine Systems

Regular monitoring of Hg concentrations in North Sea and Northern Atlantic fish has taken place for many years and is stimulated and supported by ICES and OSPAR. A comparison of the concentrations observed in muscle tissue of plaice, cod, whiting, dab, and sole is shown in Table 7. Highest average concentrations were found in whiting and dab, but the most contaminated areas are Liverpool Bay and Morecambe Bay, both in the United Kingdom. The geographical area that is probably most comparable to the Greater North Sea is the St. Lawrence Gulf. Hg concentrations in plaice and cod from both areas were very similar. The cleanest areas were apparently the Firth of Clyde, U.K. (Mathieson and

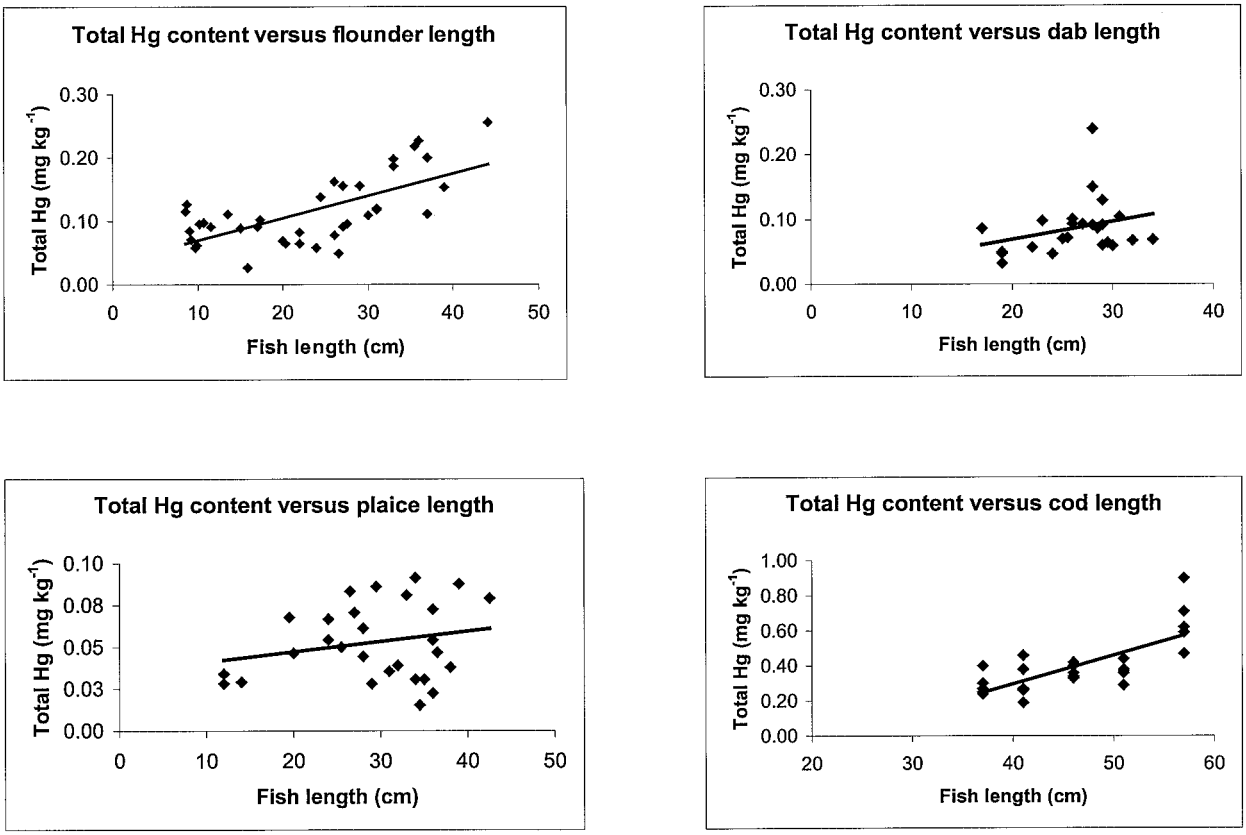

Fig. 2. Total Hg content versus fish length

McLusky 1995), as well as the Iceland zone (Gudjon Audunsson, Icelandic Fisheries Laboratories, personal communication). There, Hg concentrations in all reported fish species fit the present day background concentration range as proposed by OSPAR/ICES (1996), while for the Greater North Sea, this is only the case for plaice. Present day Hg background concentrations in the regions, related to the OSPARCOM convention, equal 0.01–0.05 mg · kg^{-1} (ww) in round fish, 0.03–0.07 mg · kg^{-1} (ww) in flat fish and 0.005–0.01 mg · kg^{-1} (ww) in mussels. These values represent concentrations found in areas remote from known point sources (OSPAR/ICES 1996).

Most of the observed Hg concentrations in fish fall within the "lower" and "medium" OSPAR categories (<0.1 and 0.1–0.3 mg · kg^{-1} ww, respectively). In mussels (*Mytilus edulis*), Hg concentrations in the North Sea and Northern Atlantic ranged from 0.002 to 0.17 mg · kg^{-1} (ww), while we found an average concentration of 0.03 ± 0.01 mg · kg^{-1} (ww) in the Scheldt estuary.

Metabolic and Toxic Aspects

The interpretation of environmental data in terms of Hg hazard requires an understanding of the pathways of mercury exposure to the different trophic levels of the ecosystem and to humans. Bioconcentration Factors (BCF values) between SPM and the water column were of the order of 4–6 logs for both inorganic Hg and MMHg. While the bioconcentration of inorganic Hg from SPM towards fish was mostly negative, MMHg further accumulated strongly (two to more than three orders of magnitude in marine fish). At higher trophic levels and—more specifically—at the level of cetaceans, Hg is not only accumulated as a function of age, but a change in speciation occurs. Hg, present as MMHg in the food of cetaceans, is readily assimilated in its organic form, but is slowly relocalized and demethylated, resulting in the formation of Se-Hg compounds (thiemanite). Particularly in liver tissue, thiemanite accumulates to extremely high, but nontoxic levels (Capelli *et al.* 1989; Hansen *et al.* 1990; Joiris *et al.* 1991; Paludan-Muller *et al.* 1993).

It is widely assumed that the principal pathway for mercury exposure in humans is through food consumption, in particular of fish. Exceptions are exposure to Hg in contaminated sites such as the Carson River Drainage Basin in Nevada, USA (Gustin *et al.* 1994), and the Katun River Drainage Basin in Altai, Siberia (Baeyens *et al.* 2003). However, the characterization of risk to human populations focuses more and more on exposure to MMHg over lifetime instead of acute exposure. To this aim, a reference dose (RfD) is defined as an estimate of daily exposure that is likely to be without an appreciable risk of deleterious effects during a lifetime (Risher and DeWoskin 1999). The RfD for MMHg has been determined by the EPA to be 0.1 μg per kg of body weight per day (Moore 2000). A person of 75 kg may thus ingest 50 μg MMHg per week. Assuming that this person consumes a 200-g portion of fish twice a week, that fish is allowed to contain 0.125 mg MMHg · kg^{-1} (ww). In our study, average

Table 7. Concentrations of Hg in fish of the North Sea and Northern Atlantic

Location	Period	Hg_T (mg kg^{-1} ww)	Reference
Plaice			
Liverpool Bay, UK	1994	av. 0.13	SIME 1996
Morecambe Bay, UK	1994	av. 0.09	SIME 1996
Southern Bight, UK	1994	av. 0.05	SIME 1996
St. Lawrence Gulf, Canada	1992–1995	0.049 ± 0.020	Gobeil *et al.* 1997
Greater North Sea	1997–1999	0.045 ± 0.023	This study
North Atlantic, French coast	1988	0.028–0.15	Cossa *et al.* 1990
English Channel	1988	0.026–0.15	Cossa *et al.* 1990
Irish coast	1994	0.05–0.09	Nixon *et al.* 1995
Firth of Clyde, UK	1992	0.011–0.019	Mathieson and McLurly 1995
Present day background concentration		0.03–0.07	OSPAR/ICES 1996
Cod			
Liverpool Bay, UK	1994	av. 0.10	SIME 1996
Belgian coast	1993	av. 0.09	Vyncke *et al.* 1996
Southern Bight, UK	1994	av. 0.07	SIME 1996
St. Lawrence Gulf, Canada	1992-1995	0.060 ± 0.023	Gobeil *et al.* 1997
Greater North Sea	1997-1999	0.053 ± 0.018	This study
Baltic Sea	1989-1996	0.002–0.365	Helcom 1996
Northern North Atlantic	1994	0.01–0.21	Stange *et al.* 1996
Irish coast	1994	0.01–0.07	Nixon *et al.* 1995
Iceland	1996	0.01–0.04	Audunsson, personal communication
Present day background concentration		0.01–0.05	OSPAR/ICES 1996
Whiting			
Liverpool Bay, UK	1994	0.27 (n = 25)	SIME 1996
Morecambe Bay, UK	1994	0.27 (n = 25)	SIME 1996
Greater North Sea	1997-1999	0.101 ± 0.021	This study
NE English coast, River Tyre	1992	0.052–0.432	Dixon and Jones 1994
Irish coast	1994	0.04–0.19	Nixon *et al.* 1995
Present day background concentration		0.01–0.05	OSPAR/ICES 1996
Dab			
Liverpool Bay, UK	1994	av. 0.20	SIME 1996
Morecambe Bay, UK	1994	av. 0.15	SIME 1996
Greater North Sea	1997-1999	0.101 ± 0.050	This study
NE English coast, River Tyre	1992	0.042–0.255	Dixon and Jones 1992
Iceland	1996	0.019–0.053	Audunsson, personal communication
Firth of Clyde, UK	1992	0.017–0.046	Mathieson and McLurly 1995
Northern North Atlantic	1994	0.01–0.02	Stange *et al.* 1996
Present day background concentration		0.03–0.07	OSPAR/ICES 1996
Sole			
Morecambe Bay, UK	1994	0.17 (n = 50)	SIME 1996
Liverpool Bay, UK	1994	0.14 (n = 40)	SIME 1996
Greater North Sea	1997-1999	0.088 ± 0.067	This study
Southern Bight	1991	av. 0.08	De Clerck *et al.* 1995
North Atlantic (French coast)	1988	0.03–0.27	Cossa *et al.* 1990
English Channel	1988	0.018–0.24	Cossa *et al.* 1990
Irish Coast	1994	0.02–0.16	Nixon *et al.* 1995
Present day background concentration		0.03–0.07	OSPAR/ICES 1996

MMHg concentrations in dogfish, pouting, and sand sole from the Greater North Sea exceeded this limit, while those in flounder from the Belgian coastal zone were very close. When considering only Hg, our results show that twice a week consumption of Greater North Sea fish does not create a major health risk. However, synergistic effects resulting from the combination of higher MMHg levels with the presence of other contaminants—such as arsenic (De Gieter *et al.* 2002) and dioxin-like compounds—are at present insufficiently known to exclude all health risks. Further study by ecotoxicologists is needed on this subject.

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Appendix

Table A1. Total Hg and MMHg concentrations in fish liver—intraspecies variability

Species	Hg ww (mg kg^{-1})	MMHg ww (mg kg^{-1})	%MMHg (mg kg^{-1})
Gurnard	0.053	0.016	30
Conger	0.253	0.200	79
Seabass	0.142	0.054	38
Dogfish	0.554	0.287	52
Dogfish	0.890	0.205	23
Dogfish	0.195	0.108	55
Dogfish	0.457	0.162	35
Dogfish	0.295	0.237	80
Dogfish	0.266	0.081	31
Dogfish	0.118	0.049	41
Dogfish	0.222	0.150	67
Dogfish	0.205	0.119	58
Dogfish	0.135	0.047	35
Pomfret	0.175	0.049	28
Pomfret	0.127	0.037	29
Thornback ray	0.013	0.005	38
Thornback ray	0.012	0.005	39
Thornback ray	0.011	0.005	46
Thornback ray	0.007	0.003	40
Thornback ray	0.034	0.016	47
Thornback ray	0.015	0.015	100
Thornback ray	0.011	0.005	44
Thornback ray	0.022	0.008	37
Thornback ray	0.028	0.013	44
Thornback ray	0.018	0.008	43
Average	0.170	0.075	46.5
s.d.	0.208	0.085	18.4

Table A2. Hg and MMHg concentrations and intraspecies variability in Belgian coastal fish

Species	*n*	Hg ww (mg kg^{-1})	Variability (%)	MMHg ww (mg kg^{-1})	Variability (%)	%MMHg	Variability (%)
Muscle							
Flounder	24	0.134	43	0.111	56	81.8	25
Plaice	13	0.063	27	0.053	34	83.8	21
Dab	11	0.069	35	0.057	40	83.1	19
Whiting	19	0.105	29	0.093	37	87	21
Liver							
Flounder	24	0.106	47	0.038	66	36.7	47
Plaice	13	0.097	60	0.037	86	35.5	45
Dab	11	0.096	81	0.030	40	38.5	38
Whiting	19	0.083	66	0.034	35	50.8	41

Table A3. Hg and MMHg concentrations and intraspecies variability in Scheldt fish

Species	*n*	Hg ww (mg kg^{-1})	Variability (%)	MMHg ww (mg kg^{-1})	Variability (%)	%MMHg	Variability (%)
Muscle							
Commercial species							
Common sole	16	0.070	44	0.022	50	31.9	43
Plaice	3	0.031		0.020		64.1	
Flounder	14	0.084	31	0.056	34	67.1	23
Eel	11	0.137	41	0.096	64	65.2	35
Seabass	7	0.091		0.054		57.8	
Herring	5	0.115		0.050		45.4	
Sprat	4	0.058		0.026		45.6	
Shrimp	1	0.043					
Zander	1	0.093					
Average		0.080		0.046		57.5	
Noncommercial species							
Bullhead	4	0.074		0.065		87.8	
Prussian carp	1	0.029		0.017			
Gudgeon	1	0.059		0.024			
Common goby	1	0.031		0.020			
Stickleback	1	0.050		0.028			
Bearded brotula	1	0.027		0.013			
Sandeel	1	0.029		0.020			
European perch	1	0.063		0.010			
Lesser pipefish	1	0.033		0.009			
Greater pipefish	1	0.102		0.045			
Roach	5	0.071		0.037		52.1	
European smelt	4	0.081		0.068		83.9	
Average		0.054		0.030		55.6	
Liver							
Flounder	6	0.051	47	0.033	39	67.8	25
Eel	7	0.171	70	0.092	48	61.6	42