Arch. Environ. Contam. Toxicol. 43, 406–417 (2002)
DOI: 10.1007/s00244-002-1193-4

ARCHIVES OF
Environmental
Contamination
and Toxicology

Total and Toxic Arsenic Levels in North Sea Fish

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Received: 21 September 2001/Accepted: 23 April 2002

Abstract. Levels of arsenic contamination in muscle and liver tissue of 25 sea fish and 4 shellfish species from the North Sea were determined. Analyses were done by both ICP-MS and HG-AFS to distinguish between nontoxic and toxic fractions of As. Highest total As concentrations were found in lemon sole, dogfish, ray, and witch. Average total As concentrations in these fish species were higher than 20 mg kg^{-1} WW. The same species as well as the other flatfishes contained the highest amounts of toxic As ($>$ 0.1 mg kg^{-1} WW). Toxic fractions (AsTox/AsT%) above 2% were found in the following six species: seabass, ling, john dory, pouting, dab, and brill. No preferential concentration in the liver compared to the muscle was observed. In a worst-case scenario (when fish has been dried or smoked and the toxic As level is high; for example 0.5 mg kg^{-1} WW), the As content of North Sea marine food may reach harmful levels. A normalization reflecting the toxic potential of sea fish was made. Shark and ray and most flatfish species have positive (high) normalized AsTox values. By ANOVA testing we compared the individual AsT concentrations of samples of the same species (intraspecies variability). Significant differences for some fish species were observed; significantly higher AsT concentrations were found in dogfish from the French coast versus the western North Sea and in common sole from the Bay of the Seine, in the north of France, versus the western North Sea.

Despite the fact that several international agreements about the reduction of pollutant loads to the North Sea have been made, relatively little attention has been paid to the contamination of the marine ecosystem, and of fish in particular, by arsenic. This is for instance demonstated by the lack of data about arsenic in the North Sea Quality Status Reports (QSRs), documents describing the state of the art of most of the dangerous contaminants in the North Sea. In the 1993 QSR only the following data about arsenic were available: dissolved arsenic concentrations in the Strait of Dover and in the Southern Bight, total arsenic concentrations in sediments of the northern and eastern areas of the North Sea, and total arsenic concentrations in mussels from the Belgian coast. No other data on arsenic levels in biota were mentioned, and this is also the case in the 2000 edition. Several reasons can be forwarded to explain why a toxic element as arsenic is so scarcely dealt with in these reports: (1) little information on background and ecotoxicological concentrations in biota exist; (2) the concentrations in fish vary considerably and are sometimes high (Michel 1993); (3) arsenic exists in a number of physiological forms in nature, all with very different toxicity characteristics. One of the consequences is that there are no unambiguous arsenic limits for edible fish.

Some authors suggest that arsenic, despite its reputation as a poison, at low concentrations might be an essential element for organisms (Uthus 1994). In the case of phytoplankton, arsenic properties, which are close to those of phosphorus, bring about a negative interference when the environment is in a state of disequilibrium, either through an excess of arsenate or through a deficiency in phosphates. Sanders (1979) has shown that a dose of arsenic just three times above that normally found in the ocean was enough to inhibit primary productivity when the concentration of phosphorus was below 0.3 nM. This influence seems particularly to affect certain diatoms and may bring about changes in the distribution of the various species. Phytoplankton detoxify themselves by reducing and methylating arsenate and excreting dimethylarsinic acid and monomethylarsonic acid, but it must be remembered that dimethylarsinic acid itself has toxic properties to such a degree that it is used in the defoliation of cotton. In higher organisms, the interference of arsenic in enzymatic mechanisms can occur, but this is difficult to quantify. Negative effects at the 300 $\mu g\ L^{-1}$ level have been noted in juvenile salmon (Nichols *et al.* 1984). Shukla and Pandey (1984) observed an alteration in the spermatogenesis in the perch *Colisa fasciatus*. Physiological modification and a large amount of necrotic tissue in the fish *Lepomis cyanellus* from a contaminated lake correlated with their concentrations of arsenic (Sørensen *et al.* 1985). Mortality occurred in *Palaemonetes argentinus* at a level above 100 $\mu g\ L^{-1}$ (Rodrigues-Capitulo 1984). Additionally, arsenic levels

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found in marine organisms are much higher than those found in terrestrial organisms (Doyle and Spaalding 1978), thus an important question relates to the ability of marine organisms to concentrate arsenic. In fact, the terms bioaccumulation or bioconcentration, strictly speaking, are imprecise when one takes into consideration the numerous chemical, mineral, and organic forms in which arsenic may be present in marine organisms. In sea water, more than 90% of the arsenic is present in mineral form, but in many organisms up to 95% takes on a much less toxic organic form. Its participation in metabolism makes it very difficult to compare with other contaminants, such as Hg, Cd, or PCBs, whose half-life in organisms is much longer. Pentreath (1977) has shown that in a plaice fed inorganic arsenic-rich food, only 5% of the contaminant remained after 10 days. Wide variations in concentrations may be expected in accordance with physiological and seasonal variations or changes in the food. Also, arsenic in the marine food chain exists in many forms. Arsenosugars and inorganic arsenic seem to be the major arsenicals in algae (Maeda *et al.* 1990; Raber *et al.* 2000), but the major fraction of the total arsenic burden in many marine animals, including fish (more than 70% according to Luten *et al.* [1982] and Ballin *et al.* [1994]), crustaceans, molluscs, and echinoderms is present as arsenobetaine. This compound is quite stable and is considered to be nontoxic. A genotoxicity study of arsenobetaine gave consistently negative results (Jongen *et al.* 1985). In addition to arsenobetaine, minor amounts of arsenocholine, trimethylarsine oxide, and tetramethylarsonium ion are found in many marine animals. These forms are also believed to be nontoxic (Sakurai *et al.* 1996). Inorganic arsenic on the other hand is highly toxic and carcinogenic (Saha *et al.* 1999). For this reason the Joint FAO/WHO Committee (1983) recommends an acceptable daily intake of 2 μg inorganic arsenic per kg body weight. A decreasing toxicity order can be set as: arsenite $>$ arsenate $>$ dimethylarsinic acid $>$ monomethylarsonic acid $>$ arsenobetaine $\approx$ arsenocholine $\approx$ trimethylarsine oxide (Hamasaki *et al.* 1995).

Until now, most studies related to arsenic speciation in marine biota focus on the analytical performances of the method, rather than on the contamination levels of the arsenic species in fish. We present a picture of the arsenic contamination levels in North Sea fish and shellfish and distinguish between the toxic and nontoxic fraction.

Materials and Methods

Sampling

In 1997 and 1998 about 300 samples of 25 different sea fish and 4 shellfish and crustacean species were randomly picked out of the catch of the day at the fish markets of Zeebrugge and Nieuwpoort. The fishes had been caught by trawling in different areas of the North Sea and the English Channel. Information about this area was supplied by the fishermen of the according boats. Table 1 lists all species investigated, along with their respective sampling area. Length and weight of the fishes was determined before subsampling of 10–20 g of muscle or liver tissue of each fish. Unfortunately, because these fish were caught for commercial purposes, the variation in length was minor. Prior to analysis, the subsamples were weighed, deep frozen, lyophilized, weighed again to determine the water content, and manually homogenized. Each collected fish and shellfish was considered as an individual sample.

Analytical Procedures

Total Arsenic in Fish: The samples were dissolved and oxidized by a mixture of HNO_3 and H_2O_2 in a microwave oven (CEM Microdigest 2000), under controlled pressure in a two-step digestion procedure described in Table 2. Lyophilized, homogenized sample (0.1–0.2 g) was weighed in a Teflon digestion bomb. Six milliliters subboiled HNO_3 (65%, Merck) was added to the sample, and the bombs were firmly closed and put in the microwave oven to digest. After cooling down, 1 ml H_2O_2 (27% p.a., Merck) was added to the solution, and the Teflon bombs were placed back into the microwave for a second digestion. The mineralized samples were diluted to 50 ml with Milli-Q water and stored in decontaminated HDPE bottles.

The digests were analyzed for total As by inductively coupled plasma-mass spectrometry (ICP-MS) (VG PlasmaQuadII). The temperature in the plasma torch amounts to 6,000°C and is high enough for atomization and ionization of the As atoms. The method is in agreement with the reference method 90/515/CEE. Certified reference materials DORM-2 (dogfish muscle), DOLT-2 (dogfish liver), and TORT-2 (lobster hepatopancreas) of the National Research Council of Canada and BCR627 (tuna fish tissue) from EEC, as well as blanks were included in every digestion batch and submitted to the same digestion and analytical procedure as the samples. To compensate for fluctuations of the instrumental stability of the ICP-MS, In, Re, Ru, and Bi were added as internal standards.

For TORT-2 certified material, an average recovery of 108% (range 103–117%) was found. For DORM-2 this recovery was 114% (range 113–115%), and for DOLT-2 102% (range 99–106%). BCR627 has only recently been certified and included in our research. Preliminary results showed a yield around 110%.

Toxic Arsenic in Fish: Our goal was to find an analytical procedure that allows us to distinguish between (1) the nontoxic fraction, which consists mainly of arsenobetaine (AB) and to lesser extent of arsenocholine (AC) and tetramethylarsonium ion (TeMA); and (2) the toxic species, *i.e.*, inorganic arsenic—arsenite (As(III)) and arsenate (As(V))—on the one hand, and organic arsenic compounds— monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)—on the other hand. For the analyses of the toxic As fraction, the same mineralized samples as for the total As determinations were used. During the digestion all labile As compounds present in fish tissue were oxidized. Stable compounds, such as AB and AC, only liberate As under very stringent conditions (high temperatures and/or very strong oxidants) and remain unchanged during the mineralization procedure used here. Additionally, AB, AC, and TeMA do not form volatile hydrides. Therefore these compounds are not detected when using hydride generation, contrarily to As(III), As(V), MMA, and DMA.

The mineralized samples were measured using a hydride generator (HG) coupled to an atomic fluorescence spectrometer (AFS) (PS Analytical Excalibur). The more labile As compounds were reduced to As (III) prior to analyses, by a mixture of HCL (30% v/v), KI (1% w/v), and ascorbic acid (0.2% w/v). The solution was pumped into a gas-liquid separator and reacted with $NaBH_4$ (1.3% w/v solution in 0.1 M NaOH) to form arsenic hydrides. These arsines were then purged from the solution using an argon carrier stream and detected by atomic fluorescence. The detection limit of this method, calculated as three times the standard deviation of a blank injected 10 times, divided by the sensitivity, was 0.1 μg L^{-1}, or converted to tissue material, 0.005 mg/kg WW.

The method was tested on the same certified materials as mentioned above. The toxic As fraction equaled 6.9% for TORT-2 (range 6.5–

Table 1. Overview of the investigated species

Order	Family	FAO English	Scientific	Month, Year, Area (# Species)	Size Range (cm)
Carchariniformes	Scyliorhinidae	Lesser spotted dogfish	*Scyliorhinus canicula*	March 1997—French Coast (10)	59.5–68.5
				October 1997—Bristol Channel (10)	61.0–68.0
				April 1998—Bay of the Seine (5)	55.0–64.5
Rajiformes	Rajidae	Thornback ray	*Raja clavata*	March 1997—English Channel (10)	54.0–71.3
				October 1997—Bristol Channel (10)	46.0–61.0
Anguilliformes	Congridae	European conger	*Conger conger*	March 1997—French Coast (1)	124.0
Gadiformes	Gadidae	Atlantic cod	*Gadus morhua*	March 1997—English Channel (5)	47.0–51.5
		Pollack	*Pollachius pollachius*	March 1997—French Coast (1)	57.0
		Pouting	*Trisopterus luscus*	March 1997—English Channel (5)	35.0–44.0
		Saithe	*Pollachius virens*	March 1997—English Channel (5)	46.4–63.0
		Whiting	*Merlangius merlangus*	March 1997—English Channel (5)	35.4–40.5
	Lotidae	Ling	*Molva molva*	March 1997—English Channel (5)	53.0–73.4
	Merlucciidae	European hake	*Merluccius merluccius*	March 1997—French Coast (1)	64.0
Lophiiformes	Lophiidae	Angler	*Lophius piscatorius*	March 1997—English Channel (10)	—
				October 1997—Northern North Sea (10)	—
Perciformes	Bramidae	Atlantic pomfret	*Brama brama*	March 1997—French Coast (2)	38.0–42.2
	Moronidae	European seabass	*Dicentrarchus labrax*	March 1997—French Coast (1)	48.0
	Mullidae	Red mullet	*Mullus surmuletus*	March 1997—French Coast (1)	50.0
Pleuronectiformes	Pleuronectidae	Dab	*Limanda limanda*	March 1997—French Coast (3)	27.0–34.0
				October 1997—Southern North Sea (5)	28.0–30.7
				November 1997—Boulogne–Lands End (5)	28.0–32.0
				March 1998—Southern North Sea (5)	28.5–32.5
				April 1998—Bay of the Seine (10)	23.5–33.5
		European plaice	*Pleuronectes platessa*	October 1997—Northern North Sea (10)	34.5–38.0
				October 1997—Boulogne–Lands End (5)	26.5–32.0
				March 1997—Unknown (2)	34.0–36.0
				March 1998—Southern North Sea (5)	32.0–36.0
				April 1998—Bay of the Seine (15)	30.0–42.0
		Lemon sole	*Microstomus kitt*	March 1997—English Channel (5)	29.0–35.0
				October 1997—Southern North Sea (5)	31.5–38.5
				November 1997—Boulogne–Lands End (5)	30.0–32.0
				November 1997—Northern North Sea (5)	29.0–33.0
				March 1998—Southern North Sea (5)	32.0–35.0
	Soleidae	Witch	*Glyptocephalus cynoglossus*	March 1998—Unknown (5)	34.5–42.0
		Common sole	*Solea solea*	March 1997—French Coast (1)	44.0
				October 1997—Bristol Channel (5)	24.9–29.6
				November 1997—Northern North Sea (5)	28.5–30.0
				November 1997—Boulogne–Lands End (5)	28.5–30.0
				April 1998—Bay of the Seine (30)	25.0–41.0
				March 1998—Unknown (5)	31.0–35.0
		Sand sole	*Pegusa lascaris*	March 1997—French Coast (4)	25.0–28.0
				October 1997—Boulogne–Lands End (5)	28.5–31.5
				March 1998—Unknown (5)	25.0–30.0
	Scophtalmidae	Brill	*Scophthalmus rhombus*	March 1997—English Channel (5)	39.0–46.0

Table 1. Continued

Order	Family	FAO English	Scientific	Month, Year, Area (# Species)	Size Range (cm)
				March 1998—Unknown (1)	47.0
		Megrim	*Lepidorhombus whiffiagonis*	March 1997—English Channel (1)	48.0
				October 1997—Boulogne–Land End (5)	34.0–41.0
				March 1998—Unknown (5)	37.0–41.0
		Turbot	*Psetta maxima*	March 1998—Unknown (1)	40.0
Scorpaeniformes	Triglidae	Grey gurnard	*Eutrigla gurnardus*	March 1997—French Coast (1)	67.0
Zeiformes	Zeidae	John dory	*Zeus faber*	March 1997—French Coast (1)	51.0
Molluscs		Great Scallop	*Pecten maximus*	October 1997—Boulogne–Lands End (5)	
				March 1997—English Channel (8)	
				March 1997—French Coast (8)	
				March 1998—Unknown (1)	
		Common whelk	*Buccinum undatum*	March 1997—Northern North Sea (1)	
				March 1998—Unknown (2)	
Crustaceans		Edible crab	*Cancer pagurus*	October 1997—Northern North Sea (1)	
				March 1998—Unknown (1)	
		Common shrimp	*Crangon crangon*	October 1997—Northern North Sea (1)	

Table 2. Digestion procedure

	Reagents	Time (min)	Power (%)	Max. Pressure (psi)
Step 1	6 ml HNO_3	0–15	100	40
		15–30	100	85
		30–45	100	135
		45–60	100	150
Step 2	1 ml H_2O_2	0–30	100	80

7.1%), 1.3% for DORM-2 (range 1.2–1.3%) and 3.3% for DOLT-2 (range 2.9–3.6%). Preliminary results suggested a value around 2.2% in BCR627. Spikes of AB to these reference material digests did not show any increase in measured concentrations. These findings correspond to a speciation study carried out by Goessler *et al.* (1998). The As species in the dogfish reference material DORM-2 were separated and identified by HPLC-ICP-MS. DMA was found to account for approximately 2% of the total As content, the MMA and inorganic As signals were below detection limit, *i.e.*, 0.03 mg kg^{-1}.

Accuracy Tests: As an additional check we carried out a speciation experiment on the certified materials, using strong anion-exchange HPLC-HG-AFS. Mobile phase used was a 20 mM KH_2PO_4/K_2HPO_4 solution adjusted to pH 6.0. Consequently, as the four compounds (As(III), As(V), DMA, and MMA) we considered the toxic As fraction, are neutral or in anionic form at this pH, a qualitative and quantitative separation and detection of these compounds can be obtained. Extraction of the certified materials was done by ultrasonication and centrifugation in a mixture of methanol and milli-Q water (1:1). The results of this experiment are summarized in Table 3. They were in a reasonable agreement with the toxic As fractions discussed herein. These findings confirmed that DMA is the only of the four toxic arsenicals present in a detectable amount in DORM-2. It accounted for 1.1% of the total As content in this reference material. This method was further evaluated by participation in a preliminary intercalibration round, organized by BCR and set up for the development of new certified biological material for As.

Several labs received a sample of homogenated oyster tissue from the Venice lagoon and were asked to make a minimum of five independent replicate determinations of several arsenicals (at least total As, AB, and DMA). Because our results were in good agreement with the average result of all labs (Table 4) and calculated *Z*-scores were satisfactory, we were allowed to participate in the second round, the actual certification. The candidate reference material was tissue from oysters collected in the Arcachon Bay. Results were presented on an international conference, organized by the INERIS and BCR (Morabito *et al.* 2001), and show again that toxic As makes up only a small part of the total As found in oysters. The toxic fraction accounted for 5.5% of the total, DMA being the most abundant (3.7%), followed by As(V) (1.4%) and As(III) (0.4%). MMA concentration was found to be below detection limit. However, AB did not account for all the remaining nontoxic As, but only for 44%. The residual fraction could not be identified, but it is suspected to contain As in the form of arsenosugars.

Results

Sea Fish

Figure 1 summarizes the total arsenic (AsT) concentrations in sea fish in 1997. Figure 2 does the same for the toxic arsenic fraction (AsTox). Regarding the AsT concentrations, the fish species studied in 1997 can be divided into three classes: (1) a class comprising fish with median AsT concentrations up to 5 mg kg^{-1} WW, including cod, brill, conger, seabass, gurnard, john dory, ling, pouting, saithe, and whiting; (2) a second class with median AsT concentrations between 5 and 20 mg kg^{-1} WW, including angler, pomfret, common sole, dab, hake, pla-

Table 3. Speciation of the toxic As fraction (mg As kg^{-1}) for reference materials

	HG-AFS		HPLC-HG-AFS				
Reference Material	Certified Total	Toxic Fraction	As(III)	DMA	MMA	As(V)	Sum
DORM-2	18.0	0.25	< DL	0.20	< DL	< DL	0.20
DOLT-2	16.6	0.54	< DL	0.84	0.035	< DL	0.88
TORT-2	21.6	1.6	< DL	0.83	0.056	0.32	1.2
BCR627	4.8	0.12	< DL	0.084	< DL	< DL	0.084

Detection limits are 0.0015 mg kg^{-1} for As(III) and MMA, 0.0020 mg kg^{-1} for DMA, and 0.0030 mg kg^{-1} for As(V).

Table 4. Speciation of As in oyster tissue from the Venice Lagoon (mg kg^{-1}, species weight)

Species	Mean Value	Number of Participating Labs	Our Value	*Z*-Score
Total As	10.0 ± 1.1	6	11.4 ± 0.3	1.2
AB	10.4 ± 3.1	6	10.1 ± 0.7	0.4
DMA	0.68 ± 0.15	7	0.51 ± 0.05	−0.9
MMA	—	—	< detection limit	

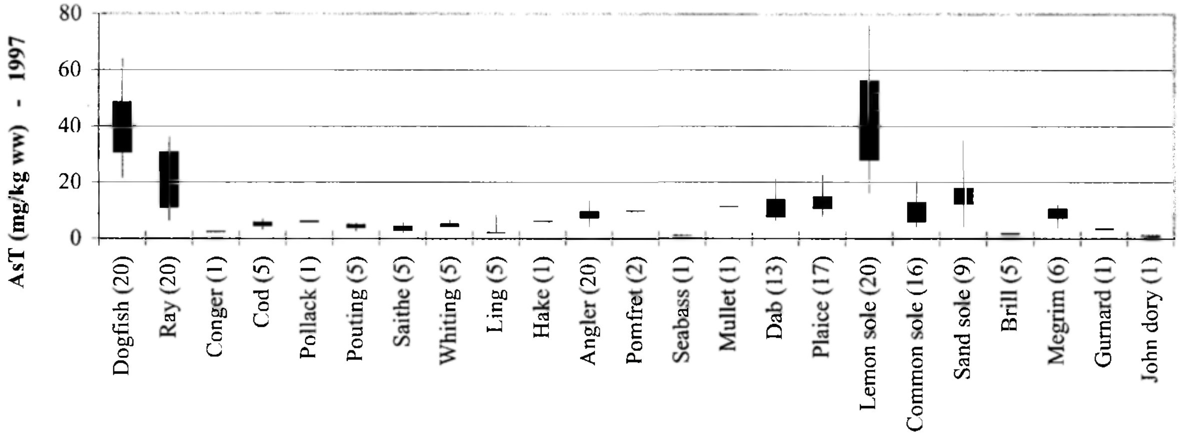

Fig. 1. AsT concentrations in sea fish 1997—minimum, maximum, 25th and 75th percentile (the numbers in brackets indicate the number of samples investigated)

ice, megrim, pollack, mullet, and sand sole; (3) a class that includes only lemon sole, dogfish, and ray, having median AsT concentrations higher than 20 mg kg^{-1} WW. The results of 1998 confirm this classification and add turbot to class 2 and witch to class 3.

For AsTox we notice that concentrations in all flatfishes (except brill) are high in 1997 as well as in 1998, compared to the concentrations in the other species. Also dogfish and ray have remarkably high AsTox concentrations. In dogfish the concentrations vary from 0.046 to 0.60 mg kg^{-1} WW, the latter value being by far the highest found in the 25 fish species over the 2 years of sampling. Besides the flatfishes, dogfish, and ray, three other species (mullet, pomfret, and turbot) have median AsTox concentrations higher than 0.1 mg kg^{-1} WW, but care should be taken because these figures are based on a very limited number of samples. Six species (seabass, ling, john dory, pouting, dab, and brill) contained more than 2% of the total As in a toxic form, but their absolute AsTox concentrations remained relatively low (median 0.09 mg kg^{-1} WW).

Shellfish and Crustaceans

It has been reported that total As concentrations in the tissues of molluscs and crustaceans are generally higher than those found in marine fish (Phillips 1990). AsT concentrations in this study indeed reach maxima up to 65.8 mg kg^{-1} WW for whelks and 40.6 mg kg^{-1} WW for crab, but AsT concentrations in scallops and shrimp remain relatively low. The lowest AsT concentrations are found in scallops, with medians of 2.0 and 3.2 mg kg^{-1} WW, respectively in 1997 and 1998 (Figure

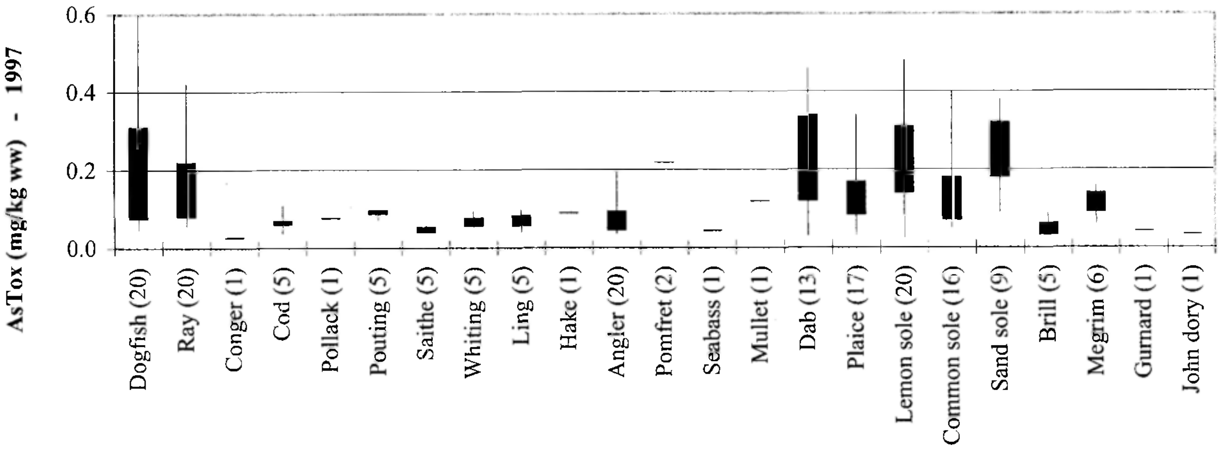

Fig. 2. AsTox concentrations in sea fish 1997—minimum, maximum, 25th and 75th percentile

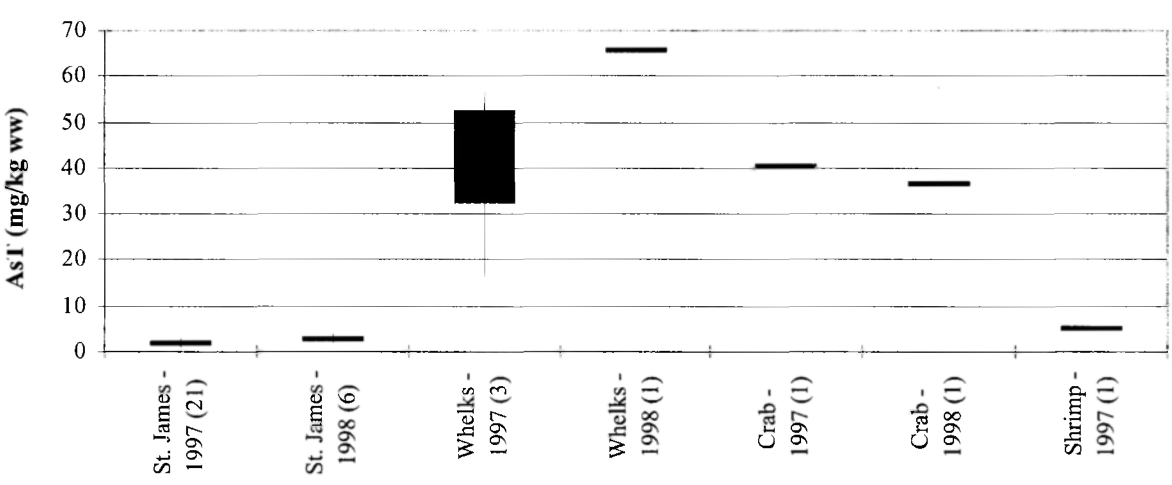

Fig. 3. AsT concentrations in shellfish and crustaceans—minimum, maximum, 25th and 75th percentile

3). AsTox concentrations on the other hand are rather comparable for the four investigated species (Figure 4). Consequently, scallops have the highest AsTox fraction. Median values are 7.1% in 1997 and 5.6% in 1998, but in individual samples up to 30% of the total As can be present as toxic As. These high percentages may be caused by two factors: (1) differences in diet, as a lot of organisms from these taxa are herbivorous; and (2) differences in the metabolical As pathway, supported by the fact that molluscs and crustaceans have a different As speciation than fish (Le *et al.* 1994; Gailer *et al.* 1995).

Although these percentages of AsTox over AsT might seem alarming, the absolute AsTox concentrations compare well with the higher concentrations found in sea fish. Median concentrations range from 0.12 mg kg^{-1} WW in scallops to 0.43 mg kg^{-1} WW in whelks.

Fish Liver

The livers of 25 fish samples were analyzed for AsT and AsTox. Suñer *et al.* (1999) stated that fish liver tissue contains more As than muscle tissue, for total As as well as for inorganic As. He suggested the existence of a detoxification (methylation) mechanism, similar to the one in mammals, which operates principally in the liver. However, our data do not support this statement; in half the cases the AsT concentration is higher in muscle than in liver (Figure 5) and in 5 of the 25 samples, this is also the case for AsTox.

Nevertheless, some peculiar relationships were observed:

1. Distribution coefficients K_d of As between liver and muscle were calculated (K_d= concentration in muscle over concentration in liver). The K_ds of AsTox were generally smaller than those of AsT, indicating a higher affinity in the liver for AsTox than for AsT. This observation may be in favor of the detoxification mechanism in the liver, as suggested by Suñer *et al.* (1999). A possible process is that toxic inorganic compounds are transported to the liver, methylated, and accumulated as a more complex but nontoxic species. Note that the latter reaction involves the transformation of simple methylated arsenicals into AC and AB, but this process has not yet been elucidated.

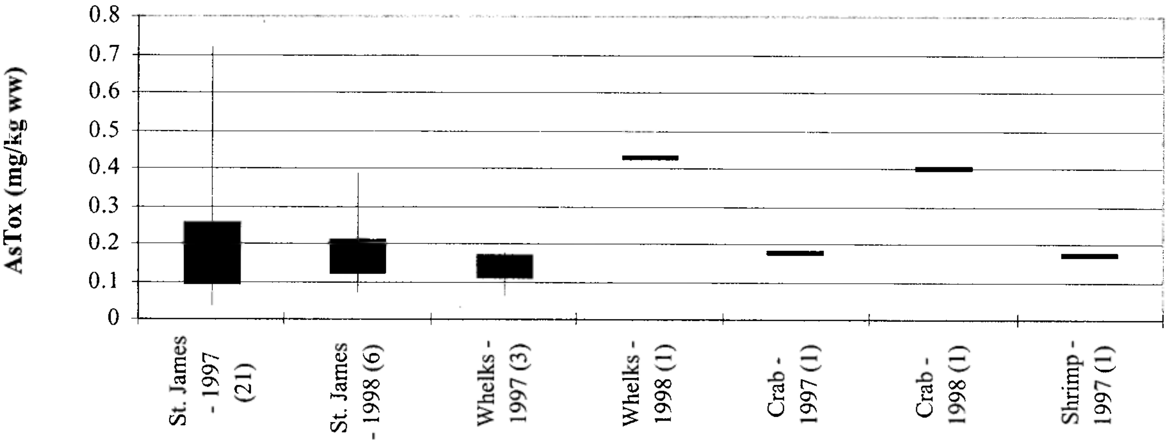

Fig. 4. AsTox concentrations in shellfish and crustaceans—minimum, maximum, 25th and 75th percentile

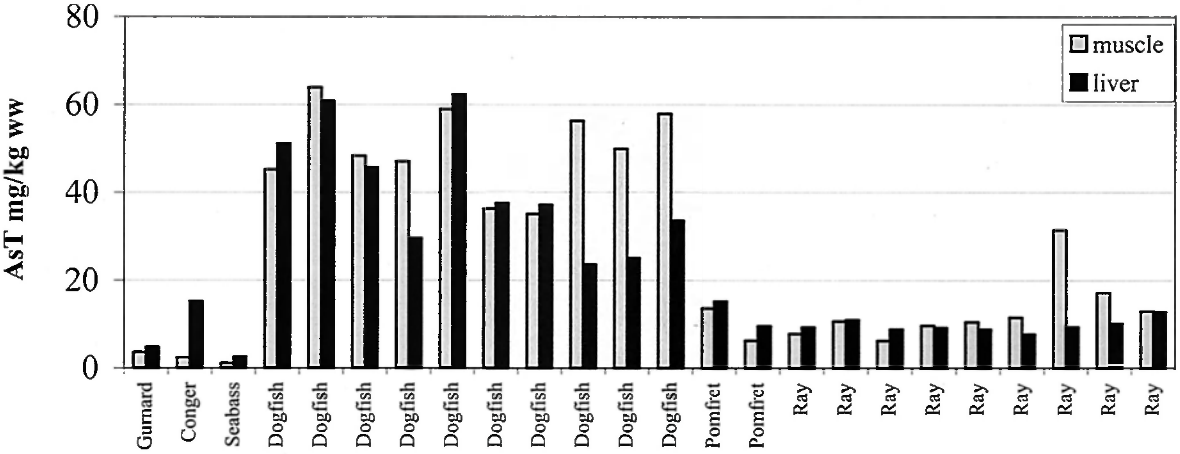

Fig. 5. Comparison between liver and muscle AsT concentrations

2. The sum of AsT concentrations, respectively AsTox, in the liver + muscle was calculated. Although the AsTox fraction is included in AsT, it is not evident that an increase or decrease in AsTox would be visible in the AsT concentration, since AsTox is only a minor fraction (previous paragraph) of the total. Nevertheless, a linear relationship between both sums is observed in a plot of the sum of AsTox versus the sum of AsT (correlation coefficient $r = 0.84$). Additionally, the ratios of AsTox (liver + muscle concentration) to the total As (liver + muscle concentration) seem to be rather constant within the same species (Figure 6). Hence, such feature suggests some kind of upper limit for the AsTox concentrations. If more toxic As compounds are accumulated by the fish, the detoxification system is triggered (in dogfish, Figure 6 suggests that this occurs when the AsTox concentration reaches 1% of the AsT).

It should be mentioned that unfortunately, the analysis method applied in this study would not provide straightforward evidence for a detoxification mechanism, as both the start products (inorganic As) as the end products (MMA and DMA) of the mechanism are detected as one.

Discussion

Comparison Between Fish Species with Respect to Human Health

Due to the lack of formal As concentration norms in fish, it is very difficult to judge the potential human risk related to the consumption of seafood. Legal limits vary between 0.1 mg kg^{-1} (Venezuela) and 10 mg kg^{-1} (Hong Kong). The Joint FAO/WHO Expert Committee (1983) has also set a limit of 0.1 mg kg^{-1} WW. However, in some countries this norm is related to the total As concentration, whereas in other countries it expresses the inorganic As fraction. Therefore the meaning of these norms is not straightforward. If we assume an acceptable daily intake of 2 μg inorganic arsenic per kg body weight or about 0.1 mg day^{-1}, we can estimate the potential danger for

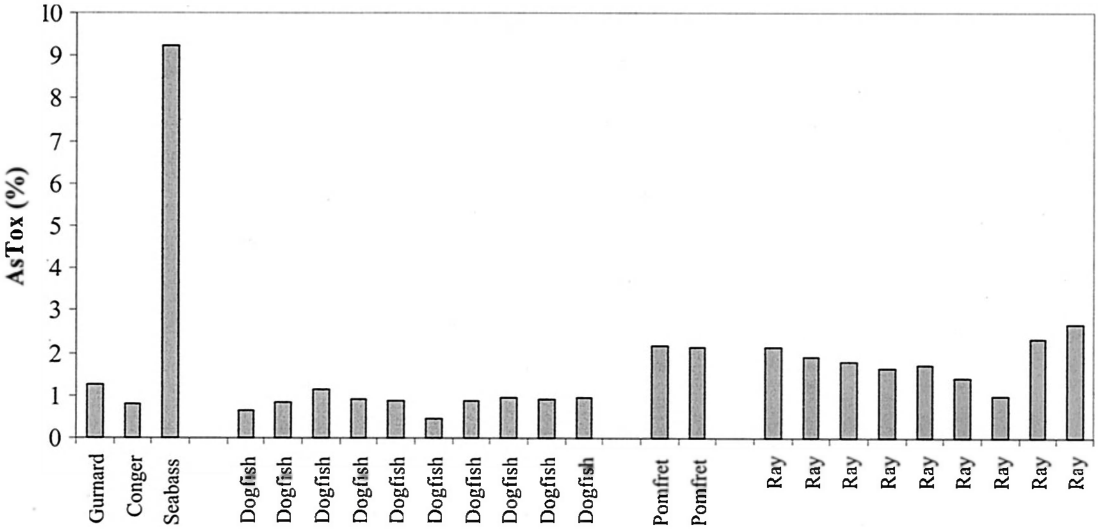

Fig. 6. AsTox fraction (liver + muscle)

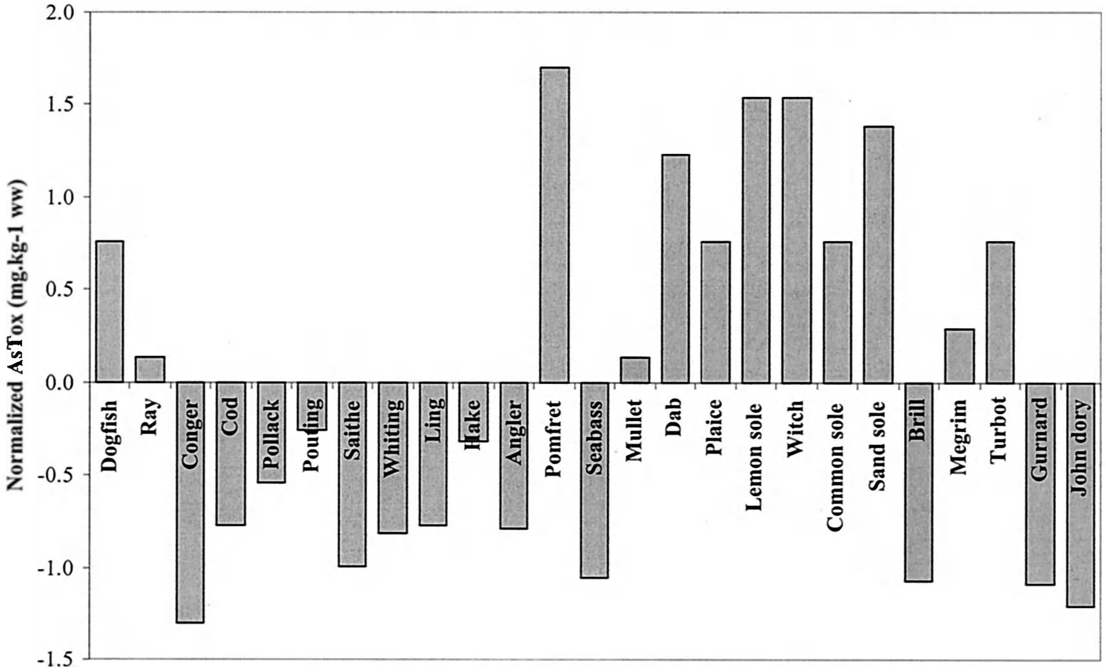

Fig. 7. Normalized AsTox concentrations for sea fish

human health. In a best-case scenario, intake consists of raw fish (wet weight) with a toxic As level beneath the average of 0.1 mg kg^{-1} WW obtained from our complete dataset of sea fishes. In such a scenario at least 1 kg of fish or shellfish can be consumed per day without any harm. A worst-case scenario is when fish has been dried or smoked and the toxic As level is amongst the highest we observed (0.5 mg kg^{-1} WW). In such a case the marine food contains about 2.5 mg AsTox kg^{-1} DW, which means that, even when ingesting 50 g of fish, the acceptable 2daily intake is exceeded. These simple calculations demonstrate that in a worst-case scenario the As content of North Sea marine food may reach harmful levels. Therefore, it would be helpful if we could make some kind of a classification of the studied sea fishes, based on their toxic As fraction. A normalization of the obtained AsTox concentrations, according to the formula $X_{\text{norm}} = (X - X_{\text{group}})/STDEV_{\text{group}}$ (X_{norm} is the normalized species concentration; X is the median species concentration; X_{group} is the group average; $STDEV_{\text{group}}$ is the standard deviation on the group average), allows us to directly identify the potentially harmful species concerning arsenic.

Despite the lack of formal arsenic norms in fish, Figure 7

Table 5. AsT concentrations versus literature data

Species	Location	AsT (mg As kg^{-1} WW)	Reference
Seafish			
Dogfish	French Atlantic Coast	7.96–25.34	Cossa 1990
	Ostkante	5.62–10.78	Ballin *et al.* 1994
	North Sea and Channel	21.3–64.0	This study
Ray	Southern North Sea	31	Luten *et al.* 1982
	North Sea and Channel	6.2–35.9	This study
Conger	French Atlantic Coast	11.7–42	Cossa 1990
	Northern North Sea	12–30	Luten *et al.* 1982
	North Sea and Channel	2.4	This study
Cod	Greenland	1.97–4.33	Ballin *et al.* 1994
	Dogger bank	3.65–7.08	Ballin *et al.* 1994
	Baltic Sea	0.50–0.64	Ballin *et al.* 1994
	Belgian Coast	2.5–5.4	De Clerck *et al.* 1990
	Northern North Sea	15.3	Luten *et al.* 1982
	No. Atlantic	0.4–2.3	Stange *et al.* 1996
	Norway	4.1	Staveland *et al.* 1993
	North Sea and Channel	3.1–7.0	This study
Saithe	Northern North Sea	1.4	Luten *et al.* 1982
	North Sea and Channel	1.8–5.7	This study
Whiting	French Atlantic Coast	2.24–11.34	Cossa *et al.* 1990
	English Channel	2.1	Luten *et al.* 1982
	North Sea	1.7	Luten *et al.* 1982
	North Sea and Channel	4.0–6.5	This study
Ling	Northern North Sea	6.0	Luten *et al.* 1982
	North Sea and Channel	2.1–8.5	This study
Angler	French Atlantic Coast	3.7–14.52	Cossa 1990
	North Sea and Channel	4.1–13.7	This study
Seabass	French Atlantic Coast	0.72–1.64	Cossa 1990
	North Sea and Channel	1.1	This study
Dab	North Sea	2.4–12.5	Cardinaals *et al.* 1985
	Southern North Sea	6.8	Luten *et al.* 1982
	Denmark	11.6	Luten *et al.* 1982
	Eastcoast England	12.6	Luten *et al.* 1982
	NE Atlantic	1.0–1.2	Stange *et al.* 1996
	North Sea and Channel	3.7–21.4	This study
Plaice	Fladenground	16.58–55.72	Ballin *et al.* 1994
	German Bight	7.39–12.72	Ballin *et al.* 1994
	French Atlantic Coast	2.08–12.04	Cossa 1990
	North Sea	3–166	Luten *et al.* 1982
	Central North Sea	31.3	Luten *et al.* 1982
	Westcoast Denmark	17.5	Luten *et al.* 1982
	Southern North Sea	9.4	Luten *et al.* 1982
	North Sea and Channel	5.9–26.0	This study
Lemon sole	Wadden Islands	25	Luten *et al.* 1982
	Dogger bank	46	Luten *et al.* 1982
	North Sea and Channel	14.9–76.1	This study
Common sole	North Sea	6.2–10.2	Cardinaals *et al.* 1985
	French Atlantic Coast	2.74–15.98	Cossa 1990
	Westcoast Denmark	10.4	Luten *et al.* 1982
	Southern North Sea	4.6	Luten *et al.* 1982
	Central North Sea	6.6	Luten *et al.* 1982
	North Sea and Channel	4.1–48.8	This study
Brill	Southern North Sea	0.8	Luten *et al.* 1982
	Westcoast Denmark	2.2	Luten *et al.* 1982
	Eastcoast England	1.9	Luten *et al.* 1982
	North Sea and Channel	1.4–2.9	This study
Turbot	Southern North Sea	6.4	Luten *et al.* 1982
	Westcoast Denmark	14.8	Luten *et al.* 1982
	Eastcoast England	4.4	Luten *et al.* 1982
	North Sea and Channel	17.9	This study
Shellfish and crustaceans			
Common shrimp	French Atlantic Coast	1.4–2.88	Cossa 1990

Table 5. Continued

Species	Location	AsT (mg As kg^{-1} WW)	Reference
	Belgian Coast	4–10	De Clerck *et al.* 1990
	North Sea and Channel	5.2	This study
Great scallop	French Atlantic Coast	2.3–5.28	Cossa 1990
	North Sea and Channel	0.99–3.6	This study

indicates which species have generally high AsTox concentrations (positive normalized values) and thus should require a more intense follow up with regard to the concern for human health.

Comparison Between Fish Species as a Function of Habitat

It appears from the normalized values that there is a certain comparability between species of a same scientific order. Some of the fish species in Table 1 listed as *Actinopterygii* or ray-finned fish, more specifically the orders *Anguilliformes* (eels and morays), *Gadiformes* (cods), *Lophiiformes* (anglerfish), *Scorpaeniformes* (scorpionfish and flatheads), and *Zeiformes* (dories), all show negative normalized values for AsTox. The *Elasmobranchii* or the class of sharks and rays, show the opposite, they have positive normalized AsTox values. Also the flatfish species all have positive normalized AsTox values, except brill.

Several studies provided evidence that organisms of the higher food chain levels, like fish, have only a limited ability for As uptake from the water column, in contradiction to lower trophic organisms (bacteria, plankton, and macroalgae) (Gailer *et al.* 1995; Langston 1984; Neff 1997). Fish accumulate As predominantly via their diet, but in contrast to Hg for example, As seems not to biomagnify. Several studies, including a three-organism food chain study (autotrophic grazer–zooplanktonic grazer–guppy) by Maeda *et al.* (1990), indeed pointed out that metabolization of As occurs instead of magnification. Also in an insect–crayfish–trout study conducted by Mason *et al.* (2000) and in a kelp–detrital feeding worms–whiting study by Edmonds and Francesconi (1981), no evidence of magnification was found. In reality, the metabolism particular to each species seems to be more important than the trophic echelon to which the species belongs. In the algae group, *Hisikia fusiformis* has seven times more arsenic than *Undaria pinnitifida* (Shinagawa *et al.* 1983). For polychaetes, *T. marioni* has an arsenic concentration 70 times greater than that of *Perinereis cultrifera* taken from the same site, whereas *Littorina littorea* has a lower arsenic concentration than the *Fucus vesiculosus* on which it feeds. The high AsTox% results described, despite relatively low AsT concentrations observed in some fish species, may be due to differences in As metabolism, but more specific information about trophic relationships, arsenic associated to the prey, and species metabolism are required to give definite answers. Differences in As concentrations between species should be directly related to their food source. In general, this study shows that all species that feed primarily on larger fish, for example cod, pollack, saithe, ling, hake, angler, seabass, john dory, and conger contain less As than the species that feed on benthic organisms and smaller fish, like the flatfishes. It is not clear why brill does not fit in this picture while its feeding pattern is comparable to that of, for example, megrim.

By ANOVA testing we compared the individual AsT concentrations of samples of the same species (intraspecies variability). Significant differences for some fish species were observed; significantly higher AsT concentrations were found in dogfish from the French coast versus the western North Sea and in common sole from the Bay of the Seine versus the western North Sea. In the early 1990s Michel (1993) also analyzed 156 individuals of the latter species, caught at seven different locations along the French coast, for total As. His results show large spatial variations, with highest average concentrations at the location with coordinates N-46°03.6; W-1°18.7 (north of Bordeaux). In northern direction, up to the Bay of the Seine, in the north of France, AsT concentrations decrease. The most northern station in Michel's (1993) study is at the same time the most southern sampling area in our study. The values we obtained for dogfish, as well as for common sole, are slightly higher than those described by Michel, but they are still of the same order of magnitude. Concentrations seem to increase again, in a northern direction, up to the Belgian and Dutch coasts. Dissolved and particulate As concentrations in the water column of Belgian and Dutch coastal waters are also consistently elevated, due to the influence of the Scheldt and Rhine (De Gieter, unpublished data). Variability in tissue As concentrations may thus also be related to variations in bioavailability caused by geographical, seasonal, and environmental differences.

Whether or not As bioaccumulation is a function of fish size, is still under discussion. Edmonds and Francesconi (1981) described a positive relationship between As concentrations and size in school whiting. De Clerck *et al.* (1990) found a similar positive relationship in flounder, but no apparent influence of length in cod. In this study, even the best correlation coefficients (r^2) found are rather low; 0.386 for AsT and 0.278 for AsTox, both for common sole. However, at least part of the lack of correlation can be attributed to the limited range in length of the investigated fishes, due to the length-specific fishing of fish for consumption. Here, too, several other parameters should also be taken into account, as bioaccumulation does not reflect exposure time alone, but is also a function of contamination level of food source, and thus changes of diet lead to changes in exposure and growth rate, as this could lead to growth dilution.

Comparison with Literature Data

A summary of total arsenic concentrations in fish reported in literature as well as our data is presented in Table 5. In general, our data compare well with those found in literature, but some small peculiarities could be noticed: dogfish, saithe, and sole show relatively higher As levels in our samples; the opposite is true for scallops. The turbot and conger results are also deviating, but these findings are based on a very limited amount of samples.

Literature data on AsTox concentrations in sea fish are rather scarce and are only reported in the framework of developing and optimizing analytical techniques. Toxic arsenic fractions of less than 10% are mentioned (Alberti *et al.* 1995; Le *et al.* 1994) coinciding with the values observed in this study.

Acknowledgments. We thank the Ministry of Public Health and the Institute for Veterinary Control for financial and practical support. In particular we would like to thank Dr. Lic. L. Moor, Dr. Lic. M. Cornelis, and Dr. P. Naassens, Dr. Schaltin, Dr. W. De Myttenaers, Dr. M. Meyns, and Dr. Mathieu for their practical help and J. Maes, Prof. F. Ollevier (KUL), and H. Hillewaert (RVZ) for the sampling at sea.

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