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Research Note

Polycyclic Aromatic Hydrocarbons, Polychlorinated Biphenyls, Chlorinated Pesticides (DDTs), Hexachlorocyclohexane, and Hexachlorobenzene Residues in Smoked Seafood

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ABSTRACT

Smoked seafoods were screened for the presence of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other organochlorine compounds. Total PAH concentrations ranged from 46.5 ng/g (wet weight) for smoked swordfish to 124.0 ng/g (wet weight) for smoked herring. Among the carcinogenic PAHs, benzo(*a*)pyrene ranged from undetectable levels for several smoked fish to 0.7 ng/g for Scottish salmon, dibenzo(*ah*)anthracene was not present in any of the samples analyzed, and benzo(*a*)anthracene was found in all samples and at particularly high levels in salmon (23.2 ng/g). Benzo(*a*)pyrene concentrations were below the tolerance limit for all samples. PCB concentrations for the different samples ranged from 2 to 30 ng/g. Chlorinated pesticides (DDTs: *p,p′*-DDE, *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDD, and *o,p′*-DDD) were detected at levels ranging from 0.2 ng/g (wet weight) in bluefin tuna to 17.5 ng/g (wet weight) in salmon. Hexachlorocyclohexane isomers (αHCH + βHCH + γHCH) were present in higher amounts in eels (6.5 ng/g) than in the other smoked fish. For 40% of the samples, PCB concentrations exceeded the limit fixed by the European Union, while pesticide levels were below the maximum acceptable limit proposed by the Food and Agriculture Organization.

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other organochlorine compounds, such as pesticides, are industrial chemicals that are widely distributed in the environment. PAHs have been the source of much concern because of their toxic potential. Because several compounds of this group have been shown to be potent carcinogens in experimental animals *(13, 14),* they are widely hypothesized to make a significant contribution to cancer in humans *(19).* PAHs undergo metabolic activation in mammalian cells to form diol-epoxides that then bind covalently to the nuclear DNA to form adducts that can lead to errors in DNA replication, resulting in mutations that can potentially initiate the carcinogenic process *(6).* Also PCBs, trichloro-2,2-bis (*p*-chlorophenyl) ethane (*p,p′*-DDT), and hexachlorocyclohexane (HCH) gamma isomer are categorized by the International Agency for Research on Cancer as probably carcinogenic or possibly carcinogenic to humans *(14).*

The presence of these pollutants in the environment leads to their presence in foods. Biomonitoring procedures have been developed to assess human exposure to PAHs, and assessments with these procedures have indicated that diet contributes substantially to nonoccupational exposure to PAHs. For nonsmokers, more than 70% of exposure to PAHs is attributable to diet *(7, 19)*; moreover, surveys carried out in a number of countries have shown that >90% of the daily exposure of humans to PCBs occurs through the diet *(20).* Among foods, fish has been shown to be a major conduit for PCBs into the human body *(3, 12),* so smoked fish can constitute a notable source of human exposure to PAHs, since it is known that the smoking of meats contributes to the formation of PAHs. For this reason, PAHs in smoked seafood have often been monitored *(11, 16, 25)* to ensure that residues are kept at a low level so that the health risk posed by their ingestion is minimized. Comparatively, little is known about organochlorine contaminants in smoked seafood, although organochlorines *(1, 5),* together with PCBs *(22),* were the main pesticides found in studies carried out to detect pesticide residues in fish.

For public health purposes, this study measured concentrations of PAHs, highly toxic contaminants whose presence in food requires continuous monitoring, in smoked seafood. In addition, concentrations of PCBs and organochlorine compounds were also measured in view of the scarcity of information about these contaminants, which are also toxic.

MATERIALS AND METHODS

Analyses for the presence of PAHs, PCBs, and other organochlorine compounds were carried out for 10 packages of smoked seafood fillets (see Table 1) purchased from major national supermarket chains selling national and imported brands.

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TABLE 1. *Concentrations of individual polycyclic aromatic hydrocarbons in smoked seafoods from different countries*

Sample type (n = 10)	Origin	Concn (ng/g [wet wt]) of PAH[a]								
		1	2	3	4	5	6	7	8	Total
Salmon	Denmark	46.6 ± 0.03	ND	9.0 ± 0.01	ND	1.2 ± 0.01	ND	ND	ND	56.8
	Scotland	50.2 ± 0.04	ND	11.7 ± 0.02	10.4 ± 0.02	23.2 ± 0.03	0.7 ± 0.01	ND	ND	96.2
	Norway	49.5 ± 0.04	ND	11.6 ± 0.01	ND	0.5 ± 0.01	ND	ND	ND	61.6
Swordfish	Italy	39.2 ± 0.02	ND	6.1 ± 0.02	ND	1.2 ± 0.01	ND	ND	ND	46.5
	Denmark	64.6 ± 0.05	5.3 ± 0.01	8.0 ± 0.01	ND	1.1 ± 0.01	ND	ND	ND	79.0
Herring	French	56.5 ± 0.04	21.2 ± 0.02	6.6 ± 0.02	ND	1.2 ± 0.01	ND	ND	ND	85.5
	Norway	49.1 ± 0.03	24.9 ± 0.02	15.1 ± 0.03	ND	1.8 ± 0.02	0.5 ± 0.01	ND	ND	91.4
	Denmark	68.1 ± 0.05	51.8 ± 0.03	3.1 ± 0.01	ND	1.0 ± 0.01	ND	ND	ND	124.0
Eel	Denmark	37.6 ± 0.02	ND	12.7 ± 0.02	ND	1.9 ± 0.01	0.3 ± 0.01	ND	ND	52.5
Bluefin tuna	Denmark	74.9 ± 0.06	24.9 ± 0.02	8.7 ± 0.01	ND	1.4 ± 0.01	ND	ND	ND	109.9
Range		37.6–74.9	ND–51.8	3.1–15.1	—	1.0–23.2	ND–0.7	—	—	46.5–124.0
Mean ± SD		53.6 ± 12.3	25.6 ± 16.7	9.3 ± 3.56	—	3.5 ± 7.0		—	—	80.3 ± 25.8

[a] Mean ± standard deviation. PAH numbers: 1, phenanthrene; 2, anthracene; 3, fluoranthene; 4, pyrene; 5, benzo(*a*)anthracene; 6, benzo(*a*)pyrene; 7, dibenzo(*ah*)anthracene; 8, benzo(*ghi*)perylene. ND, not detected.

Determination of PAHs. The analytical procedures for the extraction and purification of PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benz(*a*)anthracene, benzo(*a*)pyrene, dibenzo(*ah*)anthracene, and benzo(*ghi*)perylene) were carried out by the method of Dunn and Armour *(8)*. In brief, 60-g samples were added to 150 ml of ethanol and 7 g of potassium hydroxide and digested by gentle refluxing for 1.5 h. The mixture was extracted three times with 200 ml of isooctane (C. Erba, Milan, Italy), and the extracts were evaporated with a rotary evaporator (RE 111, Büchi, Switzerland) at 40°C and redissolved in toluene. The samples were applied to a column (florisil, 60 to 100 mesh, deactivated with 5% water and prewashed with 100 ml of toluene, which was discarded) and eluted with toluene, and 5 ml of Me_2SO (dimethyl-suloxide) was added to the eluate. The mixture was rotary evaporated to remove the toluene, leaving the samples in Me_2SO. The quantification of the PAHs in the samples was carried out with a high-performance liquid chromatography apparatus (Beckman System Gold) equipped with an ultrasphere ODS-C-18 column (inside diameter, 4.6 mm; length, 25 cm; particle size, 5 μm; Beckman). The initial mobile phase was acetonitrile/water (45:55, vol/vol) with a flow rate of 1.5 ml/min. Separation was obtained with a gradient in which the acetonitrile concentration increased from 45 to 100% in 40 min. A fluorimetric detector (excitation wavelength, 290 nm; emission wavelength, 430 nm; Perkin Elmer LS-5 Luminescence Spectrometer) and a PS2/80 IBM computer integrator were used for the determination of the peak areas. The PAHs were identified on the basis of retention time and quantified by comparison with the fluorescence response of the appropriate standard (97.3 to 99% pure, 200 μg/ml of each PAH; Supelco Park, Bellefonte, Pa.). The concentrations of the diluted standards ranged from 0.3 to 6 μg/ml. The identities of individual PAHs were confirmed with the use of stop-flow conditions to obtain the complete emission spectrum corresponding to each peak in the chromatogram. Recovery was determined with the use of spiked samples (at levels of about 60 ng/g for phenanthrene, anthracene, fluoranthene, and pyrene and of 5 to 25 ng/g for the remaining PAHs). Recovery levels ranged from 70 to 98%, and all results reported have been corrected for recovery. Precision was estimated from the results of three replicate analyses of the samples.

Determination of PCBs, DDT compounds, HCB, and HCHs. To determine concentrations of chlorobiphenyl (PCBs, 11 congeners), DDT compounds (*p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDT, and *o,p′*-DDD), hexachlorobenzene (HCB), and hexachlorocyclohexane (HCH) isomers (αHCH, βHCH, and γHCH), the following method was used. Aliquots (2 to 10 g) of the homogenized samples were ground with anhydrous sodium sulfate (Pestanal grade; Riedel de Haen, Seelze, Germany) in a mortar. The mixture was extracted with petroleum ether (40 to 60°C; SupraSolv Merck, Darmstadt, Germany) according to Erney's procedure *(9)*. The extracts were then concentrated, and subsamples were taken in order to determine tissue fat content by gravimetry. An aliquot (ca. 200 mg) of the remaining extract was dissolved in hexane (5 ml) and mixed with H_2SO_4 concentration for cleanup by the procedure described by Murphy *(18)*. After centrifugation, the hexane solution was concentrated (about 1 ml) and transferred to a glass column (inside diameter, 5 mm) filled with 1 g of florisil, which was (100 to 120 mesh; Supelco) activated at 120°C for 16 h for the separation of PCBs from other organochlorine compounds.

The first fraction eluted with hexane (12 ml) contained PCBs and some DDTs, whereas the second fraction, eluted with 10 ml of 15% ethylether in hexane, contained the remaining DDTs and other organochlorine compounds. An aliquot of the initial fraction was run on a column (inside diameter, 5 mm) packed with 125 mg of activated carbon (C. Erba) for the separation of non-*ortho* PCB congeners, 3,3′,4,4′-T_4CB (IUPAC 77), 3,3′,4,4′,5-P_5CB (IUPAC 126), and 3,3′,4,4′,5,5′-H_6CB (IUPAC 169) from other PCBs by the method of Tanabe et al. *(24)*. Analyses were carried out with a Carlo Erba HR 5300 Mega Series gas chromatograph with an automatic injection system and with an ECD-400 electron capture detector, nickel-63 (310°C). The gas chromatograph was connected to an IBM PS/2 55SX personal computer equipped with the System Gold Version 6.1 software program for integration purposes (Beckman). For all the analyses, a SPB-608 Supelco fused-silica capillary column (length, 30 m; inside diameter, 0.25 mm; film thickness, 0.25 μm) was used. Helium at a flow rate of 1 ml/min was used as the gas carrier, and nitrogen at 60 ml/min was used as the makeup gas. The temperature was programmed at 50°C for injection; this temperature was kept steady for the first 1 min and was then increased to 180°C at a rate of 15°C/min; the temperature was kept steady at 180° for 1 min and was then increased to 220°C at a rate of 4°C/min; the temperature was kept steady at 220°C for 20 min and was then increased to 275°C at a rate of 5°C/min; from this point until the end of the analytical

run, the column remained isothermal at a temperature of 275°C. The 11 individual PCB congeners were PCBs 8, 20, 28, 35, 52, 101, 118, 138, 153, 180, and 209 according to the IUPAC numbering system *(4),* as determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity, 99%). The identities of the DDT group compounds were confirmed by alkali conversion to their respective olefins and reanalysis by gas-liquid chromatography. Analytical data such as those for DDT group compounds were obtained by comparison between sample peak areas and external standard peak areas (DDT mixture, Supelco). Recovery levels were determined by adding known amounts of PCB, DDT, HCB, and HCH standards to blank samples. Recovery was within 80 to 110%. The limits of quantification were 0.1 to 0.4 ng/g on a wet weight basis for the pesticides and the PCB congeners. Residues in 10% of the samples were confirmed by gas-liquid chromatography–mass spectrometry (Fisons MD 800). Concentrations of contaminants are presented in terms of nanograms per gram on a wet weight basis.

RESULTS AND DISCUSSION

PAHs. The occurrence of PAHs in seafood can be a result of sorption caused by a contaminated environment *(3, 23, 25*) or by food preparation methods. The smoking of foods has been practiced since antiquity, not only because of the special organoleptic profiles of smoked products, but also because of the inactivating effect of smoke on enzymes and microorganisms. The technology has evolved from the simple drying and smoking of fish over a campfire to highly developed and sophisticated industrial techniques for producing large quantities of delicatessen products that are widely demanded on the market. Processing techniques vary considerably in various parts of the world, and there are even differences between the processes used by different producers in the same country. The smoke source can dramatically influence both the PAH level and the types of compounds present in the smoke and, subsequently, their deposition on the surface of smoked food *(17).* The temperature of smoke is generally very important because the amount of PAHs forming during pyrolysis in smoke was shown to increase linearly with the smoking temperature from 400 to 1,000°C *(21).* Also, hot smoking, used for treatment, brings about higher concentrations of PAHs than cold smoking, used for fermented products that are not processed thermally *(21).* Other factors, such as degree of smoking, preparation time, and the fat content of the product, also play very important roles in determining the amounts of these contaminants. In different studies, the levels of polynuclear aromatic hydrocarbons formed during smoking have been found to be higher for fish with higher fat contents *(2, 15).* However, independent of the many variables that can influence the smoking process, it is clear that the process always causes the formation of PAHs.

Various levels of PAHs have been found in different studies undertaken to quantitate these compounds in smoked fish. Lawrence and Weber *(15)* found that smoked herring had a PAH concentration of 30.5 ng/g, while in eels the total concentration of 11 PAHs was 2.1 ng/g. In a detailed analysis of smoked food, Gomaa et al. *(11)* reported total PAH levels of 9.3 to 86.6 ng/g. Concentrations of five carcinogenic PAHs (benzo(*a*)anthracene, benzo(*a*)pyrene, dibenzo(*ah*)anthracene, benzo(*b*)fluoranthene, and indeno(*1,2,3-cd*)pyrene) reached levels of 16.0 ng/g in salmon *(11).* In a study on 27 polynuclear aromatic hydrocarbon compounds in smoked trout from lakes Michigan and Superior, Zabik et al. *(25)* reported total PAH contents ranging from 132.2 to 167.9 ng/g (with a mean of 154.4 ng/g) and from 199.1 to 319.6 ng/g (with a mean of 270.9 ng/g), respectively. The levels of nine PAH carcinogens in smoked Great Lakes fish were 36.36 and 42.61 ng/g (wet weight) for Lake Michigan trout and Lake Superior trout, respectively *(25).*

The levels of individual PAHs in smoked seafood presented in Table 1 are average values for duplicate analyses of two samples per product. Total PAH concentrations for all samples ranged from 46.5 to 124.0 ng/g (wet weight), with a mean value of 80.3 ng/g (wet weight). As can be seen, phenanthrene and fluoranthene were detected in all the samples at levels ranging from 37.6 to 74.9 ng/g (wet weight) (with a mean of 53.6 ng/g [wet weight]) and from 3.1 to 15.1 ng/g (wet weight) (with a mean of 9.3 ng/g [wet weight]), respectively, while dibenzo(*ah*)anthracene and benzo(*ghi*)perylene were not present in any of the samples analyzed. The other compounds were found at variable concentrations in the different smoked products. Residues of anthracene were detected in 50% of the samples, with the highest levels being found for Danish herring (51.8 ng/g), while pyrene was found solely in Scottish salmon (10.4 ng/g). An interesting result was that the most well known carcinogenic PAH, benzo(*a*)pyrene, was absent in all of the samples except the Scottish salmon (0.7 ng/g), Danish herring (0.5 ng/g), and eel (0.3 ng/g) samples. Conversely, benzo(*a*)anthracene, another carcinogenic compound, was found in all of the samples and was present at particularly high levels in Scottish salmon (23.2 ng/g). At the present time, there are no maximum levels for individual carcinogenic PAHs in smoked food in Italy, except for benzo(*a*)pyrene, for which a tolerance limit of 1 ng/g has been established, and this is also the case for many other countries (e.g., Germany, Austria, Czech Republic, Switzerland, and Slovak Republic). In our samples, benzo(*a*)pyrene concentrations were below the tolerance limit, although the concentration of 0.7 ng/g for Scottish salmon approaches the limit.

PCBs. Table 2 lists the levels of PCBs for different samples of smoked fish. Total concentrations of 11 chlorobiphenyl congeners ranged from 2 to 30 ng/g, with the lowest levels being those for Italian swordfish (2 ng/g) and French herring (5 ng/g) and the highest values being those for Danish herring (29 ng/g), eel (30 ng/g), and Scottish and Danish salmon (26 ng/g). Chlorinated pesticides were detected in all samples at levels ranging from 0.2 to 17.5 ng/g (with a mean of 7.7 ng/g). Of the DDTs, the predominant contaminant was *p,p′*-DDE, which accounted for 48.7% of the DDTs, followed by *p,p′*-DDT (17.8%), *p,p′*-DDD (15.9%), *o,p′*-DDT (11.8%), and *o,p′*-DDD (5.8%). For total DDT concentrations (including all of these compounds), levels for the Danish and Scottish salmon, Danish herring, eel, and swordfish samples were one to five orders

TABLE 2. *Concentrations of polychlorinated biphenyls (PCBs), chlorinated pesticides (DDTs), hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB) in smoked seafoods from different countries*

Sample type (n = 10)	Origin	% lipid	Concn (ng/g [wet wt]) of agent[a]								
			PCBs	Total PCBs[b]	*p,p′*-DDT	*p,p′*DDE	*p,p′*-DDD	*o,p′*-DDT	*o,p′*-DDD	DDTs	HCHs	HCB
Salmon	Denmark	8.54	26 ± 0.03	292.3	1.6 ± 0.01	13 ± 0.03	1.4 ± 0.01	0.9 ± 0.02	0.6 ± 0.01	17.5 ± 0.05	0.9 ± 0.01	0.2 ± 0.01
	Scotland	7.77	26 ± 0.04	317.9	2.1 ± 0.02	5.5 ± 0.02	1.6 ± 0.01	0.8 ± 0.01	0.7 ± 0.01	10.7 ± 0.03	1.1 ± 0.01	2.2 ± 0.02
	Norway	5.34	17 ± 0.02	302.4	1.5 ± 0.01	3.0 ± 0.01	1.4 ± 0.02	0.8 ± 0.01	0.4 ± 0.01	7.1 ± 0.03	ND	0.1 ± 0.01
Swordfish	Italy	3.63	2 ± 0.01	55.1	0.9 ± 0.01	3.0 ± 0.02	1.0 ± 0.02	0.6 ± 0.02	0.3 ± 0.01	5.8 ± 0.02	ND	0.1 ± 0.01
	Denmark	9.86	21 ± 0.02	206.6	2.4 ± 0.02	4.0 ± 0.01	1.4 ± 0.01	2.2 ± 0.01	1.0 ± 0.01	11.0 ± 0.04	0.2 ± 0.01	0.3 ± 0.01
Herring	France	2.00	5 ± 0.01	250.0	0.3 ± 0.01	1.0 ± 0.01	0.4 ± 0.01	0.3 ± 0.03	0.3 ± 0.01	2.3 ± 0.02	0.8 ± 0.01	0.2 ± 0.01
	Norway	6.69	10 ± 0.02	149.5	0.3 ± 0.01	1.0 ± 0.01	0.2 ± 0.01	0.2 ± 0.02	0.1 ± 0.01	1.8 ± 0.01	0.6 ± 0.01	0.2 ± 0.01
	Denmark	14.04	29 ± 0.04	206.6	2.1 ± 0.02	3.0 ± 0.02	2.6 ± 0.01	1.1 ± 0.01	0.4 ± 0.01	9.2 ± 0.02	2.0 ± 0.02	0.6 ± 0.01
Eel	Denmark	19.21	30 ± 0.02	156.2	2.6 ± 0.02	4.0 ± 0.01	2.3 ± 0.02	2.2 ± 0.01	0.7 ± 0.02	11.8 ± 0.03	6.5 ± 0.04	0.6 ± 0.01
Bluefin tuna	Denmark	0.31	T	T	ND	0.2 ± 0.02	ND	ND	ND	0.2 ± 0.01	0.4 ± 0.01	0.2 ± 0.01
Range		0.31–19.21	2–30	55.1–317.9	0.2–13.0	0.3–2.6	0.2–2.2	0.2–2.6	0.1–1.0	0.2–17.5	0.2–6.5	0.1–2.2
Mean ± SD		7.74 ± 5.66	16.7 ± 11.4	215.2 ± 85.8	3.8 ± 3.6	1.5 ± 0.9	1.0 ± 0.7	1.4 ± 0.8	0.5 ± 0.3	7.7 ± 5.4	1.6 ± 2.1	0.5 ± 0.6

[a] Mean ± standard deviation. ND, not detected; T, traces.
[b] Total concentration of seven congeners (PCBs 28, 52, 101, 126, 138, 153, and 180) on the basis of lipid weight.

of magnitude higher than those for other samples, and the lowest concentration was that for Danish bluefin tuna (0.2 ng/g). Of the HCH compounds, γHCH is the most toxic and the least stable. Over time, γHCH is transformed into the more stable αHCH. The relative concentrations of these compounds for each sample tended to reflect this situation, with αHCH being more prevalent than γHCH. For total HCH concentrations (including all of these compounds), as summarized in Table 2, the concentration for the Danish eel samples (6.5 ng/g) was substantially higher than those for the other samples (0.2–2.0 ng/g), while HCH levels for Italian swordfish and Norway salmon were below the detection limit. Such variation was not seen for HCB, whose levels were of the same order of magnitude (0.2 to 0.6 ng/g) for all of the samples except the Scottish salmon samples, which had the highest concentrations (2.2 ng/g).

For pesticides, as for PAHs, the smoking process can influence the amount of contaminants. With a particular focus on the relationship between the smoking of fish and levels of organochlorine pesticides and total PCBs, the study by Zabik et al. *(25)* demonstrated 40 to >50% reductions in pesticide and total PCB concentrations. Such reductions may be a plausible explanation for the low levels of pesticides in the smoked samples; moreover, the low pesticide concentrations determined in the present study might also be due to the restriction on the use of some of the pesticides studied, such as *p,p′*-DDT and HCHs, in many countries, which would result in a decline in environmental contamination. In fact, pesticide levels for the smoked fish under study were below the maximum acceptable limits proposed by the Food and Agriculture Organization *(10)* (500 ng/g for *p,p′*-DDT and 300 ng/g for both βHCH and γHCH). With regard to PCBs, the European Union has established a maximum permissible total PCB level of 200 ng/g (lipid weight) for seven congeners (PCBs 28, 52, 101, 126, 138, 153, and 180). For our samples, the total concentration of the congener set mentioned above, which constituted 95 to 100% of all of the PCBs present, ranged from 55.1 to 302.4 ng/g (lipid weight) (see Table 2). The concentrations for 40% of the samples were above the limit established, the concentrations for 20% of the samples approached these levels, and the concentrations for the remaining samples were below the limit. On the basis of the data obtained, it can be concluded that the organochlorine pesticide content of smoked fish is unlikely to constitute a significant health hazard. Although the manufacture and use of PCBs are banned or highly restricted, they still constitute an important source of persistent chemical contaminants in the environment whose presence in food might pose a long-term human health hazard. The same conclusion can be drawn for PAHs; because of the carcinogenic nature of many of them, they might be continuously screened for, particularly in smoked food. Moreover, an international collaborative effort is clearly needed to establish maximal limits for each PAH in food, considering that it is not only benzo(*a*)pyrene that is responsible for health risks.

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