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Effects of Cooking on Levels of PCBs in the Fillets of Winter Flounder

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Contamination of surface water and sediments by toxic chemicals is a concern in many areas of the United States. Certain organic compounds and metals accumulate in the tissues of aquatic organisms posing a health threat to consumers. Exposure and risk assessments conducted to determine health risks associated with consumption of aquatic species often assume that the levels of contaminants in the edible tissue remain unchanged after preparation and cooking. This assumption may lead to overestimation or underestimation of risk because removal or transformation of toxic constituents in the tissue may occur by thermal decomposition, volatilization, dissolution in aqueous tissue fluids or lipids that drip off the tissue, or extraction into cooking oil.

Polychlorinated biphenyls (PCBs) have several properties which make them toxic in the environment. They can significantly bioacumulate and concentrate in the fatty tissues of organisms. PCBs are of particular interest because of their known toxicity, their widespread presence, and their persistence in the environment. U.S. EPA classifies PCBs as a probable human carcinogen. (U.S. EPA 1988; U.S. EPA 1997) The effects of cooking on PCB levels and other contaminants have been studied by several researchers. Some scientists have found no significant changes in PCB concentrations, while others have reported both decreases and increases in PCB levels following cooking of fish tissue. (Smith 1973; Skea 1979; Cinchy 1979; Zabik 1979; Zabik 1982; Puffer 1983; Armbruster 1989; Trotter 1989)

The objective of this study was to quantify the effect of preparation and cooking on levels of PCBs found in fillets of winter flounder. The cooking methods used in this study were deep fat frying, pan frying with butter, and broiling.

MATERIALS AND METHODS

Twenty-one winter flounder were caught by otter trawl from New Bedford Harbor, Massachusetts on February 21, 1991. The intent was to collect fish that had been in the estuary for approximately 3 to 4 years before beginning their annual migration. Age classification was determined by length.

The initial processing of the fish consisted of filleting and sectioning the fillets.

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The weight and length of the fish were recorded for all fish before filleting. The fish were also examined for abnormalities (e.g., fin rot and tumors). During filleting, care was taken not to disrupt the viscera. All laboratory utensils coming in contact with the sample were cleaned with soap and distilled water, then rinsed with methanol and dichloromethane. Glassware was also baked and rinsed with solvent between samples. Polyethylene gloves were worn at all times when handling fish or tissue.

A top and bottom fillet were obtained from each fish using a stainless steel filleting knife. Top and bottom fillets were assigned for precooking and cooking treatments in an alternating fashion to reduce bias which may result from the choice of fillet. The fillet used for precooked analysis was placed in prelabeled glass jar immediately after filleting. The fillet used for cooking was cut on a dorsal-ventral plane into three subsamples of approximately equal size. The anterior (nearest the head) section was Section I, the middle section was Section II, and the posterior section was Section III. An equal number of the three different sections were used for each of the three cooking methods (i.e., seven of each section from the 21 fish).

The fish were cooked thoroughly but not overcooked. Pre- and post-cooking weights of the treatment sections were recorded to determine weight loss and precooking equivalent weights. Moisture and lipid content were determined on all raw samples.

Deep fried samples were cooked for approximately 1 minute in 200 mL of pure vegetable oil in a fryer that had been preheated for 5 minutes. Pan fried samples were cooked for approximately 1 minute per side in 1 tablespoon of lightly salted butter in a 9-inch non-stick frying pan that had been preheated. Broiled samples were cooked for approximately 2 minutes on a broiling pan in an oven that had been preheated for broiling. All utensils were thoroughly cleaned between samples. Fresh vegetable cooking oil was used for each fish. Cooked samples were homogenized and stored until sample extraction.

After thawing, approximately 5 to 25 g of tissue was removed and placed in a Teflon jar for extraction. This subsample was fortified a known and recorded amount of the surrogate compound dibromooctafluorobiphenyl (DBOFB). Matrix-spike samples were fortified with a known amount of each of the PCB congener analytes and the surrogate compound. Sodium sulfate was added to absorb water. The sample was macerated twice for 2 minutes, using dichloromethane as the extraction solvent. The sample was centrifuged between extractions and the extract decanted. After the two maceration/extraction steps, dichloromethane was added to the sample jar and shaken for 30 min. Again, the sample was centrifuged and the extracts combined.

The extract was passed through 20 g of alumina for preliminary cleanup and concentrated using a Kuderna-Danish apparatus, followed by gentle evaporation

with nitrogen gas. The extract was then purified by gel permeation high-performance liquid chromatography (HPLC). The HPLC analyte fraction was concentrated by nitrogen evaporation, solvent exchanged for isooctane, and reduced to a final volume of 200 µL. The sample was then fortified with 50 ng of the recovery internal standard tetrachloro-m-xylene (TCMX), and submitted for analysis by gas chromatography/electron capture detection (GCECD).

The samples were analyzed using a gas chromatograph with hydrogen as the carrier gas. PCB congener concentration data were determined in nanograms per gram on a raw (precooked) wet-weight basis. The extraction sample wet weights were corrected to raw wet weight for the cooked samples, using pre- and post-cooking sample weight ratios from the same fish and cooked section. Samples were quantified relative to the surrogate compound DBOFB. The recovery internal standard TCMX was used to determine surrogate recoveries in the samples. Samples were analyzed for 17 PCB congeners.

The equation used to determine the fractional change in PCBs resulting from cooking was derived by Skea (1979):

$$F = \frac{(C_r \times M_r) - (C_c \times M_c)}{C_r \times M_r}$$

where: F = the fractional change in PCBs
C_r = concentration of PCBs in raw fillets
M_r = mass of raw fillet
C_c = concentration of PCBs in cooked fillets
M_c = mass of cooked fillet

Visual inspection of the raw data indicated that the distribution was skewed. Therefore, the data were transformed using the natural logarithm. A three-way analysis of variance (ANOVA) was performed to evaluate differences of cooking treatment, section (i.e., I, II, or III) and fillet (i.e., top or bottom) on the transformed data. A one-way ANOVA was performed on treatment effects, and differences between cooking treatments evaluated with Fisher's Least Significant Differences (LSD) test. The transformed data were also tested against the null hypothesis (H,) of no difference between cooked and uncooked samples by conducting two-tailed t tests.

The same approach was used to evaluate the effects of cooking on individual congeners. Cases in which a congener was less than the detection limit in the raw tissue were deleted from the data sets. When both the cooked and the raw fillet were below detection limits, the fractional change was recorded as 0%. The results presented in below include only those samples with quantifiable concentrations in the raw and cooked fillets. There were few cases where a congener was quantified in the raw fillet but was below detection in the cooked

fillet. These samples were initially excluded from analysis. For these samples, assuming that the fractional change was 100% would overestimate PCB reduction because a small change in congener concentration might have resulted in a less-than-detectable amount, but not complete removal. Congeners for which some cooked samples were below detection limits included Cl_2(08), Cl_3(18) Cl_4(44), Cl_9(206), and Cl_{10}(209).

RESULTS AND DISCUSSION

Deep-fried fillets showed approximately a 40% weight loss, apparently resulting from loss of water, while broiled and pan fried fillets showed a weight loss of approximately 15% and 7%, respectively. The moisture content for the uncooked fish ranged from 80% to 85%. The lipid content for the uncooked fish ranged from 0.8% to 5% with an average of 2%.

The 21 fish collected showed a large range in PCB concentrations. Total PCB concentration, based on the 17 congeners, ranged from 0.014 to 4.0 µg/g wet weight in the precooked samples. The PCB congener distributions were similar for most fish; Cl_5(118), Cl_6(153) and Cl_6(138) were the most abundant of the 17 congeners. The distribution of congeners in the fish looked more similar to the distribution in Aroclor 1254 alone, based on the relative amounts of PCB congeners by levels of chlorination.(Alford-Stevens, 1989)

The transformed data were analyzed by a three-way ANOVA for treatment, section, and fillet effects (i.e., top or bottom) on total PCB concentration. In this three-factor ANOVA, only the treatment effects were significant ($\alpha = 0.05$). A one-factor ANOVA of treatments indicated that the differences in cooking treatments between deep frying and either broiling or pan trying were significant. The individual cooking results were also tested with two-tailed t-tests to determine whether the fractional change resulting from cooking was statistically significant. The Total PCB levels decreased 47% when fillets were deep fried (Table 1). This decrease in PCB concentrations was found to be significant ($a = 0.05$). However, no statistically significant difference ($a = 0.05$) was observed in total PCB levels in broiled and pan fried fillets. Fisher's Least Significant Differences test indicated that the deep-frying treatment was significantly different from the pan-frying and broiling methods.

Individual congeners were also evaluated for the effects of cooking on congener levels (Table 2). The initial analysis excluded those cases for which congener concentrations were found below detection limit in the raw or cooked samples. significant fractional increases of approximately 25% in congener level ($\alpha = 0.05$). Increases in congener levels for congeners Cl_5(105), Cl_5(118) Cl_6(138) and Cl_9(206) were also significant for broiled samples ($\alpha = 0.05$).

These results indicated a significant decrease (47%) in total PCB levels following deep frying. In contrast, pan frying and broiling did not result in a statistically

Table 1. Fractional change in total PCB levels resulting from cooking

Cooking Method	Mean Fractional Change (SD)(percent change)	t-Test Probability
Broil	0.85 (SD=0.40) (+17%)	0.17
Deep Fry	1.6 (SD=0.48) (-48%)	<0.001
Pan Fry	0.46 (SD=0.46) (+15)	0.09

Table 2. Changes on specific congeners resulting from cooking

CONGENER	DEEP FRIED		PAN FRIED		BROILED	
	%	Probability	%	Probability	%	Probability
Cl_2(08)	74	<0.001	4	0.82	29	0.07
Cl_3(18)	57	<0.001	21	0.24	18	0.05
Cl_3(28)	47	<0.001	-5	0.65	-6	0.53
Cl_4(44)	50	<0.001	11	0.19	21	0.11
Cl_4(52)	53	<0.001	1	0.93	1	0.90
Cl_4(66)	42	<0.001	-13	0.27	-17	0.11
Cl_5(101)	51	<0.001	-6	0.50	-7	0.41
Cl_5(105)	45	<0.001	-25	0.03	-26	0.02
Cl_5(118)	43	<0.001	-24	0.04	-29	0.09
Cl_6(128)	59	<0.001	-5	0.61	-1	0.89
Cl_6(138)	46	<0.001	-20	0.08	-21	0.03
Cl_6(153)	47	<0.001	-13	0.23	-14	0.16
Cl_7(180)	57	<0.001	2	0.89	1	0.88
Cl_7(187)	58	<0.001	4	0.70	3	0.70
Cl_8(195)	54	<0.001	-6	0.62	-3	0.81
Cl_9(206)	68	<0.001	21	0.09	22	0.05
Cl_{10}(209)	51	0.01	10	0.45	-2	0.95

The t tests indicated a significant reduction of specific congener levels ranging from 42% to 74% for deep-fried fillets. Congeners Cl_2(08), Cl_3(18), Cl_4(44), Cl_9(206), and Cl_{10}(209) were found below detection limit in some deep-fried fillet samples. In pan-fried fillets, congeners Cl_5(105) and Cl_5(118) had the only

significant difference in total PCB. The factors that contribute to changes in PCB levels in cooked fillets are complex. This study was designed to prevent any bias from position of the fillets by sectioning them into three subsamples. However, this analysis was not able to demonstrate a relationship between lipid content in the three fillet sections and PCB levels. Other variables include total surface area of the fillet, thickness, of the fillet, and interactions of these variables with the cooking methods. Pan-fried fillets have less surface area exposed to air, which might reduce volatilization of PCBs compared with broiled fillets. The deep frying process creates unique cooking conditions that accelerate drying of the fillets compared with broiling and pan frying.

Zabik et al. (1982) suggested that extractability of PCBs was related to a high percentage of lipid in the tissue. Their early work with lake trout indicated significant reductions in total PCBs due to cooking when expressed on a mass basis. The lake trout had a high amount of lipid (20-30%) in the fillets. In contrast, carp used in their 1982 study had 7.7 ± 3.2% lipid. The winter flounder used in this study had lipid levels ranging from 0.8 to 4.5% (mean = 1.8%). While increased lipid content in fish flesh may be associated with greater PCB losses during cooking, there are also data to the contrary. Trotter et al. (1989) reported increases in PCB levels in bluefish (11.8% lipid) following cooking. Smith et al. (1973) reported decreases in PCB levels in salmon following cooking, where precooked lipid levels ranged from 2.7% to 3.6%. Moreover, most weight loss following cooking is due to losses in moisture, either by vaporization or in drippings. There generally is not a significant change in lipid levels on a gravimetric basis following cooking. Deep frying, however, increases the measurable amount of lipid in cooked tissue by absorption of the cooking oil.(Zabik 1982)

It should also be emphasized that the range in PCB concentrations encountered in winter flounder fillets was very large. This variability would result in some statistical error. Nevertheless, deep frying appears to significantly reduce PCB levels. This reduction may be explained by the evaporation of water and PCBs from the fillets resulting from the high temperature of the cooking oil. Another possible explanation to this reduction may be that the cooking oil itself could be acting as an extraction solvent. This is, however, unlikely due to the short period of time that the fillets are in contact with the oil.

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