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Effect of Preparation and Cooking on Contaminant Distributions in Crustaceans: PCBs in Blue Crab.

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EXECUTIVE SUMMARY

This study quantitated the changes in the distribution of PCBs in blue crab caused by boiling or steaming. Since blue crabs are cooked whole, there is the potential for residue transfer from highly contaminated organs such as the hepatopancreas during cooking. Thus crabs were boiled with and without the hepatopancreas. Since levels of PCBs from crustacean to crustacean may vary more than the effect of cooking, quantitating loss must be based on the best estimate of residues in an individual crab's tissues. The basic assumption os this study was that the concentration of PCBs in the right and left claw muscles would be the same.

The first study developed relationships between the total cooking losses of the crab and the cooking losses of the claw as well as the body muscle. These relationships had R^2 of >0.95. Using crab from a noncommercial area (Berrys Creek, a tributary of the Hackensack River, New Jersey), the relationship of the ppm PCBs in the body muscle to that of the claw was determined based on the analyses of 13 crabs. Duplicate body muscle samples showed excellent agreement and the recovery of the internal standard met quality assurance specifications. PCB patterns were found to best match a 1:1 ratio of Aroclor^R 1248/1254. PCBs were determined using capillary-column GC and quantitation was based on total peak area minus the area of the interfering DDE peak. The relationship determined had an R^2 value of 0.87. The mean level of PCBs in the claw was 0.227 ppm and in the body muscle 0.349 ppm. This is close to data reported from the same area although some of the high values were greater than had been previously reported for 5 samples.

Using crabs collected simultaneously, crabs were boiled from the frozen state with and without the hepatopanceas. Crabs from the same location were obtained approximately 6 months later to study the effect of steaming. The hepatopancreas was removed from these crabs. Cooking per se reduced the PCBs by more than 20%. There was no significant differences in the cooking losses from the claw for any of the procedures with losses ranging from 23.3 to 25.4%. Removing the hepatopancreas significantly increased the PCB loss from the body muscle of boiled crab (36.4% loss for crabs boiled with the hepatopancreas removed versus 31.0% for crabs boiled with the hepatopancreas intact). Boiling crab resulted in significantly greater PCB losses from the body muscle than steaming when the hepatopancreas was removed in both procedures (36.4 versus 33.9%).

About 80% of the PCBs lost from boiled crab was recovered in the cooking medium. Thus continuous reuse of boiling water or the use of the boiling liquid for stock could increase the amount of PCBs ingested.

INTRODUCTION/ PROBLEM STATEMENT

Polychlorinated biphenyls (PCBS) are widespread, stable, environmental contaminants that pose a threat to humans due to possible biological harmful effects. While there is no clear cut evidence that low levels of PCBs in humans causes specific health effects, results from enzyme induction and animal toxicity studies indicate that exposure to PCBs should be minimized (1).

Because of the resistance of these neutral organic chemicals to chemical and biological breakdown, they enter the food chain, accumulating especially in the lipid of organisms, concentrating as they pass from one trophic level to the next. Aquatic environments, in particular enclosed lakes, are particularly susceptible to this because of the airborne movement of PCBs and deposition into water. Highly industrialized, highly populated coastal states often have significant levels of chemical pollutants in the sediments of their bays and coastal waters, which are important commercial sources of crustaceans such as blue crabs.

Many states have issued advisories concerning consumption of certain species of fish and shellfish to limit human exposure to potential harmful effects from consumption of PCBs. Most people consume seafood from a wide range of locations and therefore the potential danger from consuming one contaminant in harmful levels is reduced. The sport or commercial fishermen and their families who consume large amounts of fish or shellfish from one location are at a greater risk than the general population, especially if this location is one with significant contamination.

The biological assimilation of PCBs and general values of PCBs levels in crab have been reported by several researchers. Young (2) summarized levels of PCBs in crab to be 0.19 mg/kg (1254) in organisms taken off Palos Verdes Peninsula, CA. Comprehensive monitoring of PCB levels found in the inner New York Bight were shown by Reid et al. (3) to have 0.09-0.11 ppm in rock crab harvested off the Northern New Jersey coastal area. PCB levels found were not consistently related to the levels in sediments or to areas of contamination input. More recently, Wenner (4) reported that sand crabs collected from Santa Monica Bay, Oceanside and San Clemente, CA sites had levels of 0.03 to 0.38 ppm PCBs. Rock crab caught off the Northeastern U.S. coast were found by Farrington and Boehm (5) to have 0.043 0.15 ppm PCBs. Hauge et al. (6) reported crabs from the Northeastern waters of New Jersey to have mean PCB levels of 0.33 ppm in muscle (maximum muscle PCBs were 0.40 ppm, N=5) and 5.38 ppm in the hepatopancreas (maximum hepatopancreas PCBs were 8.27 ppm, N=5). Based on 18 crabs, these same authors reported PCBs in combined muscle and hepatopancreas to average 1.84 ppm (maximum PCBs were 3.86 ppm).

Preparation and cooking techniques have resulted in loss of PCBs and other organic toxicants from certain species of fish (7-10). In a study by Zabik (11) which evaluated PCB levels in chicken, stewing or pressure cooking chicken pieces reduced PCBs by 50 to 70%, while half of the organochlorine residue transferred to the cooking media. This may result in residue contaminating foods such as rice or pasta cooked in this type of broth (12).

States such as New Jersey use decisions concerning the issuance of advisories and other measures to protect consumers from potentially contaminated fish and shell fish based on contaminant levels found in raw samples. As a result of the studies which have indicated contaminant level reductions through preparation and or cooking techniques, the state of New Jersey's Department of Environmental Protection (NJDEPE), in conjunction with Michigan State University, decided to investigate cooking's potential to reduce PCB level in blue crab. Sound technical information obtained from this study could then be used to help the NJDEPE assess the human health risk of exposure to the specific pollutant, PCB's and reevaluate their basis for issuance of consumer advisories. It is also possible that if lower levels of contaminants in the edible portion of blue crab were found, the perceived risk of the public consuming blue crab would be lessened, thus restoring consumer confidence and benefiting recreational and commercial fishermen.

Thus, the primary purpose of this study was to quantitate the distribution of PCBs in raw blue crab claw and body muscle and to quantitate changes in PCBs during boiling or steaming. Since blue crabs are cooked whole, the potential for residue transfer from highly contaminated organs such as the hepatopancreas (14) to muscle exists. The second objective of this study was to make a comparison of PCBs in the claw and body muscle from crabs boiled with and without the hepatopancreas removed. It was hoped that based upon data generated from this study, guidelines to consumers for preparation/cooking of blue crab to maximize PCB reduction could be developed.

PROJECT DESIGN AND METHODS

Crustaceans pose a particular challenge for quantitation of residue reduction because they are typically cooked whole from the live state. Since individual variation from crustacean to crustacean may be greater than the residue loss or transfer during cooking, quantitating loss must be based on the best estimate of residues in the individual crab's tissues. Some alterations in the typical cooking method thus were made in order to obtain the necessary cooking and PCB loss data. For the basic study design, the left claw of the blue crab was removed prior to cooking and was used to determine raw muscle cooking loss and PCB data. The remainder of the crab was cooked and the right claw muscle and the muscle from the body cavity were used to obtain data for losses due to changes in weight and PCB level. In this study, the term whole crab will refer to the crabs which were left whole with the exception of removal of the left claw unless stated otherwise.

Patterns of Weight Loss Due to Cooking

The initial phase of this study involved determination of cooking weight loss patterns. Two dozen male blue crabs of similar weight (183-228 g) were obtained from a commercial fishery in Boston, Massachusetts and shipped live with cool packs via overnight delivery. While blue crabs are typically cooked whole from the live state, it was not practical to work with live whole crabs for this study. In order to provide information to compare cooking losses for fresh and frozen blue crab, three live blue crabs were anesthetized with chloroform, and weighed, and the left claw was removed. The crabs were reweighed and cooked for 18 minutes/lb in 6 liters of deionized boiling water. Each crab was cooled in ice water for 10 minutes, drained for five minutes and reweighed.

Twelve blue crabs were frozen from the live state in a blast freezer (-20°C). These crabs were paired according to weight and assigned random numbers. In each pair one crab (A) was used to obtain raw tissue data and the other (B) was used to obtain the cooked crab data for the following: whole body total weight loss, hepatopancreas, claw muscle, body muscle and a composite of gills, heart and stomach. In the raw crabs (crab A in each pair), the left claw was removed from one of each pair and the remainder of the crab was thawed in a Kenmore microwave (Model 567.882,1480, 900 watts, 2450 MHZ) for 80 seconds in order to facilitate the shell removal. The crab was cut open along the top edge of the upper shell and the hepatopancreas located in this area was removed (Figure 1). The hepatopancreas has a distinctive dark yellow color but lacks a clear shape in the slightly thawed crab that makes it difficult to completely separate it from the crab. Highly active enzymes and tissue

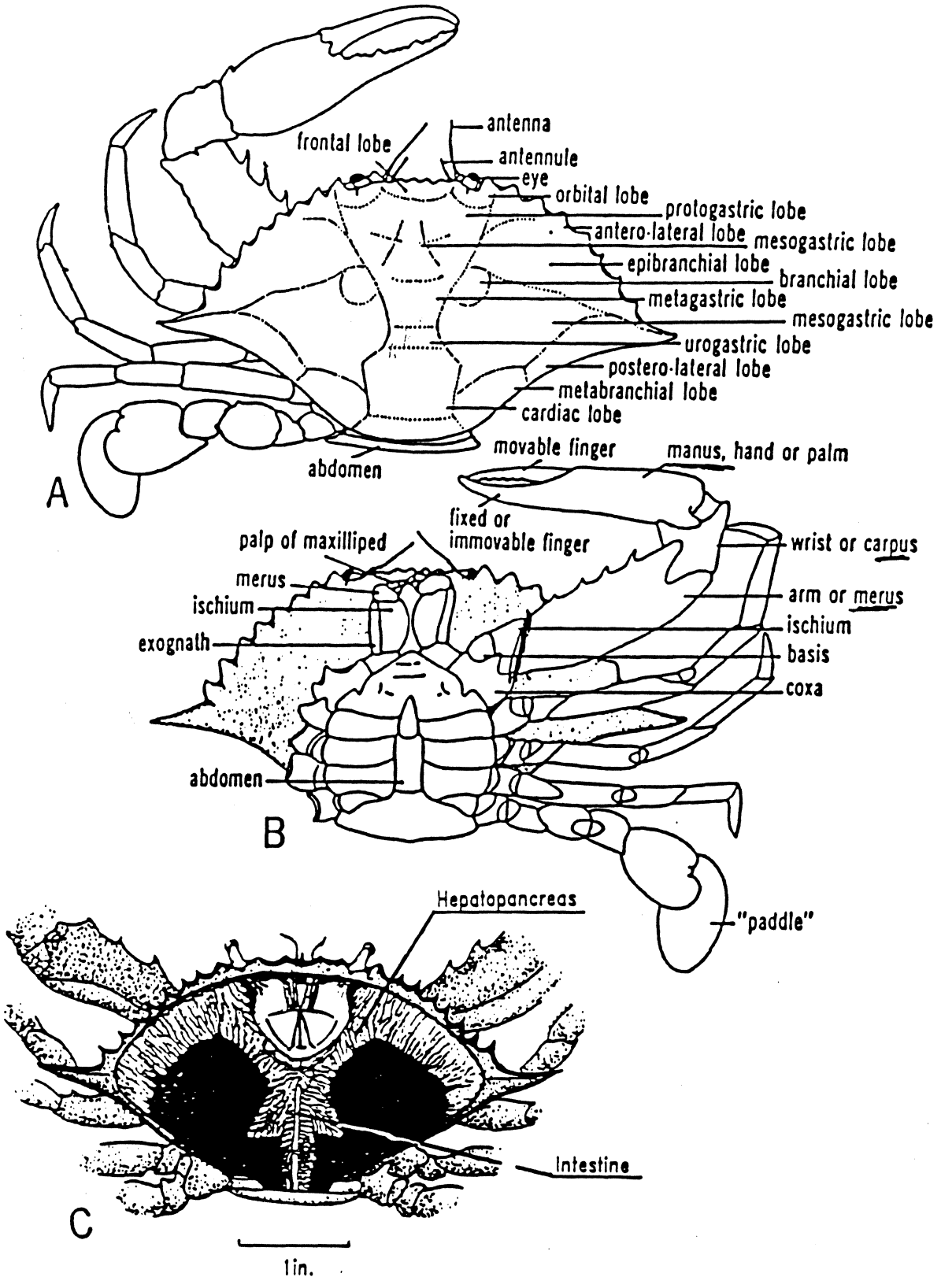

Figure 1. Diagram of Blue Crab. A & B are exterior top & bottom, C interior showing Hepatopancreas.

damage due to freezing are most likely responsible for liquification of this organ. The visible hepatopancreas was removed and then the stomach, heart and gills were removed, and combined. The crab was then turned over and cut up the middle and the remaining visible hepatopancreas was removed. The body muscle was then taken out of the crab body cavity. Finally, the whole right and left claws were weighed and the muscle from the each of the claws was removed.

The remaining crab from each pair (crab B) which was to be cooked was weighed, the left claw was removed and the rest of the crab reweighed. Since additional time was required to cook the crab from the frozen state, these crabs were cooked 30 minutes/lb in six liters deionized boiling water. The crab was then cooled on ice for 10 minutes, drained five minutes and weighed. It was then opened, wrapped in gauze, drained an additional five minutes. and reweighed. The most consistent total cooking loss data was obtained using the weight from the second draining period to calculate results. It appeared that the hepatopancreas was lost into the boiling water since very little of it could be seen remaining in the cooked crab. However, it is possible that some of the "disintegrated" hepatopancreas transfered to other portions of the crab. Percentage changes in weight with cooking were calculated for all six crab pairs and wide variation in sample values resulted. It was thought that in the raw crab, frozen body fluid was associated with the tissue from the different composites which may have resulted in some of the wide variations. It was decided to repeat the study to try to improve the data reproducibility.

Seven more blue crab pairs were shipped live to MSU and then frozen as previously described. Mean weight for these crabs used raw and for cooking studies was 211.88 g (range 175.5-248.1 g). However, the procedure was altered to allow all the raw tissue to thaw at room temperature before being excised and combined. This improved the reproducibility of the total cooking loss data. Nevertheless, the total percentage loss was close to that of the first study. These cooking loss values were then used to develop relationships to estimate the cooking losses for the right claw and body muscle, based upon the total cooking losses which could be calculated for each crab analyzed for the PCB cooking loss study.

Development of PCB Ratios

The PCB ratio study and PCB cooking loss study was initiated by obtaining, forty male blue crabs from the New Jersey Department of Environmental Protection (NJDEPE),which collected the crabs in an area in northern New Jersey where no commercial crabbing takes place (Berrys Creeks, a tributary to the Hackensack River). Immediately upon collection, the crabs were

frozen and shipped on dry iced via overnight delivery. The blue crabs were stored at -25°C until needed. These samples were smaller than the blue crab obtained commercially for the cooking loss study. Mean weight was 167.5 g with a range of 110.2 to 246.5. Weights of claw muscle were as low as 2 grams in some small samples which was less that half the weight of the claw muscle obtained from the commercially obtained blue crab for the cooking weight loss study.

In order for the left claw muscle to be used to estimate the raw PCBs in the right claw muscle and the body muscle, a PCB ratio study (Body Muscle/Left Claw) was conducted. It was assumed that the raw left claw muscle PCB level could be used to estimate that of the right raw claw muscle. The ratio of PCBs in the body muscle to the left claw muscle were then determined to obtain a relationship equation to estimate the raw body muscle PCB values for the PCB cooking study.

Boiling Whole Crab with the Hepatopancreas Retained

Eight frozen blue crab were boiled whole after the raw left claw was removed and retained determination of PCBs in the raw muscle. The weight of the frozen, whole blue crab was recorded before and after the left claw was removed. The crab was cooked for 30 minutes/lb based on the weight without the left claw. Width of the crab from point to point was recorded and the crab was placed in rapidly boiling deionized water (6 liters). The crab was cooled 10 minutes in an ice/water slurry and drained for 5 minutes on a wire rack and paper towels. By gently rocking the crab back forth on the paper towels, any extra water in the shell was removed. The crab was then weighed and the dorsal (top) portion of the shell was then cut open along the outside periphery. The crab was covered with gauze and inverted to drain on a wire rack for 5 more minutes. This crab weight was the value used to determine cooking losses. The whole raw left claw and the excised left claw muscle were weighed and the muscle was ground in an Omnimixer for 30 seconds, placed into a glass jar and frozen at -20°C until analyzed for PCBs. The small amount of remaining hepatopancreas located on the top of the cooked crab was removed and saved. The gills, heart and stomach were then removed, weighed and frozen in foil (composite). The ventral exterior (bottom shell) of the crab was then cut down the middle, starting between the eyes, and the remaining hepatopancreas was removed, combined and weighed with the upper portion of the hepatopancreas. This combined portion was wrapped in aluminum foil and frozen at -25°C. The body muscle was then removed, weighed, ground and frozen as previously described. Finally, the right claw was weighed and the muscle was excised, weighed and ground in the same manner as the body muscle and left claw muscle. Total PCBs in the remaining cooking water were determined after analysis of the crab tissue was completed. For

this, approximately 100 ml of cooking water was frozen at -25°C in glass jars and stored until analyzed.

Boiling Crab with the Hepatopancreas Removed

The hepatopancreases of eight frozen blue crabs were removed prior to boiling for this portion of the study. The weight of the frozen, whole blue crab was recorded before and after the left claw was removed. The crab was then thawed in a Kenmore Microwave (Model 88213) for 80 seconds. The upper shell of the thawed crab was cut open as previously described for the cooked crab and the upper portion of the hepatopancreas was removed. The lower shell was then cut down the middle starting at the mouth (between the eyes) and the crab was allowed to thaw enough to effectively remove the bottom hepatopancreas. The crab was weighed and the cook time was calculated (30 minutes/lb). The crab with the hepatopancreas removed was wrapped in gauze to insure the crab would remain intact during boiling. It was then cooked and cooled in the same manner as the whole crab. Draining time was reduced to 5 minutes since the crab shell was already cut open. The raw left claw, body muscle, right claw muscle and cooking media were treated as previously described.

Steaming Crab with the Hepatopancreas Removed

Additional blue crabs were obtained by the NJDEPE as previously described and eight were cooked by steaming. The procedure used to prepare the crab for the boiling study in which the crab hepatopancreas was removed was the same as that used for steaming the blue crab. A slight variation in the procedure included bringing 2 liters deionized water to a rolling boil and placing the prepared blue crab on a wire rack suspended a few centimeters above the water level.

PCB Analysis

Extraction and cleanup of samples for PCB analysis was performed using the procedure outlined by Ribick et al. (13) as modified by Giesy et al. (14). An internal standard, 0.50 µg of 2,4,6-trichlorobiphenyl (#30), was added to a 2 to 10 g sample, depending upon the available muscle sample size. In order to dry the muscle, an amount of anhydrous Na_2SO_4 (stored at 130°C) equal to four times the sample weight was mixed into the sample. The resultant dry powder was extracted with 200 ml of dichloromethane at a flow of 3 to 5 ml/minute in a 2 cm i.d. glass column. The volume of solvent was reduced under a vacuum with a Model 110 Rotovapor at 31°C and the volume was adjusted to 8.0 ml with 1:1 (v/v) mixture of hexane and dichloromethane.

Lipids were removed from the extract by gel permeation chromatography (GPC). Five ml of the extract was loaded

quantitatively onto an automated GPC apparatus equipped with a column packed with 60 g of SX-3 Bio-Beads. The 1:1 hexane and dichloromethane mobile phase was pumped through the column at 5 ml/minute. A 140 ml fraction was collected after a 170 ml dump cycle. The volume of solvent of the collected fraction was reduced using a Zymark TurboVap evaporator at 31°C. The sample was quantitatively transferred with hexane to an acid/silica gel column. This 1.0 cm column was prepared with 1.0 g Na_2SO_4, 5.0 g of silica gel, 1 g of H_2SO_4 (40% w/w basis) treated silica gel, and 1 g of Na_2SO_4. After the column was rinsed with 20 ml hexane, the sample was quantitatively transferred in hexane to the column. The sample collection was begun after the five ml of 0.5% toluene in hexane was added to the column. Forty five additional ml of the toluene/hexane solvent were added to elute the sample. To the eluant, 0.5 ml of isooctane was added and its volume was then reduced. The sample was quantitatively transferred to 2 ml sample vials with volume reduction via a gentle stream of purified nitrogen after each transfer and rinse. The final volume was 0.5 ml.

Concentrations of total PCBs were determined by gas chromatography (GC) with electron capture detection (GC-ECD). A Perkin-Elmer, model 8500, equipped with a ^{63}Ni detector operated at 340°C and a split injector at 235°C. A DB-5 column, 30 m X 0.24 mm i.d., with 0.25 µm film thickness was used with helium at 20 psig as the carrier gas. The Perkin-Elmer oven was held at 215°C for a 25 minute run time.

All quantitation was based on peak areas relative to the area of the internal standard, congener #30. The coeluting *p,p'*-DDE peak was omitted from all calculations. Area integration was performed by Perkin-Elmer software. The concentration of total PCBs were determine by summing the concentrations of all of the individual peaks detected and corrected for recovery of the internal standard. Preliminary analysis of crab samples indicated that the Aroclors[R] 1248 and 1254 were the dominant PCB sources in the blue crab samples (Figure 2).

The research protocol called for the determining PCBs in the claw, body muscle, and broth of the boiling study only. A duplicate body muscle sample and procedural blanks were run for every third crab extracted (approximately 10% of total samples). Duplicates of the claw muscle were not done because of the consistently small sample size. PCBs in the raw body muscle of the cooked crab were estimated from the PCBs in the claw using the ratio of PCBs in the raw body muscle/claw relationship. PCBs were expressed as ppm wet muscle, ppm dry weight as well as micrograms in the raw muscle, cooked muscle and broth (boiling study). The latter data were used to calculate percentage losses.

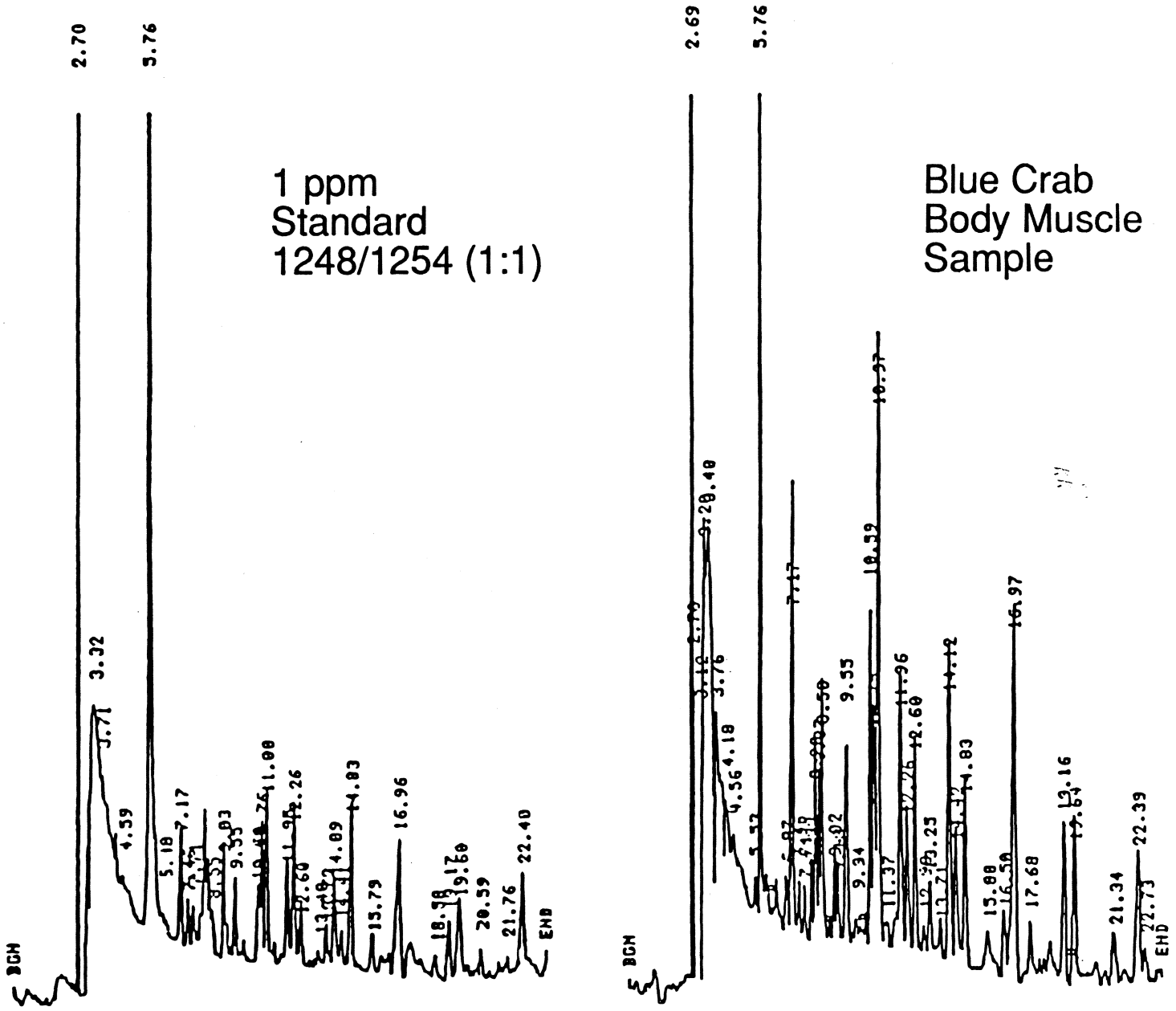

Figure 2. Sample gas chromatographs of standard and body muscle.

Data Calculations, Assumptions and Equations

PCB levels were calculated from total area minus the area of the DDE peak using the linear equation of the PCB standards and were corrected for recovery of the internal standard (2,4,6-trichlorobiphenyl #30). The PCB congener pattern from the crab best correlated with a 1:1 ratio of Aroclor 1248 and 1254 (Figure 2). PCBs are expressed as ppm wet weight unless otherwise noted.

estimated ppm PCBs raw right claw = ppm PCBs raw left claw (1)

estimated ppm PCBs raw body muscle = ppm PCBs raw claw x the relationship of ppm PCBs raw body muscle to ppm PCBs raw claw (2)

estimated raw claw weight (g) = cooked claw weight (g) times the relationship of claw cooking loss to total cooking loss (3)

estimated raw body muscle weight (g) = cooked body muscle weight times the relationship of body muscle cookingloss to total cooking loss (4)

µg PCBs raw claw = ppm PCBs raw claw times estimated raw claw weight (5)

µg PCBs cooked claw = ppm PCBs cooked claw times cooked claw weight (6)

µg PCBs raw body muscle = estimated ppm PCBs raw body muscle times estimated raw body muscle weight (7)

µg PCBs cooked body muscle = ppm PCBs cooked body muscle times cooked body muscle weight (8)

µg PCBs lost = (µg PCBs raw body muscle + 2(µg PCBs raw claw)) - (µg PCBS body muscle + PCBs cooked claw)) (9)

µg PCB broth = (ppm PCBs broth x weight of broth) (10)

The basic assumption was that the PCBs in the raw claws would be the same so that the PCBs which were analyzed for the raw left claw could be used to represent the PCBs in the raw right claw (Equation 1). PCBs in the raw body muscle were based on the ratio of PCBs analyzed using crab from the same location as those used in the cooking studies according to equation 2. Raw claw and body muscle weights were calculated from the relationships of cooking losses in the crab muscles to the total

crab cooking loss (Equations 3, 4). To calculate PCB loss during cooking, µg of PCBs were calculated for the raw and cooked claw and body muscle, respectively (Equations 4-8). Values for whole crab consist of the PCBs in the body muscle and in two claws. The total µg of PCBs lost were calculated using Equation 9 while the µg of PCBs in the broth was calculated for the crabs which were boiled (Equation 10).

Statistics

Statistics used were the Analysis of Variance and Tukey's Honestly Significant Difference Test (15). The relationships between total cooking losses and cooking losses of the body muscle and claw as well as the relationship of the ppm PCBs in the body muscle to ppm PCBs in the claw were determined using PlotIT (16).

Quality Assurance

Sampling procedures were carefully followed. Refrigerators and freezers were found to keep temperatures of 3-4° C and -24 to -26° C, respectively, The instruments used to clean up samples and determine PCBs remained in good working order throughout the study.

Data Quality: The detection limit was determined to be 0.01 ppm of a 1:1 mixture of 1248/1254 by analyzing the mixture as compared with hexane using five replications. The mean of the peak height generated by the 0.01 ppm standard was greater than 2.5 times that of the noise. The quantitation limit was set at five times the detection limit. All blanks contained levels of PCBs less than the method detection limit and thus were considered acceptable. The R^2 value for standard curves which were run with every set of gas chromatograph runs was always greater than 0.99. PCB standards included 0.01, 0.1, 1 and 10 ppm Aroclor^R 1248/1254 (1:1 mixture). PCB quantitation was based on peak area.

A 50µg sample of 2,4,6-trichlorobiphenyl (#30) was added to each sample as an internal standard. Recoveries of the internal standard were as follows:

Ratio study -- 85.77 ± 5.79%
Boiled crab -- 77.63 ± 5.54%
Steamed crab -- 79.40 ± 3.16%.

Precision of analysis was determined by running 10% of the crab body muscle samples in duplicate. No claw samples were run in duplicate due to the small sample size. Variability in the duplicate samples based on the lower ppm in each pair averaged 2.01% with a standard deviation of 1.12(range= 0.76%-4.35% N=9).

RESULTS AND DISCUSSION

Establishment of Patterns of Weight Loss Due to Cooking Among Individual Tissues of Blue Crab

When a comparison between cooking losses resulting from cooking the blue crab from the live and frozen state was made (Table 1), a slight percent gain in weight resulted with a mean and standard deviation of 1.12 and 0.19, respectively. Cooking crab from the frozen state showed a consistent cooking loss rather than gain (8.70±5.01%). The freezing process disrupts tissues, which could account for additional losses. It was found in subsequent studies that additional weight loss could have occurred in the crab cooked if the crab shells were opened and drained for five minutes before a final weight was recorded. This would have allowed any cooking water which was trapped in the shell to drain. This additional draining step was used to obtain all blue crab total cooking loss data in the study.

Table 1. Comparison of cooking weight loss in blue crab boiled from the live and frozen state.

	Live crab percent weight change (gain)	Frozen crab percent weight change (loss)
	+ 1.15	- 9.55
	+ 0.92	- 6.78
	+ 1.29	- 4.17
		-10.38
		-17.36
		- 3.94
Mean, Std. Dev.	+ 1.12 ± 0.19	- 8.70 ± 5.01

Mean weight for seven blue crab pairs used to determine cooking losses was 211.88 g (range 175.5-248.1 g). The weight of the left and right claws of a single blue crab, and the left or right claws of blue crabs paired by weight and size, differed enough to result in large variations for claw cooking loss data. The data in Table 2 shows variation of 21-47% when the right claws of the raw and cooked crabs within a pair are compared. The ability of blue crabs to regenerate lost claws results in crabs which may have one very tiny claw that is in the process of growing and one full size claw. This could account for these

large claw size variations.

A large weight loss of the hepatopancreas (86.36± 4.74%) resulted from boiling the crab. Partially thawing the crab in order to remove the hepatopancreas, showed that the hepatopancreas was less intact and of a more liquid consistency than that of the anesthetized dissected crab. The hepatopancreas also contains highly active enzymes that could have resulted in self digestion of this organ. The lack of integrity of the hepatopancreas would help explain why the hepatopancreas was so easily lost during the boiling process.

Cooking weight losses of the body muscle were 24.44± 7.51% and were similar to total losses (25.05± 4.39). The higher total weight loss found in this portion of the study compared to the fresh/frozen study (Table 1) was due to recording weights after the additional 5 minute drain period after the crab shell was opened. The cooking weight loss of total crab and body muscle as well as claw cooking weight loss results presented in Table 2 were used to determine the following relationships (16):

% Total Cooking Loss = 0.976377 times % Body Muscle Cooking Losses R^2 = 0.955 (11)

% Total Cooking Loss = 0.620428 times % Claw Cooking Losses R^2 = 0.951 (12)

Table 2. Percent cooking weight losses in whole body, hepatopancreas (H), claws, and body muscle (BM) between pairs of blue crabs of similar weight.[1]

Pairs	Claw	H	BM	Total Loss
1	21.42	83.16	15.31	16.22
2	32.02	88.02	27.39	25.01
3	55.30	88.12	34.94	27.39
4	23.66	81.31	23.95	23.95
5	47.27	84.44	14.40	26.25
6	39.80	95.47	30.34	30.34
7	47.21	83.87	24.72	26.25
Mean	38.10	86.36	24.44	25.06
S.D.	±12.84	±4.74	±7.51	±4.39

[1]Frozen crabs allowed to thaw before preparation.

Development of PCB Ratios

The size of the crabs used to determine the ratio of PCBs in the raw tissues averaged 14.0 cm with a range of 12.2 to 16.3 cm. Mean weight was 167.5 g with a range of 110.2 to 246.5. Mean solids of the claw and body muscle were quite similar (Table 3). Recoveries of the internal standard in the PCB ratio study averaged 85.77% with a range of 73.20 to 97.22%. The ppm expressed on either a wet or dry weight basis showed expected biological variability (Table 4). Yet the ratio of PCBs in body muscle to PCBS in the claw muscle was much more consistent. The average ratio for PCBs expressed on a wet weight basis was 1.54 ± 0.30 and on a dry weight basis was 1.57 ± 0.35. This points out that the body muscle consistently carries a greater PCB burden than the claw muscle. These values were used to calculate the following relationship for PCBs expressed as ppm wet weight basis in the body muscle to that in the claw (16) ($R^2=0.866$):

ppm PCBs body muscle = 1.604 times ppm PCBs claw (13)

Table 3. Weights and percent solids of crabs used in ratio study.

Crab Number	Crab Size	Crab Weight[1]	Left Claw Muscle Weight	Body Muscle Weight	Left Claw Solids	Body Muscle Solids
	cm	g	g	g	%	%
1	14.8	177.3	9.06	13.46	18.66	17.56
2	14.8	207.1	11.70	43.00	21.69	20.41
3	14.0	156.2	17.88	20.00	14.63	15.74
4	14.0	136.9	6.82	20.57	12.54	14.23
5	14.2	200.6	8.94	27.10	17.12	17.61
6	15.7	241.7	11.72	36.00	19.86	18.46
7	14.8	220.7	11.76	30.16	21.09	19.57
8	12.8	136.0	6.01	12.98	23.93	20.92
9	16.3	246.5	12.09	41.97	18.22	18.93
10	14.4	174.5	7.77	20.58	20.39	18.29
11	12.9	136.8	6.49	17.85	22.53	22.54
12	13.0	127.0	4.34	14.37	22.49	19.06
13	12.3	120.8	5.16	11.90	20.92	23.45
14	14.0	140.3	4.19	23.13	18.02	19.22
15	12.2	110.2	5.11	16.25	19.08	23.30
Mean	14.0	167.5	9.45	23.29	19.41	19.29
S.D.	± 1.2	± 43.5	± 4.37	± 10.27	± 3.05	± 2.59

[1]Left claw was not removed for this weight.

Table 4. PCB content in raw left claw (LC) and body muscle (BM) of blue crabs used for the ratio study (BM/LC).

Crabs	Tissue	PPM wet wt.	PPM dry wt.	Ratio wet wt.	Ratio dry wt.
1	BM	0.192	0.941	1.78	1.89
	BM	0.184	0.903	1.71	1.82
	LC	0.108	0.497		
2	BM	0.280	1.776	1.33	1.24
	LC	0.210	1.432		
3	BM	0.237	1.669	1.44	1.27
	LC	0.165	1.315		
4	BM	0.145	0.743	1.96	2.11
	LC	0.074	0.352		
5	BM	0.319	1.526	1.34	1.53
	LC	0.239	0.997		
6	BM	0.445	2.350	1.88	1.80
	LC	0.237	1.302		
7	BM	0.129	0.708	1.28	1.43
	LC	0.101	0.495		
8	BM	0.617	3.341	2.02	2.17
	LC	0.306	1.538		
9	BM	0.247	1.411	1.42	1.55
	LC	0.174	0.909		
10	BM	0.223	1.169	1.45	1.71
	LC	0.154	0.685		
11	BM	0.367	1.565	1.22	1.09
	LC	0.302	1.441		
12	BM	0.379	1.973	1.22	1.14
	BM	0.370	1.927	1.19	1.11
	LC	0.312	1.730		
13	BM	1.095	4.911	1.92	1.64
	LC	0.570	2.986		
AVG BM PCBs		0.349±0.24	1.794±1.10		
AVG LC PCBs		0.227±0.13	1.206±0.69		
AVG Wet Wt Ratio				1.54±0.30	
AVG Dry Wt Ratio					1.57±0.35

Cooking Studies -Levels of PCBs in Blue Crab Boiled Whole, and Boiled or Steamed with the Hepatopancreas Removed Prior to Cooking

While the blue crabs which were cooked whole (left claw off) in the boiling study had greater average weights and sizes than the two groups of crabs used for the studies in which crabs were boiled or steamed (left claw off) with the hepatopancreas removed, values did not differ by more than one standard deviation (Table 5). The large standard deviations found for the crab weights are typical of those found for biological specimens.

Table 5. Comparison of weights[3] and sizes of blue crab used in the boiling and steaming cooking studies, with and without the hepatopancreas (H).

	Boiled Whole[1]	Boiled- H Out[1]	Steamed ~~Simmered~~-H Out[2]
Weight (g)	159.90	142.40	138.19
Std. Dev.	22.31	39.45	32.51
N	9	8	10
Size (cm)	13.6	13.1	13.1
Std. Dev.	0.9	1.2	1.3
N	7	8	10

[1]Crabs collected Fall, 1990, [2]Crabs collected Spring, 1991,
[3]These weights are for wholes, not crabs with the left claw removed.

For all three cooking studies, the left claw of each crab was removed and the left claw muscle analyzed for PCBs. These results were used to estimate PCB levels in raw tissues from the right claw by assuming that the PCB levels are equal to that of the left claw Equations (1-2). Also, the PCB levels in the raw body muscle were estimated by inserting the relationship in Equation 13 into Equation 2. Raw tissue weights for the right claw and body muscle were estimated from the cooked tissue weights (Equations 3-4), using the cooking weight loss factors developed from Equations 11 and 12.

The results of the PCB analyses for the raw left claw, the cooked right claw and the cooked body muscle are given in Table 6. These values are expressed as ppm of wet tissue. The results of each of the PCB analyses is given in the Appendix. Since PCBs

Table 6. Effect of cooking on PCB levels in the claw and body muscle of blue crab whole.

		PCBs				
		ppm wet tissue		µg per muscle		PCBs µg % Loss[1,2]
Muscle Tissue	N	Raw	Cooked	Raw	Cooked	
Boiled with Hepatopancreas						
Claw	8	0.192 ±0.065	0.222 ±0.065	1.70[3] ±0.70	1.26[4] ±0.45	24.98a ± 5.68
Body Muscle	8	0.307[5] ±0.104	0.278 ±0.098	7.10[6] ±4.00	4.87[7] ±2.63	31.02b ± 2.68
Whole (2 x Claw) + Body Muscle	8					29.03e ± 2.97
Boiled without Hepatopancreas						
Claw	7	0.168 ±0.074	0.187 ±0.080	1.26 ±0.49	0.94 ±0.38	25.38a ± 2.07
Body Muscle	7	0.269 ±0.119	0.218 ±0.090	6.13 ±2.95	3.91 ±1.89	36.38c ± 2.07
Whole	7					33.10f ± 1.78
Steamed without Hepatopancreas						
Claw	10	0.270 ±0.039	0.260 ±0.044	1.27 ±0.23	0.98 ±0.18	23.25a ± 1.64
Body Muscle	10	0.434 ±0.063	0.336 ±0.060	5.89 ±0.88	3.89 ±0.60	33.88d ± 3.10
Whole						30.63e ± 2.20

[1]Averages with different letters are significantly different, $p<0.05$ for comparision of cooking methods for a single tissue, i.e., claw, body muscle or whole (15).
[2]Values for whole crab calculated using Equation 9.
[3]Calculated using Equation 5(using 1,3,12).
[4]Calculated using Equation 6.
[5]Calculated using Equation 2 (using 13).
[6]Calculated using Equation 7 (using 2,4,11,13).
[7]Calculated using Equation 8.

are often expressed as ppm of solids, the solids content of each of the tissues analyzed for PCBs and the ppm PCBs expressed on a solids basis also are included in the Appendix.

The estimated value for the ppm of PCBs in the raw body muscle calculated using Equations 2 and 13 are also given in Table 6. These values ranged from an average of 0.269 ppm for the crabs that were boiled with the hepatopancreas to 0.434 for the crabs that were steamed without the hepatopancreas. These are similar to the ppm PCBs found when the body muscle of blue crabs harvested from the same area at the same time (boiling studies) or from the same area harvested at a later time (steaming study) were analyzed (Table 4). Hauge et al (6) had reported that in 1986-87 the body muscle of blue crabs had an average of 0.33 ppm with the highest value in the 5 samples analyzed being 0.40 ppm. The average ppm in the raw body muscle of the crabs which were boiled either with or without the hepatopancreas was estimated to be slightly less than this value, i.e., 0.31 or 0.27 ppm, respectively. For these crabs the raw claws, had been analyzed to contain mean PCB values of 0.19 and 0.17 ppm, respectively. The raw claw PCB values from the second lot of crab obtained from New Jersey had a mean of 0.27 ppm so tha estimated PCB value of the raw body muscle was 0.43 ppm which is higher than the highest value reported by Hauge et al(6). Three of the 13 crabs analyzed for the ratio study (Table 4) also had analyzed PCB values for body muscle greater than the maximum values reported earlier (6) even though the mean value for ppm PCBs in the body muscle was similar.

During the boiling process, 86% of the hepatopancreas is lost when the crab is boiled whole (Table 2). High levels of PCBs in the hepatopancreas of the blue crab have been found (6) which is more than an order of magnitude higher than levels in crab muscle for crabs from northeastern New Jersey. The ppm PCBs in the cooked body muscle of the crab boiled with the hepatopancreas was slightly greater than the ppm PCBs in the cooked body muscle of crabs boiled after the hepatopancreas had been removed. Therefore it is possible that during boiling a crab with the hepatopancreas intact, some of the PCBs are redistributed throughout the body muscle in addition to any loss into the cooking medium.

To calculate loss of PCBs during cooking by boiling with or without the hepatopancreas or steaming without the hepatopancreas, micrograms of PCBS in each raw and cooked tissue were calculated using Equations 5-8. These values are included in Table 6. The %PCBs loss in the whole crab was based on the change in micrograms of PCBs in two claws plus that of the body muscle which was calculated using Equation 9 (Table 6). Calculating PCB losses based on micrograms in the raw and cooked sample is more accurate than just comparing the level of PCBs

expressed as ppm wet or dry tissue since cooking losses and particullarly, losses of moisture and fat are not consistant for various cooking methods.

Analyses of variance for the effect of cooking method on the loss of PCBs from the claw, from the body muscle, and from the whole crab established significant differences for losses from the body muscle and whole crab only. Losses for the claw ranged from an average of 23.25% for the crabs that were steamed without the hepatopancreas to 25.38% for the crabs that were boiled without the hepatopancreas. This shows that more than 1/5 of the PCBs in the raw crab were lost by all cooking methods.

Crabs boiled with the hepatopancreas intact lost 31.0% of the PCBs in the body muscle. Removing the hepatopancreas before cooking resulted in significantly greater cooking losses (Table 6) with boiling producing significantly greater ($p<0.05$) losses (36.4%) than steaming (33.9%). The same significant differences were found for the comparison of the PCB losses from the whole crab although the percentages lost were slightly lower than for the body muscle. This could be due to the significantly ($p<0.05$) higher cooking losses from crabs which were boiled as compared to those which were steamed (Table 7). Nevertheless, reduced the PCBs in the body muscle of the blue crab by more than 30% so that this loss should be taken into account in evaluating the potential hazard of ingesting blue crab from New Jersey or any other contaminated waters. These results are consistent with other studies that evaluated preparation and cooking methods as a means of reducing toxic residues in fish and poultry (8-11).

The greater percentage PCB losses in the body muscle of crabs that were boiled after removal of the hepatopancreas, as compared with losses in crabs that were steamed after removal of the hepatopancreas, may be due to the contact of the crabs with the large amount of boiling water (6 liters). There is less opportunity for loss of PCBs from the body muscle into the steaming cooking water.

Levels of PCBs in Cooking Medium

Analysis of the PCB content of the cooking medium from crabs boiled whole and with the hepatopancreas removed prior to cooking (Table 8) showed no difference in total average mass of µg PCB lost for the crabs (2.68 ± 1.64 ug and 2.53 ± 1.18 ug, respectively). Also, no differences in the total mass of PCBs in the cooking broth occurred for crabs boiled whole and with the hepatopancreas removed (2.12 ± 1.34 ug and 2.06 ± 0.99 ug, respectively). Complete data for the micrograms of PCBs lost and recovered in the broth are in the Appendix. High recoveries of

Table 7. Percent total cooking weight losses for crabs boiled whole, and boiled and steamed with the hepatopancreas removed prior to cooking.

	N	Wet Weight Basis
Boiling with Hepatopancreas in Crab	8	21.24 ± 7.67[a]
Boiling with Hepatopancreas Removed Prior to Cooking	7	19.37 ± 8.61[a]
Steaming with Hepatopancreas Removed Prior to Cooking	10	12.21 ± 4.97[b]

[1]All means with the same letter are not significantly different, $p<0.5$

Table 8. Mean and standard deviation for PCB content of cooking medium for crabs boiled whole and after the hepatopancreas was removed.[1]

Boiling Condition	Total PCBs lost from crab (ug)	PCBs recovered in broth (ug)	% Loss recovered in broth
With Hepatopancreas	2.68 ± 1.64	2.12 ± 1.34	78.2 ± 2.5
Hepatopancreas Removed	2.53 ± 1.18	2.06 ± 0.99	80.9 ± 2.0

[1]Equations 9 and 10 were used to calculate ug PCBs lost and ug PCBs recovered in the broth.

the PCBs lost from the cooked crab were found in the broth, 78.2 ± 2.5% and 80.9 ± 2.0%, for crabs cooked whole and with the hepatopancreas removed, respectively. It is possible that some of the PCBs recovered from the cooking medium is due to the PCBs in the crab from portions which were not analyzed (shell, body fluids, body organs, etc.).

Application of Data to Risk Assessment

Cordle and workers (17) discussed data related to the risk assessment of ingestion of PCBs and arguments for establishment of either a 2 or 1 ppm tolerance for PCBs in fish and shellfish. Clearly, any tolerance established or advisories developed should consider the level of residue in the food as consumed. Only 1 of the 38 crabs analyzed had levels of PCBs in the raw tissues which exceeded 1 ppm (body muscle of crab 13 in Table 4).

The EPA cites PCBs as classification -- B2; probable human carcinogen in its Integrated Risk Information System which was last updated in 1991. The basis for this listing is hepatocellular carcinomas in three strains of rats and two strains of mice and inadequate yet suggestive evidence of excess risk of liver cancer in humans by ingestion and inhalation or dermal contact.

As one method to assess the risk of injesting crab from this waterway, we backcalculated the average PCB concentration in cooked crabs that would result in a cancer risk of 10^{-6}. The EPA generally regards carcinogenic risk to be of concern at levels greater than the 10^{-4} to 10^{-6} range. Using the exposure and risk assessment equations developed for the EPA Superfund program (18), the following relationships can be calculated:

Carcinogenic Risk = Exposure Intake x Oral Slope Factor

Exposure Intake = Risk/Oral Slope Factor

If we use a conservative risk of 10^{-6} and the oral slope factor of 7.7 (mg;kg-day)-1 which is obtained from the 1991 IRIS of EPA, the intake can be calculated as:

Intake = 1.3×10^{-7} mg/kg-day PCBs.

The EPA relates Intake to risk as follows (18):

$$\text{Intake (mg/kg-day)} = \frac{CF \times IR \times FI \times EF \times ED}{BW \times AT}$$

Where:

CF = Contaminant Concentration in Fish (mg/kg)
IR = Ingestion Rate (kg/meal)
FI = Fraction Ingested from Contaminated Source (unitless)
EF = Exposure Frequency (meals/year)
ED = Exposure Duration (years)
BW = Body Weight (kg)
AT = Averaging Time (period over which exposure is averaged - days)

Then CF can be calculated as:

$$CF = \frac{Intake \times BW \times AT}{IR \times FI \times EF \times ED}$$

According to Javitz (19), the mean consumption of crab other than king is 0.254 g per day. If we use this value for the IR, an intake value of 1.3×10^{-7}, a 70 kg man for an average body weight for BW, 70 year lifetime for carcinogenic effect x 365 days per year for AT, a value of 1.0 for FI (assumes all crabs consumed come from contaminated sources), an EF of 365 days,and an ED of 9 years (national median time (50th percentile) at one residence (18)), the CF would be 0.28 mg/kg PCBs or 0.28 ppm. Only the average ppm in the cooked body muscle of crabs steamed exceeds this value. Considering the contamination level in the body muscle of the steamed crabs, the years of exposure could be about 7.5.

The New Jersey Department of Environmental Protection may wish to use an ED of 30 years (nation upper-bound time (90th percentile) in one residence) which could be considered as a reasonable maximum exposure or 70 years which is a conventional lifetime estimate. Moreover, an EF of 365 days is approximately 7.6 times the average fish/shellfish consumption pattern of 48 days per year (19).

Since high percentages of the PCBs lost during the boiling of crab are found in the cooking water, the NJDEPE may wish to eastablish advisories for limited reuse of cooking water. Disposal issues related to this cooking water could also be addressed.

CONCLUSION AND RECOMMENDATIONS

While an effort was made in this study to obtain Blue crabs from an area thought to be highly contaminated with PCBs, levels found in the edible portions of the blue crab (body muscle and claw) were still low. However, based upon the results of this study, recommendations for consumers interested in reducing PCB ingestion could be made. Cooking the blue crab by boiling or steaming always reduced the PCB content of the crab tissue by greater than 20%. A preparation technique involving removal of the hepatopancreas from the top and bottom of the crab interior increased PCB loss for the body muscle. Since this loss was small compared to the overall PCB loss from cooking, it would only be necessary when the crabs were thought to contain very high residue levels. Those who consume large numbers of blue crab may also benefit by the further reduction in PCBs by removal of the hepatopancreas prior to cooking.

It is possible that since crabs cooked from the frozen state had larger overall cooking weight losses than those cooked from the live state, the PCB losses may be greater in these frozen crabs. However, this was not verified by our study. If crabs are cooked from the live state it is possible to remove the hepatopancreas after the crab has been boiled for short time to kill but not fully cook the crab (blanching). Since this is time consuming and messy, it should only be recommended in the case of highly contaminated crabs or for people who are particularly concerned with PCB reduction in their food.

When cooking blue crabs, the cooking medium should be discarded and not be used for preparing other foods. While cooking does reduce residues, this advantage is lost if the cooking broth is further used to prepare soups, sauces, etc. The actual average ppm of the cooking medium was found to be low (0.039 and 0.031 ppm, whole and without the hepatopancreas, but any further concentration of the broth or boiling of many crabs in the same volume of water could lead to potential problems.

This study only considered total PCBs. Since areas of contamination often have more than one contaminant present, the levels of other contaminants such as dioxins and other persistant xenobiotics should be addressed to be able to give the best guidelines to consumers. Even within the polychlorinated biphenyl compounds, differences in toxicity of specific congeners is know so further studies looking at coplanar or congener specific PCBs may be warrented.

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APPENDIX

Table A. Comparison of PCB ppm based on wet weights of the right claw (RC) and body muscle (BM) blue crab when cooked by boiling whole, with the hepatopancreas removed prior to boiling and steaming.

	Steamed LC	Steamed RC	Steamed BM	Boiled LC-Out[1]	Boiled RC-Out[1]	Boiled BM-Out[1]	Boiled LC-Whole	Boiled RC-Whole	Boiled BM-Whol
	0.336	0.311	0.413	0.123	0.121	0.151	0.265	0.359	0.432
	0.225	0.201	0.261	0.151	0.147	0.176	0.282	0.258	0.357
	0.215	0.228	0.297	0.099	0.100	0.123	0.198	0.217	0.268
	0.274	0.248	0.312	0.114	0.137	0.167	0.098	0.110	0.129
	0.231	0.229	0.286	0.160	0.278	0.251	0.156	0.217	0.261
	0.316	0.285	0.359	0.215	0.229	0.274	0.201	0.214	0.273
	0.290	0.336	0.442	0.313	0.299	0.382	0.118	0.133	0.170
	0.267	0.259	0.336				0.214	0.271	0.335
	0.287	0.290	0.373						
	0.261	0.217	0.283						
AVG[1]	0.270	0.280	0.336	0.168	0.187	0.218	0.192	0.222	0.278
Std. Dev.	0.039	0.044	0.060	0.074	0.080	0.090	0.065	0.079	0.098

[1]Refers to crabs which had the hepatopancreas removed prior to cooking

Table B. Comparison of average percent soluble solids for the left claw (LC), right claw (RC) and body muscle (BM).

	Boiled Whole[1]	Boiled- Hepatopancreas Removed[1]	Steamed-Hepatopancreas Removed[2]
Raw			
LC Soluble Solids	18.73	21.71	13.67
Std. Dev.	4.40	1.79	1.11
Cooked			
RC Soluble Solids	19.82	23.22	15.43
Std. Dev.	2.10	3.79	1.21
Cooked			
BM Soluble Solids	19.58	22.07	16.70
Std. Dev.	2.58	1.18	1.34
N	8	7	10

[1]Crabs collected Fall, 1990, [2]Crabs collected Spring, 1991

Table C. Comparison of PCB ppm based on dry weights of the right claw (RC) and body muscle (BM) of blue crab when cooked by boiling whole, with the hepatopancreas removed prior to boiling and steaming.

	Steamed LC	Steamed RC	Steamed BM	Boiled LC-Out[1]	Boiled RC-Out[1]	Boiled BM-Out[1]	Boiled LC-Whole	Boiled RC-Whole	Boiled BM-Whole
	2.300	1.962	2.716	0.625	0.648	0.744	2.078	2.298	2.484
	1.586	1.225	1.396	0.777	0.605	0.806	1.235	1.294	1.756
	1.770	1.422	1.972	0.457	0.430	0.553	0.926	1.017	1.227
	2.043	1.735	1.967	0.524	0.646	0.761	0.550	0.543	0.692
	1.700	1.453	1.558	0.739	1.197	1.182	1.235	1.053	1.722
	2.700	1.888	2.317	0.914	1.084	1.197	1.056	1.157	1.336
	2.040	2.371	2.656	1.288	0.974	1.588	0.653	0.581	0.870
	2.062	1.959	2.055				0.852	1.379	1.446
	1.923	1.730	2.141						
	1.756	1.293	1.576						
AVG[1]	1.988	1.704	2.035	0.761	0.797	0.976	1.069	1.166	1.442
Std. Dev.	0.328	0.358	0.447	0.280	0.286	0.359	0.466	0.549	0.562

[1]Refers to crabs which had the hepatopancreas removed prior to cooking

Table D. Comparison of PCB percent losses in the right claw (body muscle (BM) and whole crab (WC) when cooked by boiling whole, with the hepatopancreas removed prior to

	Steamed RC	Steamed BM	Steamed WC	Boiled RC-Out[1]	Boiled BM-Out[1]	Boiled WC-Out[1]	Boiled RC-Whole	Boiled BM-Whole	Boiled WC-Whole
	24.43	33.72	30.02	22.39	34.94	30.95	27.65	29.56	28.70
	24.15	36.16	33.95	25.06	39.03	35.04	23.86	30.92	29.09
	24.76	31.36	29.43	25.39	36.70	33.23	17.98	30.04	26.25
	20.08	35.88	30.74	27.70	32.66	31.20	24.57	36.18	32.25
	21.01	33.96	29.87	28.19	39.46	35.34	27.58	28.36	28.06
	23.42	37.19	32.85	25.46	36.89	33.92	19.84	30.13	26.35
	24.33	27.10	26.32	23.49	34.98	31.99	22.12	29.01	26.92
	24.82	34.00	31.44				36.23	33.97	34.59
	22.28	32.23	29.12						
	23.20	37.23	32.51						
AVG[1]	23.25[2a]	33.88[2b]	30.63[2e]	25.38[2a]	36.38[2c]	33.10[2f]	24.98[2a]	31.02[2d]	29.03[2g]
Std. Dev.	1.64	3.10	2.20	2.07	2.41	1.78	5.68	2.68	2.97

[1]Refers to crabs which had the hepatopancreas removed prior to cooking

[2]Averages with different letters are significantly different, $p<0.05$ for comparison of cooking methods within a single tissue type (RC, BM, WC)

Table D. Comparison of PCB percent losses in the right claw (body muscle (BM) and whole crab (WC) when cooked by boiling whole, with the hepatopancreas removed prior to cooking.

	Steamed RC	Steamed BM	Steamed WC	Boiled RC-Out[1]	Boiled BM-Out[1]	Boiled WC-Out[1]	Boiled RC-Whole	Boiled BM-Whole	Boiled WC-Whole
	24.43	33.72	30.02	22.39	34.94	30.95	27.65	29.56	28.70
	24.15	36.16	33.95	25.06	39.03	35.04	23.86	30.92	29.09
	24.76	31.36	29.43	25.39	36.70	33.23	17.98	30.04	26.25
	20.08	35.88	30.74	27.70	32.66	31.20	24.57	36.18	32.25
	21.01	33.96	29.87	28.19	39.46	35.34	27.58	28.36	28.06
	23.42	37.19	32.85	25.46	36.89	33.92	19.84	30.13	26.35
	24.33	27.10	26.32	23.49	34.98	31.99	22.12	29.01	26.92
	24.82	34.00	31.44				36.23	33.97	34.59
	22.28	32.23	29.12						
	23.20	37.23	32.51						
AVG[1]	23.25[2a]	33.88[2b]	30.63[2e]	25.38[2a]	36.38[2c]	33.10[2f]	24.98[2a]	31.02[2d]	29.03[2g]
Std. Dev.	1.64	3.10	2.20	2.07	2.41	1.78	5.68	2.68	2.97

[1]Refers to crabs which had the hepatopancreas removed prior to cooking

[2]Averages with different letters are significantly different, $p<0.05$ for comparison of cooking methods within a single tissue type (RC,BM,WC)

Table E. PCB content of cooking medium for crabs boiled whole and after the hepatopancreas was removed.[1]

	Crabs Boiled Whole			Crabs Boiled After Removal of the Hepatopancreas		
	Total PCBs lost from crab (µg)	PCBs recovered in broth (µg)	% Loss recovered in broth	Total PCBs lost from crab (µg)	PCBs recovered in broth (µg)	% Loss recovered in broth
	2.22	1.73	77.59	3.00	2.44	81.36
	3.56	2.89	81.06	2.41	2.00	82.99
	2.28	1.78	78.40	0.99	0.81	82.03
	1.63	1.31	80.20	2.16	1.69	78.33
	1.53	1.16	76.13	1.79	1.40	78.64
	2.57	2.00	77.97	4.78	3.95	82.64
	1.32	0.97	73.50	2.60	2.10	80.82
	6.33	5.09	80.52			
AVG	2.68 ±1.64	2.12 ±1.34	78.17 ±2.52	2.53 ±1.18	2.06 ±0.99	80.90 ±1.96

[1]Equations 9 and 10 were used to calculate µg PCBs lost and µg PCBs recovered in the broth