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Polycyclic Aromatic Hydrocarbons in Seafood from the Gulf of Alaska Following a Major Crude Oil Spill

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More than ten million gallons of Prudo Bay crude oil spilled into Prince William Sound, Alaska, when the supertanker EXXON VALDEZ ran aground on Bligh Reef March 24, 1989 (Baker et al. 1989). The oil spread over thousands of square miles of prime commercial fishing waters, causing State and Federal agencies to initiate immediate controls to ensure that seafood contaminated with this crude oil did not enter commercial channels. As a consequence, the 1989 herring fishery was closed for the season, and other fisheries, including the salmon fishery, were closely monitored.

Whenever there was visible evidence of oil in an area, the Alaska Department of Fish and Game (ADFG) closed that area to commercial fishing. Salmon harvested from open areas during the remainder of that season were screened organoleptically by Alaska Department of Environmental Conservation (ADEC) and Federal Food and Drug Administration (FDA) investigators as they were being off-loaded from the vessels. Those catches found to exhibit oil contamination were rejected at the site of delivery. Those lots found negative organoleptically were sampled, the samples were sent to the ADEC laboratory in Palmer, Alaska, a portion of the tissue removed, frozen, and sent to the FDA laboratory in Bothell, Washington for gas chromatographic/mass spectrometric analysis for the presence of polycyclic aromatic hydrocarbons (PAHs). The PAHs were selected for analysis because they are constituents of crude oil and some are carcinogenic (Jones and Leber 1979).

During the 1990 herring fishing season, ADFG collected samples at the various catch sites prior to the season opening. After initial organoleptic analysis, samples from these test catches were sent to the FDA laboratory in Bothell, Washington, for gas chromatographic/mass spectrometric analysis for the possible presence of PAHs in the edible tissue. Only when there was no evidence of oil contamination was the herring fishery allowed to open. In addition, samples were collected during the course of the harvest season and similarly analyzed. The 1990 salmon fishery was controlled in a like manner. Some pre-opening test catches were analyzed, both organoleptically and analytically to determine if the

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salmon showed any signs for oil contamination. After the fishery was allowed to open, catches continued to be monitored organoleptically by ADEC and FDA investigators. Negative organoleptic samples were sent to the ADEC, Palmer Laboratory, selective tissue portions removed and sent to the FDA laboratory where they were further analyzed for PAH levels by gas chromatography/mass spectrometry.

In 1989, following the spill, and again in 1990, representative samples of other finfish of some commercial significance were also sent to the FDA laboratory for gas chromatographic/mass spectrometric analysis for PAHs in the edible tissue. These included halibut, rockfish, and cod. Since the natural habitat of these finfish would be expected to put them in closer proximity to the oil, it was felt they would be good indicators of PAH uptake.

The primary objectives of the sampling and analyses were:

1. To compare PAH levels in various species of salmon from two designated control areas (areas geographically remote from the oil spill) to those taken from areas that were impacted by the oil spill (five impacted areas were initially selected for this study).

2. To compare 1989 PAH levels in the various species of salmon caught within the spill area to levels of the same PAHs in salmon from the 1990 catch, to note trends, and to determine the necessity of continued monitoring.

3. To determine if the organoleptic findings, which indicated that the sampled seafood was safe for consumption, was confirmed by a lack of PAHs in the edible tissue, as determined by gas chromatography/mass spectrometry.

MATERIALS AND METHODS

With the exception of salmon, all samples of seafood were taken from Prince William Sound sites that had been impacted by the oil spill. The initial plan for monitoring the salmon called for sampling from five areas impacted by the oil spill: Cook Inlet, Seward, Cordova, Kodiak, and Valdez (some samples received were identified as being from Chignik and some merely as from Prince William Sound) and two control areas that were outside the spill area, Sitka and Bristol Bay. Sitka was selected as representative of an area with an open ocean front and Bristol Bay as representative of an area with considerable inboard and outboard boat traffic. A sample history was recorded for each sample collected. The data included date caught, specific location of the catch, environmental conditions at the catch site, and condition of the animals in the catch (visible oil or not.) Although the fish samples were collected at the commercial off-loading sites, the area in which they were caught was identified and recorded.

Herring samples, consisting of approximately 2 kg of whole in-the-round herring, were frozen and sent by the ADFG investigator directly to the FDA laboratory in Bothell, Washington. The animals were thawed, eviscerated, heads removed,

and the remainder macerated in a food processor to prepare the analytical portion. Frozen halibut, rock fish, and cod were sent to the FDA by the ADEC laboratory personnel and consisted of 0.5 to 1 kg sections of the animal or a fillet. The entire edible portion was macerated in a food processor to obtain the analytical sample.

All salmon samples collected by ADEC and FDA investigators were initially submitted to the ADEC laboratory in Palmer, Alaska. After screening the collection data, fish meeting predetermined criteria were selected, a 5 by 8-cm section of skin and the underlying tissue was taken from just below the dorsal fin and these sample portions were shipped individually frozen to the FDA laboratory. Each sample portion was thawed then macerated in a food processor to prepare the analytical portion.

The samples were analyzed for each of the 25 PAHs listed in Table 1 by a method that was developed by researchers at the Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, in Seattle, Washington.

Table 1. A list of the polycyclic aromatic hydrocarbon standards used in the screening procedure.

1. d8-Naphthalene	15. 1-Methylphenanthrene
2. Naphthalene	16. Fluoranthene
3. 2-Methylnaphthalene	17. Pyrene
4. 1-Methylnaphthalene	18. Benz[a]anthracene
5. Biphenyl	19. Chrysene
6. 2,6-Dimethylnaphthalene	20. Benzo[b]fluoranthene
7. Acenaphthylene	21. Benzo[k]fluoranthene
8. d10-Acenaphthene	22. Benzo[e]pyrene
9. Acenaphthene	23. Benzo[a]pyrene
10. 2,3,5-Trimethylnapthalene	24. d12-Perylene
11. Fluorene	25. Perylene
12. Dibenzothiophene	26. Indeno[1,2,3cd]pyrene
13. Phenanthrene	27. Dibenz[a,h]anthracene
14. Anthracene	28. Benzo[g,h,i]perylene

The method is summarized in a journal publication (Krahn et al. 1988) and is described in detail in a technical memorandum (Krahn et al. 1988). A brief description of the method follows:

Granular sodium sulfate and 100 ng each of the deuterated internal standards (Supelco), d8-naphthalene, d10-acenaphthene, and d12-perylene were added to 5 g of macerated sample. The mixture was then blended with 50 mL methylene chloride (OminiSolve, EM Science), centrifuged, and the supernatant decanted into a beaker. The sample mixture was blended again with 50 mL methylene chloride and the combined supernatants poured through a 20 x 400-mm column containing 10 cc of F-20, 80-200 mesh alumina topped by 20 cc of Davidson 923, 100-200 mesh silica gel and 5 cc of sodium sulfate. The column was eluted with

25 mL methylene chloride and the eluate evaporated in a Kuderna-Danish concentrator to a final volume of 300 μL.

The eluate was further purified through a high performance, size exclusion chromatographic (SEC) system consisting of a 7.8 x 50-mm guard column and two 22.5 x 250-mm columns of 100 Å Phenogel (Phenonenex) connected in series and eluted with methylene chloride at 7 mL/min. A 200-μL aliquot of the eluate was loaded into the SEC system, the first 104 mL of eluate discarded, then 28 mL collected. All the PAHs listed in Table 1 had been experimentally determined to elute in the collected fraction.

The 28-mL fraction was concentrated to approximately 50-70 μL, then 2 uL (equivalent to approximately 100 mg sample) was injected into a gas chromatograph/mass spectrometer (Hewlett Packard 5790/5970A). The injection port was operated at 300°C, in the splitless mode, the initial column temperature was 50°C, and it was programmed to increase at 3.5°C/min to a final temperature of 300°C. The column transfer line was operated at 290°C.

The mass spectrometer, selected ion monitoring acquisition file was set as follows to detect the parent ion, along with two additional significant ions, of each targeted PAH that emerged within a specific chromatographic retention time interval:

16.0-20.0 minutes: 102, 127, 128, 136, 137 m/z.
20.0-24.5 minutes: 115, 141, 142 m/z.
24.5-32.0 minutes: 76, 141, 152, 153, 154, 156, 162, 164 m/z.
32.0-37.0 minutes: 76, 155, 163, 165, 166, 170 m/z.
37.0-43.0 minutes: 152, 176, 178 m/z.
43.0-47.0 minutes: 189, 191, 192 m/z.
47.0-54.0 minutes: 101, 202, 203 m/z.
54.0-63.0 minutes: 114, 226, 228 m/z.
63.0-71.0 minutes: 125, 252, 253, 260, 264 m/z.
71.0-80.0 minutes: 138, 139, 276, 277, 278 m/z.

The acquisition file dwell time was the same for each ion within a timeset and totaled 400 for each timeset.

The level of a PAH was determined by comparing the parent ion abundances of the detected PAH and a proximal internal standard, both in the sample solution, relative to their abundances in the reference standard solution (NIST/NOAA #C524).

Compound identity was confirmed by comparing the SIM spectra of the sample peak with that of the corresponding standard peak. The abundance ratios of three significant ions in the sample peak were required to match within $\pm$ 20% of the corresponding ions in the standard peak. In addition, the sample peak t_R was required to match that of the standard within 0.2 min.

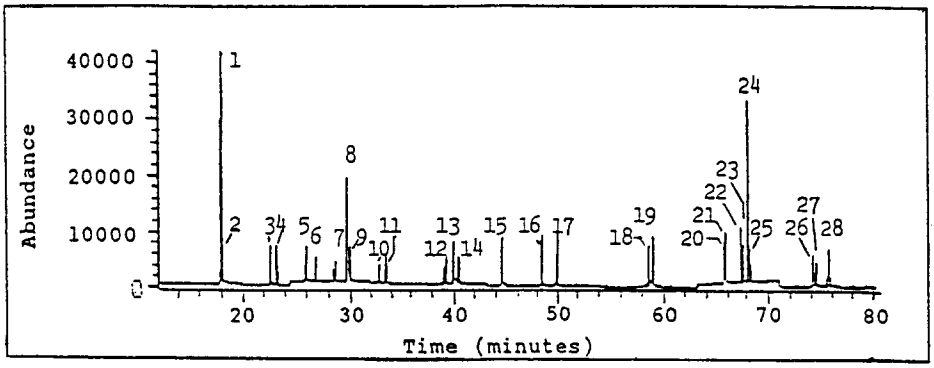

Figure 1. A SIM total ion chromatogram of the internal and reference standards. Two ng of each of the deuterated internal standards was injected and 0.41 to 0.55 ng of each of the 25 PAH standards (4-5 μg/kg for 100-mg samples.) The compounds are identified in Table 1.

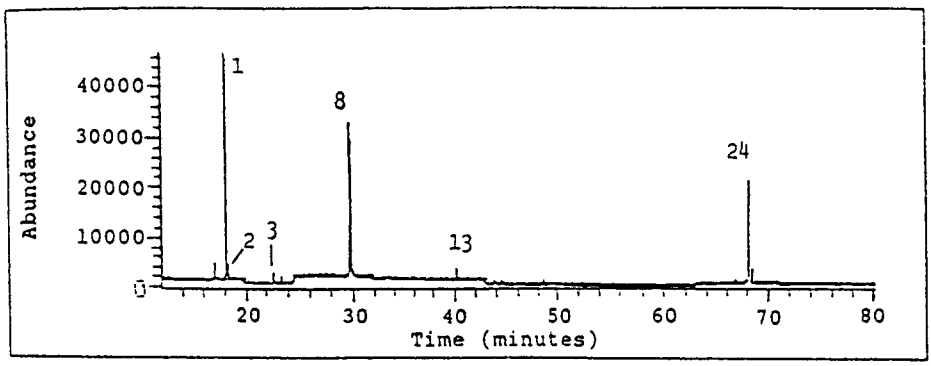

Figure 2. A SIM total ion chromatogram of the extract from a sample of pink salmon that is typical of the samples analyzed. Peaks 1, 8, and 24 are the added internal standards. The sample contained trace levels of naphthalene (#2), 2-methylnaphthalene (#3), and phenanthrene (#13).

A reagent blank and recovery was included in every other batch of 6 samples. A duplicate 5-g portion of one of the samples in the batch was spiked with a mixture of the 25 PAHs for the recoveries. The spike levels of the individual compounds ranged from 0.83 to 1.1 μg/kg in each determination. Any PAHs detected in the unspiked portion were corrected for in determining the recoveries.

RESULTS AND DISCUSSION

A chromatogram of the 25 reference standards along with the 3 deuterated internal standards is presented as Figure 1, and a chromatogram typical of most of the fish analyzed is presented as Figure 2. As noted, the fish contained trace levels of naphthalene, 2-methylnapthalene, and phenanthrene.

Quantitative results of the samples spiked at 0.83 to 1.1 μg/kg were variable, however the recoveries did establish that approximately 1 μg/kg could be detected and confirmed throughout the screening period. Recoveries of the 25 compounds at 5 μg/kg and higher were more reliable (85 to 125 %). Therefore, PAHs which were detected below 5 μg/kg are reported as a trace.

The data in Table 2 suggests that the range of PAHs found may be independent of species except perhaps for shrimp. For example, of the 290 samples of salmon analyzed, no PAHs were detected in 13%, trace levels were found in 84%, and levels of individual PAHs from 5 to 8 μg/kg were found in 3% (9 samples). Of the 43 samples of herring tested, no PAHs were detected in 9%, trace levels were detected in 79%, and levels of individual PAHs from 5 to 12 μg/kg were found in 12% (5 samples.) The PAH levels found in cod, rockfish, and halibut, if anything, are less than those for salmon and herring. By contrast, 3 of the 5 samples of shrimp tested showed levels of individual PAHs in excess of 5 μg/kg indicating that perhaps shrimp may be more susceptible to contamination. However, it is difficult to draw definitive conclusions as the sample size was exceedingly small, and the maximum level of contamination, 12 μg/kg, was low.

Table 2. A comparison of individual PAH levels in samples of several seafood species.

		None Detected		Trace		5-12 μg/kg	
Species	Number of Fish	No.	%	No.	%	No.	%
Salmon	290	38	13	243	84	9	3
Herring	43	4	9	34	79	5	12
Cod & Rockfish	55	26	47	28	51	1	2
Halibut	9	4	44	5	56	0	0
Shrimp	5	0	0	2	40	3	60
Total	402	72	18	312	78	18	4

There is no apparent difference in the levels of PAHs found in salmon taken from the 7 impacted areas versus the levels found in salmon from the two control areas, Bristol Bay and Sitka, as evident from the data presented in Table 3. These results indicate that either there was no contamination attributable to crude oil exposure or that the contaminants were rapidly metabolized and did not accumulate in the edible tissue.

PAH levels found in various species of salmon caught within the spill area in 1989 were compared to the PAH levels in these species of salmon caught in the same areas in 1990, Table 4. Although the levels are low for both catch seasons,

a trend seems to be emerging which suggests that contamination may be increasing with time. For example, no PAH residues were detected in 14% of the samples from the 1989 catch, trace levels were found in 85%, and only 1% (3 samples) contained more than 4 μg/kg of individual PAHs. By contrast, none of the samples from the 1990 catch were free of PAHs, trace levels were found in 87%, and 13% (6 samples) contained more than 4 μg/kg of individual PAHs. Whether the apparent trend is real and represents a potential problem, is unknown and remains to be determined.

Table 3. A comparison of individual PAH levels in samples of salmon from various catch areas.

	Number	None Detected		Trace		5-12 μg/kg	
Catch Area	of Fish	No.	%	No.	%	No.	%
Seward	16	7	44	9	56	0	0
Cook Inlet	66	11	17	54	82	1	1
Cordova	20	2	10	17	85	1	5
Kodiak	88	8	9	76	86	4	5
Valdez	36	1	3	33	92	2	5
PWS	31	2	6	29	94	0	0
Chignik	11	0	0	10	91	1	9
Bristol Bay	11	5	45	6	55	0	0
Sitka	11	1	9	10	91	0	0

Table 4. A comparison of individual PAH levels in samples of salmon from each of two catch seasons.

	Number	None Detected		Trace		5-12 g/kg	
Year	of Fish	No.	%	No.	%	No.	%
1989	221	31	14	187	85	3	1
1990	47	0	0	41	87	6	13

The primary intent of the survey was to determine if the seafood from areas impacted by the oil spill were fit for human consumption as indicated by sensory analyses. As shown in Table 2, no PAHs were detected in 18% of the 402 samples analyzed, trace levels were found in 78%, and the remaining 4% (18 samples) had individual PAHs in the 5 to 12 μg/kg range. The PAHs and

measurable levels found in those 18 samples are shown in Table 5. The low levels and sparse numbers of PAHs found do indeed indicate that the harvested and processed seafood was safe for consumption as indicated by the sensory screening.

Table 5. Record of the 18 samples in which individual PAHs exceeded trace levels.

Sample	PAH #*	μg/kg	Sample	PAH #*	μg/kg
Salmon	2	5	Herring	3	5
Salmon	2	6	Herring	3	5
Salmon	3	5	Herring	13	8
Salmon	2	5	Herring	3+4	7+5
Salmon	3	5	Herring	2+3+4	10+12+6
Salmon	3	5	Rockfsh	3+4	9+5
Salmon	28	8	Shrimp	2	7
Salmon	13+16	7+5	Shrimp	2+3	6+7
Shrimp	PAHs 13+16+17+18+19+26+28 @ 12+12+9+6+6+6+5 μg/kg				

* The PAH#s correspond to those listed in Table 1.

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