International Fishmeal & Oil Manufacturers Association

POLYAROMATIC HYDROCARBONS (PAHs) IN FISH OILS

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EXECUTIVE SUMMARY

Animal tests have shown that Polyaromatic Hydrocarbons (PAHs) are carcinogenic. The degree of carcinogenicity depends upon the molecular structure of the PAH. PAHs have been found in a variety of animal and vegetable oils and fats. IFOMA, in collaboration with the Unilever Research Laboratory in the Netherlands, measured PAHs in a variety of fish oils taken from different species of fish in different geographical areas in 1989 in order to evaluate the levels in fish oils.

The more responsible sectors of the edible oils and fats industry have designated that samples with more than 1ppb of benz(a) pyrene or 7ppb or total heavy PAHs have to be pretreated with active carbon in order to reduce the PAH content in the oils to acceptable levels.

34 samples of fish oil were analysed, representing commercial samples from around the world. Only six of these samples exceeded the Action Limit. Contamination appeared to be of a spasmodic nature and is not necessarily related to species or fishing zone.

Introduction

Animal tests have shown that Polyaromatic Hydrocarbons are carcinogenic. The degree of carcinogenicity depends upon the molecular structure of the PAH (see Appendix 1 for outline molecular structure).

PAHs have been found in a variety of animal and vegetable oils and fats. The level of contamination varies considerably from batch to batch, but analytical investigations have indicated that some types of oil are more likely to contain high levels of PAHs than others. IFOMA, in collaboration with the Unilever Research Laboratories in the Netherlands, has measured PAHs in a variety of fish oils taken from different species of fish in different geographical areas in order to evaluate the levels in fish oils.

The more responsible sectors of the edible oils and fats industry have designated that samples with more than 1ppb of benz(a) pyrene or 7ppb of total heavy PAHs has to be pretreated with active carbon in order to reduce the PAH content in the oils to acceptable levels.

Methodology

The method of analysis used by the Unilever Research Laboratory is caffeine complexing for isolating PAHs from the edible oil (Appendix 2) followed by reversed phase HPLC with diode array detection. Table 1 shows duplicate and triplicate analysis of certain samples of fish oil; this illustrates good reproducibility by the laboratory.

In a collaborative study organised by Unilever with various laboratories using the same method undertaken in 1989, the following repeatability (within a single laboratory) and reproducibility (between laboratories) were observed for fish oil.

mean batch level (µg/kg)	repeatability CV_r (%)	reproducibility CV_R (%)
4.87	13.1	22.4
3.08	6.5	17.6
0.98	24.6	29.4

Results

34 samples of fish oils, collected from IFOMA members during 1989, were analysed (Table 2), representing commercial samples from around the world. Six of these samples exceeded the industry Action Limit. Three samples from the North Sea, two samples from the South East Atlantic and one from the South East Pacific exceeded the Action Limit. The majority of samples were well below the Action Limit. This indicates that contamination can be of a spasmodic nature and is not necessarily related to species or fishing zone.

Samples 20, 21 and 22 clearly demonstrate the efficacy of active carbon in reducing PAHs to acceptable levels. Samples 16 and 17 showed that refining, bleaching and deodorising only have a marginal effect on reducing PAHs.

Discussion

Fish oil processing involves cooking the fish in an indirect steam jacketed cooker, squeezing the fish in a press and separating the oil from the liquors coming from the press by means of centrifuges. Consequently, unlike certain drying processes for oilseeds, there is no direct contact of flue gases with the oil. Consequently, these gases are not a source of contamination.

It would appear that the occasional parcel of fish oil having higher than the industry Action Limits of PAHs is on a random basis, not necessarily related to species or fishing zone. The possibility exists of PAH contamination during handling and shipment, which is a problem in common with all types of animal and vegetable oils.

By the analytical technique it is possible to distinguish two major groups of PAH sources, namely:

- Pyrogenic sources, like diesel exhausts, car exhausts and all other kinds of burning processes
- Petrogenic sources, like all kinds of mineral oils

The Unilever database on fish oil indicates that all sources, or combinations of the above sources, are present in fish oils. Fish themselves are susceptible to environmental contamination, mainly from pyrogenic sources but also from petrogenic sources.

TABLE 1 - REPEATABILITY OF ANALYSIS

SAMPLE NO.	SPECIES	B(a)P (ppb)	TOTAL HEAVY PAHs (ppb)
3	Menhaden	0.2	1.4
	"	0.2	1.4
30	Anchovy	0.2	1.4
30-duplicate	"	0.3	2.0
9	Sand eel	2.5	10.1
9-duplicate	"	2.9	11.3
10	Mackerel	0.1	0.8
10-duplicate	"	0.1	0.8
19	Sardine	0.3	2.0
19-duplicate	"	0.3	2.0
27	Anchovy	<0.1	<0.8
27-duplicate	"	0.1	0.8
28	Anchovy	1.2	5.7
28-duplicate	"	1.2	5.7
33	Cod liver oil	0.2	1.4
33-duplicate	"	0.2	1.4
33-triplicate	"	-	-

TABLE 2

FISHING ZONE	SPECIES	SAMPLE NO.	PROCESSING OF OIL	B(a)P (ppb)	TOTAL HEAVY PAHs (ppb)
Gulf of Mexico	Menhaden	2	Crude	0.3	2.0
Gulf of Mexico	Menhaden	3	Crude	0.2	1.4
Gulf of Mexico	Menhaden	4	Crude	0.3	2.0
Gulf of Mexico	Menhaden	5	Crude	0.2	1.4
North Sea	Sand eel	6	Crude	1.6	7.1
North Sea	Sand eel	7	Crude	1.7	7.5
North Sea	Sand eel	8	Crude	0.7	3.8
North Sea	Sand eel	9	Crude	2.5	10.1
North Sea	Mackerel	10	Crude	0.1	0.8
North Sea	Herring (mainly) with sand eel	31	Crude	0.4	2.4
Norwegian Sea	Mackerel (plus other species)	11	Crude	0.6	3.3
Norwegian Sea	Capelin	13	Crude	1.0	5.0
Norwegian Sea	Capelin	14	Crude	0.7	3.8
Norwegian Sea	Capelin	15	Crude	0.8	4.2
N.E.Atlantic	Capelin	16	Crude	0.5	2.9
N.E.Atlantic	Capelin (same as sample 16)	17	Refined, bleached, deodorised	0.3	2.0
N.E.Atlantic	Cod liver oil	33	Medical grade	0.2	1.4
N.W.Atlantic	Menhaden	1	Crude	0.7	3.8

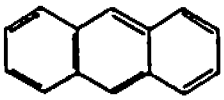
(cont.)

TABLE 2 (cont.)

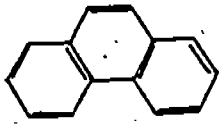
FISHING ZONE	SAMPLE	SAMPLE NO.	PROCESSING OF OIL	B(a)P (ppb)	TOTAL HEAVY PAHs (ppb)
S.E.Atlantic	Anchovy	28	Crude	1.2	5.7
S.E.Atlantic	Anchovy	29	Crude	0.3	2.0
S.E.Atlantic	Maasbanker	32	Crude	1.0	5.0
S.E.Atlantic	Pilchard	34	Crude	2.9	11.3
Mid E.Pacific	Anchovy	30	Crude	0.2	1.4
S.E.Pacific	Horse mackerel	12	Crude	0.0	0.0
S.E.Pacific	Sardine	18	Crude	0.1	0.8
S.E.Pacific	Sardine	19	Crude	0.3	2.0
S.E.Pacific	Sardine (?spps.uncertain)	20	Crude	7.0	17.0
S.E.Pacific	Sardine (same as sample 20)	21	Bleached and active carbon	0.2	0.3
S.E.Pacific	Sardine (same as sample 20)	22	Active carbon	0.2	0.4
S.E.Pacific	Sardine/horse mackerel	23	Crude	0.2	1.4
S.E.Pacific	Sardine/jack mackerel (70/30)	24	Crude	<0.1	<0.8
S.E.Pacific	Anchovy	25	Crude	0.1	0.8
S.E.Pacific	Anchovy	26	Crude	0.1	0.8
S.E.Pacific	Anchovy	27	Crude	<0.1	<0.8

Light and Heavy PAHs

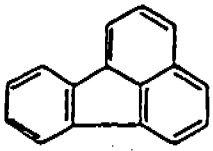
Light PAH: 3-4 aromatic ring



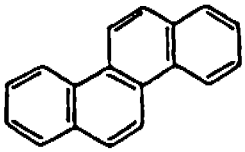
Anthracen



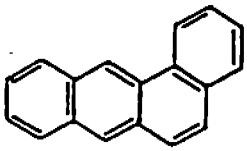
Phenanthren



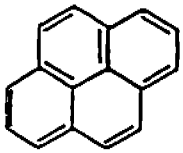
Fluoranthen



Chrysen

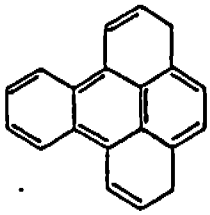


Benz(a)anthracen
(1,2-Benzanthracen)

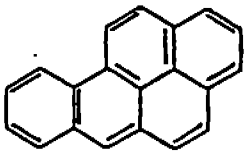


Pyren

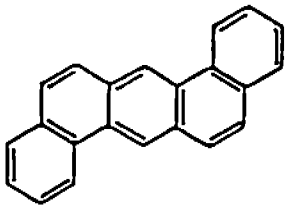
Heavy PAH: 5-7 aromatic ring



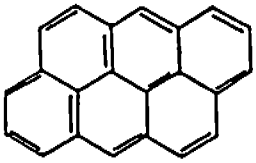
Benz(e)pyren
(1,2-Benzpyren)



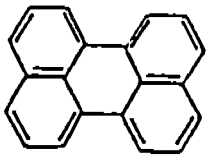
Benz(a)pyren
(3,4-Benzpyren)



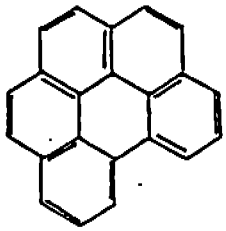
Dibenz(a,h)-anthracen
(1,2,5,6-Dibenzanthracen)



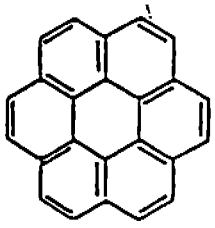
Anthanthren



Perylen



Benz(g,h,i)-perylen
(1,12-Benzperylen)



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ISOLATION PROCEDURE FOR PAHs

1. **Reagents:**

1. 1. Cyclohexane Uvasol, Merck art.2822

1. 2. Glass wool DMCS treated, Alltech

1. 3. Formic Acid p.a. 98-100%, Merck art.264

1. 4. Sodiumchloride p.a., Merck art.6404

1. 5. Sodiumsulphate anhydrous p.a. (granular), Merck art.6639

1. 6. Caffeine, Merck art.2584

1. 7. Silica 100-200, active 60A, ICN, art.02747, deactivated by adding 15g of water to 100g of silica.

1. 8. 90% formic acid: fill up 100ml H_2O to 1 liter with formic acid.

1. 9. 40% formic acid: fill up 600ml H_2O to 1 liter with formic acid.

1. 10. Caffeine-formic acid solution: Dissolve 150g of caffeine in 90% formic acid, an fill up to 1 l.

2. **Procedure:**

2. 1. Fill a 2.5 l separating funnel with 1.5 l distilled water and 30g of NaCl (1.4).

2. 2. Dissolve 50g of oil in 200ml of cyclohexane (1.1) in a 500ml separating funnel.

2. 3. Wash the cyclohexane phase by shaking with 100ml 90% formic acid (1.8) for 1 min. Remove the formic acid layer (lower phase). Repeat the extraction procedure twice.

2. 4. The PAHs in the cyclohexane layer are removed by complexing with caffeine-formic acid (1.10). Add firstly 100ml caffeine-formic acid solution and shake for 2 min. Transfer the lower water layer into the 2.5 l separating funnel containing the salt solution. Repeat the extraction twice with 60ml caffeine-formic acid solution.

2. 5. Shake the combined salt-caffeine-formic acid phase in the 2.5 l separating funnel for 2 min.

2. 6. Remove the PAHs from the water phase by shaking with 250ml cyclohexane for 2 min. Because the cyclohexane phase is the upper layer, firstly transfer the water phase in a conical flask, and transfer the cyclohexane phase to a 1 l separating funnel. Transfer the water phase in the 2.5 l separating funnel and repeat the extraction once.

2. 7. Wash the combined cyclohexane phases twice with 150ml 40% formic acid (1.9). Draw off the lower (formic acid) layer.

2. **8.** Transfer the cyclohexane phase, containing the PAHs, into a conical flask containing 30g anhydrous granular Na_2SO_4 (1.5). Dry for approximately 30 min.

2. **9.** Filter the dried cyclohexane, using a funnel containing a little plug of glass wool (1.2), into a 1000ml round-bottomed boiler flask.

2. **10.** Evaporate the cyclohexane at 35°C using a Rotavapor until approximately 2ml (NOT until dry).

2. **11.** Prepare a silica column using 5g of silica (1.7).

2. **12.** Transfer the cyclohexane concentrate on the silica column and elute with 110ml cyclohexane. Collect the eluent into a 250ml round-bottomed boiler flask.

2. **13.** Evaporate the cyclohexane at 35°C using a Rotavapor until approximately 2ml (NOT until dry).

2. **14.** Transfer quantitatively the concentrate into a micro vial, and evaporate until dry under a gently stream of nitrogen (15ml/min).