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n-Alkanes and Polynuclear Aromatic Hydrocarbons in Fresh-Frozen and Precooked-Frozen Mussels

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Heavy oil pollution has been found in sea water and coastal environments not only near industrial petroleum districts and places of oil spillage but also in other places where crude oil and/or refined products can be carried to by winds, streams, etc.

Marine oil pollution may not only affect productivity and quality of marine organisms but may ultimately affect the health of the human population as there is a possible health risk from consumption of sea food contaminated by oil-derived carcinogens such as polycyclic aromatic hydrocarbons (PAHs). In the marine habitat, many orga-nisms readily accumulate n-alkanes and PAHs from the environment and store them at a relatively high level in their tissues (Murray *et al.* 1991; Singh *et al.* 1992), and studies have been carried out on the accumulation and depuration of toxic organic pollutants in marine organisms (Murray *et al.* 1991; Narbonne *et al.* 1992; Ogata and Fujisawa 1985).

As a part of a continuous monitoring program of the foods imported to the Canary Islands (Galindo *et al.* 1986, Brito *at al.* 1990) this paper presents the results obtained in the determination of n-alkanes and PAHs in fresh-frozen and precooked-frozen mussels, *Perna canaliculus*, commercialized in these islands.

MATERIALS AND METHODS

Analytical reagent grade purity chemical (Merck, Darmstadt) were used throughout the study. n-Hexane was distilled, treated with sulfuric acid, and re-distilled under reflux. Milli-Q water was extracted with purified n-hexane prior to use.

Samples of shell-free fresh-frozen and precooked-frozen mussels were bought every fifteen days from September 1992 to May 1993 in the markets throughout the island of Tenerife, wrapped in aluminium foil and stored at -20°C until analysed.

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Table 1. Procedence and handling of the analysed samples

Procedence	Trade mark	Package	Sample number
New Zeeland	A	Plastic bag/original	1,2, 31-34
New Zeeland	B	" " "	3-6
Unknown	C	Plastic bag/re-packed	7-11, 16-19
New Zeeland	D	Plastic bag/original	12-15
New Zeeland	E	" " "	20-30

All analyses were carried out in a clean room where a composite sample for each sampling date was prepared from about 10 specimens, homogeneized, freeze-dryed, and weighed (5-15g). Then the internal standard 9-methyl-phenanthrene (100% recovery) was added to each composite sample for the purposes of instrument calibration and percent recovery calculation. Subsequent analysis included alkaline hydrolysis (8% KOH in ethanol), addition of saturated aqueous sodium chloride to avoid emulsion formation, and extraction with 3x50 mL of n-hexane. The organic layer was first washed with 10% HCl (3x100 mL) and then with purified water until neutral, dried over sodium sulfate and concentrated to 1-2 mL in a rotary evaporator. The aliphatic and aromatic fractions were then separated by column chromatography on florisil-alumina-silica (de-activated at 3%, 5% and 5% with purified water) using n-hexane and n-hexane-dichloromethane as the eluents. Both fractions were taken almost to dryness in the rotary evaporator and disolved into n-hexane (aliphatic fraction) or toluene (aromatic fraction). Both fractions were analysed by gas chromatography with flame ionization detector (Varian 3400 provided with an 8200 automatic injector) on SPB-5 and SPB-20 0.32mmx15m columns (He carrier 1.4 $mL.min^{-1}$, N_2 make-up 30 $mL.min^{-1}$, splitless/split injection, 90°C for 2 min, 8°C/min until 280°C, hold for 15 min), calibration was carried out using Aldrich standards for n-alkanes, pristane and PAHs. Tables 2 and 3 show the percent recovery and detection and determination limits for n-alkanes and PAHs according to the method used.

RESULTS AND DISCUSSION

Values for paraffinic hydrocarbons and pristane, and PAHs concentrations in every sample of fresh-frozen and precooked-frozen *Perna canaliculus* are given in Tables 4 and 5, respectively, expressed on a dry weight basis.

Shell-free mussels were sold packed in plastic bags, and most of them were packed directly in the original country but in some cases they seemed to have been re-packed by the importer, Table 1. However, no significant differences in the concentrations of alkanes and PAHs were found between originally packed and

Table 2. Detection and determination limits for the *n*-alkanes studied

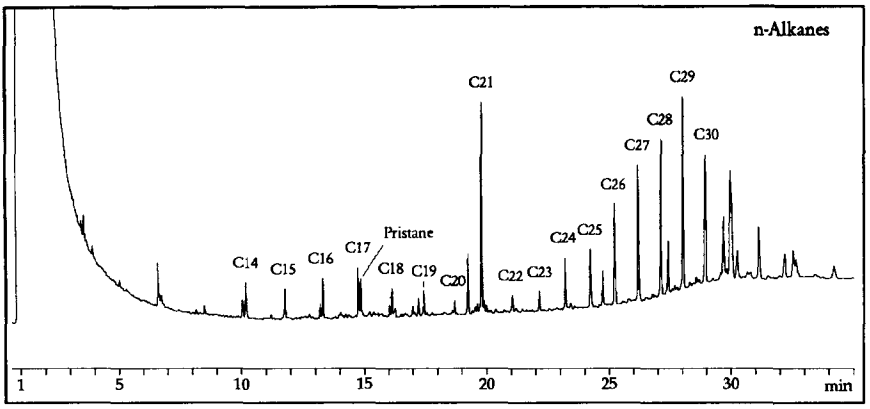
Compound	Recovery	Detection Limit (μg/mL)	Determination Limit (μg/mL)	r^2
C_{15}	76	1.22	2.58	0.987
C_{16}	89	0.45	1.50	0.994
C_{17}	89	0.36	1.00	0.998
C_{18}	94	0.20	0.66	0.999
C_{19}	94	0.29	0.97	0.998
C_{20}	93	0.26	0.85	0.998
C_{21}	89	0.25	0.85	0.998
C_{22}	89	0.37	1.24	0.997
C_{23}	90	0.54	1.81	0.993
C_{24}	94	0.43	1.42	0.996
C_{25}	94	0.25	0.83	0.998
C_{26}	88	0.35	1.15	0.997
C_{27}	88	0.66	2.19	0.992
C_{28}	83	0.71	2.35	0.988
C_{29}	83	0.91	3.04	0.985
C_{30}	84	0.73	2.42	0.987
Pristane	90	0.07	0.25	0.999

Table 3. Detection and determination limits for the PAHs studied

Compound	Recovery (%)	Detection Limit (μg/mL)	Determination Limit (μg/mL)	r^2
Phenanthrene	79	0.23	0.50	0.997
Anthracene	73	0.34	0.80	0.993
4,5-Methylenephenanthrene	44	1.22	2.51	0.944
Fluoranthene	84	0.18	0.60	0.994
Pyrene	86	0.18	0.59	0.993
Benz[*a*]anthracene	86	0.51	0.97	0.994
Chrysene	85	0.32	0.77	0.994
Benzo[*b*]fluoranthene	82	0.42	0.91	0.993
Benzo[*e*]pyrene	87	0.46	0.86	0.994
Benzo[*a*]pyrene	75	0.77	1.30	0.989
Perylene	72	0.86	1.49	0.986
Coronene	85	1.07	1.70	0.995

Table 4. Concentration levels (μg/g, dry weight) of *n*-alkanes in mussels *Perna canaliculus* (nd= not detected/determined).

Sample	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	Pristane
1	16.105	0.291	11.647	0.101	5.619	0.044	2.462	0.021	1.048	nd	0.855	0.777	0.495	nd	0.518	1.699	0.992	0.405
2	27.726	0.391	17.338	0.252	7.524	0.033	2.723	nd	0.878	nd	0.491	nd	0.234	0.226	0.081	0.189	nd	0.429
3	18.061	0.935	12.877	0.364	7.572	0.156	3.812	0.054	1.648	0.064	0.904	nd	0.399	0.257	0.184	0.318	0.134	0.544
4	14.709	0.797	10.564	0.248	5.847	0.055	2.679	nd	1.052	nd	2.646	1.967	0.477	nd	0.187	0.095	0.596	0.277
5	163.123	8.374	114.379	2.260	55.143	0.327	22.067	nd	7.175	nd	4.010	nd	1.329	0.653	0.562	1.041	0.454	2.208
6	nd	4.428	14.006	1.330	11.690	0.313	6.876	nd	2.905	0.139	0.856	nd	0.705	0.266	0.212	0.208	0.086	0.925
7	63.412	2.687	52.507	0.934	33.108	0.075	16.679	0.302	7.122	0.189	3.359	0.663	1.158	1.128	nd	0.559	0.267	1.015
8	36.097	1.766	30.718	0.509	20.405	0.074	10.844	0.062	5.282	0.047	1.963	0.228	0.781	0.228	0.332	0.515	0.196	0.253
9	nd	6.087	nd	2.644	13.052	0.616	8.294	0.289	3.944	0.302	8.778	nd	3.339	0.449	0.870	1.415	0.882	1.374
10	46.710	2.514	34.131	0.812	17.969	0.148	7.488	nd	2.715	nd	1.761	nd	0.691	nd	0.449	0.927	0.564	0.633
11	95.397	5.942	101.683	1.376	64.721	0.393	34.764	0.170	17.425	0.109	4.060	nd	2.763	0.856	1.396	2.492	0.867	1.487
12	14.901	0.845	12.236	0.322	7.422	nd	3.804	nd	1.459	nd	0.758	0.190	0.294	0.349	0.134	0.246	nd	0.495
13	15.170	0.814	11.760	0.282	7.628	0.046	3.702	0.021	1.453	0.015	0.590	nd	0.300	0.082	0.125	0.265	0.085	0.439
14	27.314	1.459	22.198	0.605	15.158	0.088	6.585	0.064	2.537	nd	1.055	0.277	0.541	0.592	0.271	0.419	0.190	0.792
15	80.745	4.505	65.614	1.658	43.369	0.358	21.526	0.105	8.526	0.093	3.445	nd	1.973	1.216	0.826	1.434	0.395	2.105
16	0.233	0.110	0.510	0.264	0.310	0.109	0.143	0.083	nd	nd	nd	0.242	0.164	0.509	0.192	0.689	0.322	0.817
17	0.286	0.136	0.625	0.410	0.468	0.203	0.291	0.270	0.252	0.306	0.296	0.779	0.486	0.864	0.462	0.882	0.708	0.319
18	0.045	0.105	0.037	0.037	0.089	0.020	0.008	0.030	0.006	0.033	0.022	0.125	0.107	0.096	0.078	0.131	0.104	0.057
19	0.173	0.057	0.142	0.053	0.113	0.023	0.013	0.013	0.009	0.012	0.007	0.105	0.004	0.108	0.081	0.183	0.109	0.077
20	0.079	0.140	0.115	0.278	0.180	0.085	0.038	nd	0.045	0.034	0.036	0.044	0.056	0.120	0.107	0.301	0.251	0.373
21	0.050	0.088	0.069	0.154	0.126	0.042	0.035	nd	0.003	0.013	0.016	0.018	0.023	0.042	0.047	0.039	nd	0.233
22	0.148	0.200	0.243	0.427	0.300	0.168	0.080	0.064	0.126	0.102	0.103	0.100	0.114	0.147	0.088	0.158	0.055	0.483
23	0.095	0.118	0.111	0.244	0.178	0.055	0.039	0.316	0.028	0.025	0.379	0.203	0.178	0.360	0.321	0.680	0.306	0.247
24	0.341	0.128	0.442	0.085	0.241	0.040	0.113	0.022	nd	0.016	0.036	0.210	0.035	0.241	0.076	0.362	0.078	0.211
25	0.085	0.074	0.076	0.037	0.056	0.025	0.032	0.017	0.029	0.029	0.067	0.066	0.102	0.167	0.141	0.425	0.209	0.186
26	0.067	0.037	0.062	0.026	0.032	0.016	0.013	0.006	0.015	0.012	0.054	0.031	0.045	0.082	0.066	0.154	0.086	0.096
27	0.095	0.086	0.093	0.111	0.069	0.072	0.035	0.015	0.048	0.046	0.121	0.147	0.240	0.431	0.391	0.838	0.414	0.137
28	0.077	0.122	0.049	0.073	0.020	0.035	nd	0.011	nd	0.029	0.042	0.068	0.048	0.130	0.098	0.329	0.104	0.314
29	0.075	0.115	0.055	0.178	nd	0.051	nd	0.037	nd	0.083	21.493	nd	0.060	0.903	0.402	0.848	0.347	0.519
30	0.081	0.108	0.028	0.061	nd	nd	nd	nd	nd	nd	0.525	0.285	nd	0.821	0.444	0.729	0.321	2.367
31	0.083	0.133	0.043	0.051	0.012	0.042	0.009	0.010	0.010	0.019	0.066	0.159	0.161	0.558	0.372	0.440	0.335	0.229
32	0.187	0.256	0.108	0.060	0.017	0.053	0.012	0.012	0.013	0.027	0.056	0.089	0.055	0.296	0.120	0.168	0.125	0.334
33	0.097	0.121	0.053	0.060	0.007	0.044	0.013	0.016	0.016	0.021	0.749	0.294	0.026	0.373	0.137	0.264	0.208	0.080
34	0.120	0.212	0.125	0.189	0.080	0.087	0.023	0.022	1.496	0.076	0.118	0.208	0.122	0.825	0.248	0.567	0.278	2.255



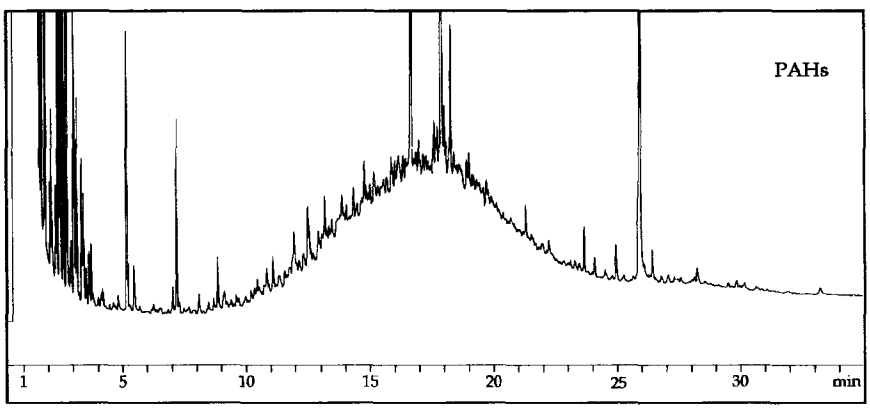


Figure 1. Typical chromatograms of the n-alkane and PAHs fractions of contaminated samples

re-packed samples.

The concentration of n-alkanes in these mussels varied from 163 μg $C_{14}.g^{-1}$ for sample n° 5 to not detected/not determined (nd) for several alkanes in several other samples. Even though no bimodal distribution could be observed on the chromatograms of the paraffinic fraction, and no odd-to-even predominance in the alkanes could be established, the biogenic source of hydrocarbons in these mussels is supported by the presence of pristane in significant concentrations. On the other hand, the anthropogenic contribution of hydrocarbons is evident from the presence of the unresolved complex mixture (UCM) (Figure 1) (Farrington and Tripp, 1977), in some samples belonging mostly to re-packed mussels (samples 7, 8, 17-19) and to one of the production zones (samples 32-34). In the former samples,

contamination may be due to bad re-packing conditions, in the later ones due to bad depuration processes. Besides, samples bought from July to December 1992 show an accumulation of the shorter even-chain alkanes $n < 20$, while samples from January to May 1993 present an accumulation of the odd-chain alkanes $n > 20$ (Figure 2).

The presence of a deformed baseline (Unresolved Complex Mixture) in the chro-

Table 5. Concentration levels (ng/g, dry weight) of PAHs in mussels (*Perna canaliculus*)

Sample	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1	20	nd	nd	130	nd	23	7	nd	100	nd	nd	nd
2	14	4	18	59	19	6	nd	nd	70	nd	nd	nd
3	13	3	8	50	11	nd	28	2	345	nd	nd	8
4	8	nd	68	77	18	26	nd	nd	38	nd	nd	nd
5	18	6	97	15	35	11	nd	9	33	28	38	nd
6	6	nd	21	13	5	nd	nd	nd	20	13	nd	nd
7	2	20	18	1757	22	nd	21	nd	48	nd	339	846
8	1	3	4	52	1	2	nd	6	20	nd	nd	5
9	65	27	186	171	168	73	54	nd	nd	nd	nd	nd
10	5	nd	18	38	nd	nd	nd	nd	87	40	nd	nd
11	23	19	24	2492	12	nd	37	nd	nd	nd	33	32
12	4	5	9	41	9	nd	11	3	126	69	nd	nd
13	19	22	36	22	8	nd	13	4	210	81	nd	nd
14	7	5	14	99	12	nd	23	3	161	88	4	nd
15	7	28	19	70	10	36	22	5	207	76	5	8
16	32	21	87	1319	2	nd	25	2	40	nd	8	79
17	31	16	nd	641	nd	1	19	7	100	55	nd	nd
18	3	4	16	5	nd	3	nd	nd	nd	nd	nd	2
19	36	19	38	894	nd	nd	23	nd	452	33	26	17
20	5	1	13	1	158	nd	nd	2	20	33	3	13
21	5	3	18	31	nd	nd	9	3	336	57	nd	nd
22	8	nd	5	35	3	nd	19	2	244	nd	17	1
23	5	2	10	30	nd	nd	5	3	228	72	nd	nd
24	15	17	29	17	6	nd	11	3	67	74	nd	nd
25	1	nd	8	20	19	nd	nd	nd	nd	70	2	nd
26	6	nd	10	87	92	nd	14	4	246	190	nd	nd
27	2	nd	27	nd	8	nd	nd	10	20	25	nd	59
28	23	27	46	27	9	20	17	5	333	64	15	4
29	15	2	19	2	5	nd	nd	nd	nd	10	3	29
30	13	7	5	62	73	nd	7	3	96	80	nd	nd
31	2	2	nd	35	nd	nd	18	3	nd	nd	nd	22
32	7	6	24	39	1	nd	16	3	100	nd	nd	nd
33	3	14	10	38	2	nd	8	nd	306	84	214	68
34	nd	nd	5	5	5	nd	nd	nd	nd	nd	nd	14

A1..... Phenanthrene
A2..... Anthracene
A3..... 4,5-Methylenephenanthrene
A4..... Fluoranthene
A5..... Pyrene
A6..... Benz[*a*]anthracene
A7..... Chrysene
A8..... Benzo[*b*]fluoranthene
A9 ..... Benzo[*e*]pyrene
A10..... Benzo[*a*]pyrene
A11..... Perylene
A12..... Coronene

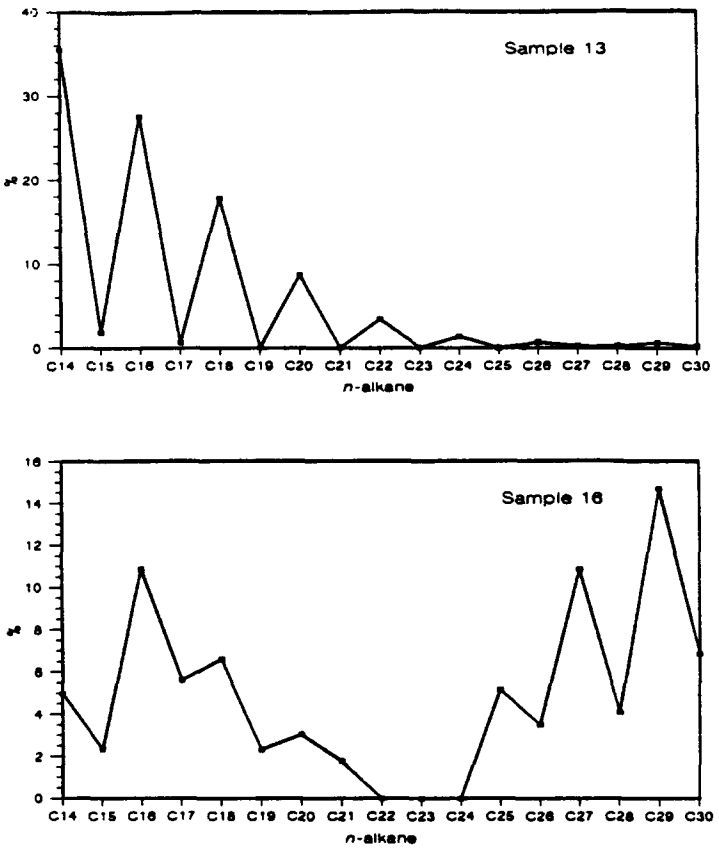


Figure 2. Variation of the content of n-alkanes in samples 13 and 16

matograms of the aromatic fraction of some samples (Figure 1) also supports an anthropogenic contamination of these samples (Farrington and Tripp, 1977). Even though the concentrations for most of the PAHs quantified are low (Table 5 some samples show relatively high levels of fluoranthene (samples 7, 11, 16, 19) and the concentrations of benzo[*a*]pyrene in samples 13-15, 17, 23-25, 28, 30, 33 are in the range 55-80 $ng.g^{-1}$, values similar to those reported by Dunn and Stich (1975) for areas of relatively high contamination. Sample 26 shows a concentration as high as 190 $ng.g^{-1}$ of benzo[*a*]pyrene. Among these more contaminated samples one can find samples of mussels packed in the country of origin and samples of mussels re-packed by the importer; thus, one can think that the contamination in the former is due to bad deputarion processes, as mussels to be sold in Spain must be subjected to depuration (Real Decreto 263/1985), and the latter may be contaminated due to bad re-packing conditions.

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