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Bioassay-Directed Chemical Analysis of Genotoxic Components in Coastal Sediments

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■ Organic extracts of coastal sediments collected off-shore from Barcelona were submitted to a three-level bioassay-directed chemical fractionation, including gel permeation chromatography (GPC) and normal-phase (NP) and reversed-phase (RP) liquid chromatography (LC). The chemical characterization, directed by the *Salmonella* microsome mutagenicity assay (TA98+S9, TA98NR+S9, and TA98/1,8DNP+S9), was carried out by capillary GC (CGC) coupled to selective detection systems and by CGC–MS techniques. The mutagenic activity recovered among the GPC fractions was concentrated (78–98%) in the second fraction (GPC-2), which was further fractionated by NP-LC. The mutagenicity among the 35 NP-LC-collected fractions exhibited a three-modal distribution, the highest level being recovered in the intermediate-polarity fractions (10–15%), which were further fractionated by RP-LC. Detailed chemical analysis of the mutagenic fractions led to the identification of 140 aromatic compounds, 57 of them classified as mutagenic or belonging to mutagenic chemical classes, among them, 1-nitropyrene, 6-nitrochrysene, and 6-nitrobenzo[*a*]pyrene were positively identified for the first time in a coastal sediment.

Introduction

The role of coastal sediments as sinks for refractory organic compounds of urban–industrial origin is well documented (*1*, *2*). The occurrence of xenobiotics in this compartment deserves special attention from the ecotoxicological point of view because they may constitute a particular threat for benthic organisms, or even for the marine food web through their mobilization to the water column. In this respect, chemical contaminants associated with sediments receiving industrial and urban wastes have been implicated, for example, in the ethiology of tumors in bottom-feeding freshwater organisms (*3*, *4*).

The determination of genotoxic agents in the environment can be accomplished by the use of the *Salmonella* microsome assay. This is one of the best validated short-term bioassays for mutagenicity testing (*5*), and as a consequence, a large database of assayed chemicals is available (*6–8*). Moreover, it has already been applied to screen multicomponent mixtures extracted either from pollution sources (*9–12*) or from environmental compartments (*13–18*).

However, due to the complex chemical composition of the environmental matrices, the assignment of the chemicals responsible for their mutagenicity is not an easy task. Furthermore, multiple interactions between components lead to a variety of synergistic, antagonistic, or toxic effects (*19*) that further complicate the toxicity assessment. Consequently, several chemical class fractionation schemes according to acid–base properties or polarity of chemicals have been applied previous to mutagenicity testing.

In this regard, significant levels of mutagenic activity have been found in the intermediate (*20*) or polar fractions of river sediments (*21*), although the responsible genotoxic components were not identified. West et al. (*22*) identified complex mixtures of polycyclic aromatic compounds (PACs) in two mutagenic fractions isolated from a heavily polluted river. Further fractionation of the polycyclic aromatic hydrocarbons (PAHs) according to the number of aromatic rings demonstrated that the mutagenicity was recovered in the four- to five-ring PAH fractions (*23*). Similar attempts undertaken to correlate the mutagenic activity and chemical composition of polar fractions isolated from coastal polluted sediments have illustrated the difficulty of identifying the genotoxic components (*24*, *25*).

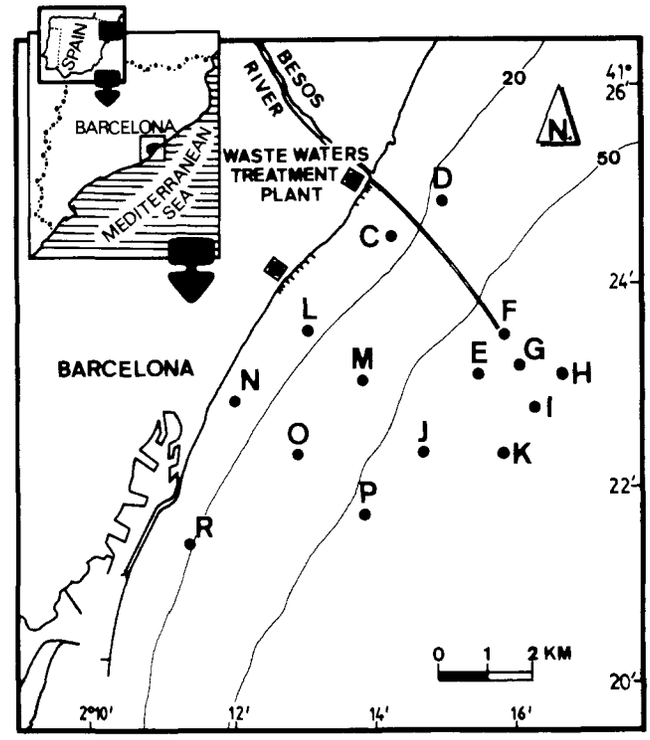

Figure 1. Sampling site locations.

In order to overcome this problem, Schuetzle and Lewtas (*26*) have developed an approach known as the bioassay-directed chemical analysis, which consists of a multilevel fractionation scheme coupled to a short-term bioassay, focusing the analytical target onto the most mutagenic fractions. These are submitted to several levels of fractionation in order to simplify as much as possible their composition with the aim of correlating mutagenicity and chemical composition. This approach has been successfully applied to the determination of genotoxic components from diesel exhaust emissions (*27*), synfuels (*28*), and atmospheric suspended particulate matter (*29*), enabling the identification of strongly mutagenic nitro- and amino-substituted aromatic compounds.

The aim of the present work is to extend this strategy to the determination of mutagenic agents present in coastal sediments receiving a high load of pollutants from wastewater discharges and the disposal of sewage sludges. Sediments were collected off-shore from (Figure 1), in an

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area affected by the discharges of two heavily polluted rivers (*30*, *31*), urban–industrial wastewaters, and sewage sludge outfalls, together with the diffused input of atmospheric deposition. Previous studies in this area have already shown significant levels of mutagenicity in the polar fractions of the dissolved and particulate wastewater phases, as well as in the riverine and coastal sediments, although the precise sources of this activity remained unknown (*24*, *25*).

Sediments collected from characteristic depositional environments were extracted, sequentially fractionated using a multistep fractionation procedure, including semipreparative gel permeation chromatography (GPC) and normal-phase high-performance liquid chromatography (NP-LC). Collected fractions were tested against mutagenicity using the *Salmonella* microsome bioassay in the presence of metabolic activation (+S9 mix) and analyzed by capillary gas chromatography–mass spectroscopy (CGC–MS) in the electron impact (EI) and negative ion chemical ionization (NICI) modes. Those fractions exhibiting the highest levels of mutagenicity were further fractionated by reversed-phase high-performance liquid chromatography (RP-LC) and analyzed by CGC–MS.

Special emphasis was placed on the chemical characterization of mutagenic fractions by an extensive application of CGC–MS in the NICI mode, which provides high sensitivity and selectivity for molecules amenable to ionization by electron capture mechanisms, which usually correspond to those of higher environmental concern (*32*, *33*).

Experimental Section

Sediment Samples. Sediment samples were collected with a van Veen grab from three different areas (Figure 1): (a) the Besòs river mouth (stations C and D), (b) the sewage disposal site (stations E–G), and (c) an area between the Besos river and the Barcelona harbor representative of the urban littoral (stations L–O and R). That small river (mean flow 2–5 $m^3\ s^{-1}$), which influences stations C and D, receives many urban and industrial partially treated effluents along its highly industrialized basin. During the summer the whole river flow is subject to physicochemical treatment in the same plant that operates on most of the Barcelona wastewater. The resultant primary treated liquid effluents and the sewage sludge (77 $\times 10^3$ tons $year^{-1}$ dry wt) are dumped by submarine outfalls, respectively, to 0.6 and 4 km from the shoreline, the latter corresponding to sampling site F, and influencing sites E and G. A smaller secondary treatment plant, located in front of sampling site L, dumps secondary treated effluents, occasionally chlorinated, directly into the coastline. The sewage sludges are transferred to the main treatment plant. Stations M and O can be considered as representative of the background pollution in the coastal area and stations H–K and P were selected for assessing the advective transport of the sewage sludge from its disposal site (F). Sampling site R is affected by the harbor activities.

Chemicals and Reagents. *n*-Hexane, acetonitrile, and methanol were HPLC grade (SDS), whereas dichloromethane and dimethyl sulfoxide (DMSO) were nanograde (Baker) and spectrograde (Merck), respectively. The derivatization reagent *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Fluka. DDEs, DDDs, HCHs, chlordane, and dieldrin were obtained from Polyscience Corp. Benzo[*c*]quinoline, 9*H*-fluoren-9-one, 2-aminofluorene, 1-nitropyrene, 2,4-dibromophenol, acridine, 2- and 4-nitrophenols, and 1,8-naphthalenedicarboxylic acid anhydride were purchased from Aldrich and Sigma. The 4*H*-cyclopenta[*def*]phenanthren-4-one, 7*H*-benz[*de*]anthracen-7-one, benz[*a*]anthracen-7,12-dione, 6-nitrobenzo[*a*]pyrene, and 6-nitrochrysene were a gift of Professor M. L. Lee (Brigham Young University).

Sample Handling. The sampled superficial sediments (0–5 cm) were wrapped in aluminum foil and frozen at −20 °C until analysis. Portions of the freeze-dried sediments (20–60 g) were homogenized and analyzed for total organic carbon (TOC) in a Carlo-Erba elemental analyzer. Alternatively, about 20–60 g was extracted with dichloromethane by sonication (5 × 30 mL). Similar extraction procedures have demonstrated a quantitative recovery of the mutagenicity from different particulate materials (*34*). The organic extracts were concentrated to dryness in a rotary evaporator, weighted, reconstituted with dichloromethane, and filtered through a glass microfiber filter (Whatman GF/F).

Chemical Fractionation. (a) GPC. Filtered organic extracts were injected (50–100 mg every injection), via a 150-μL loop fitted in a high-pressure valve (Rheodyne), into a 500 × 10 mm i.d. column packed with BioBeads SX-12 styrene–divinylbenzene copolymer (200 mesh) (BioRad). A high-pressure pump (Knauer) delivered, isocratically, the mobile phase (dichloromethane) at a flow rate of 1 mL min^{-1}, and the effluent was monitored with a UV variable-wavelength detector set at 254 nm (Varian). The following fractions were collected: GPC-1, containing lipidic and high molecular weight biogenic compounds (9–15 min); GPC-2, enriched in PAC (15–28 min); GPC-3, containing elemental sulfur and highly polar compounds (28–35 min) (*35*). A total of 30 injections were performed to fractionate the whole amount of each extract.

(b) NP-LC. GPC-2 fractions were injected (15 mg every injection), via a 50-μL loop fitted in a high-pressure valve (Rheodyne) into a 300 × 7.8 i.d. mm column packed with 10 μm μ-Porasil silica (Waters Assoc.). The eluent was monitored with a UV diode-array (220–370 nm) (Applied Biosystems) and fluorescence (λ_{ex} = 280 nm and λ_{em} = 390 nm) (Perkin Elmer) detectors coupled in series. A binary gradient of hexane, dichloromethane (DCM), and acetonitrile (ACN) at a flow rate of 4.5 mL min^{-1} was delivered by two high-pressure pumps (Kontron AG). The mobile-phase programming scheme started with 100% hexane for 5 min, a linear gradient of 4% DCM min^{-1} to 100% DCM, held at 100% DCM for 10 min, a linear gradient of 20% ACN min^{-1} to 100%, and held at 100% for 15 min, and then step-changed to 100% DCM and 100% hexane, which were held for 10 min each. Fractions were automatically collected (Gilson) every 2 min, yielding a total of 36 per injection, the latter corresponding to the methanolic back-flushing step, performed every 10 injections (*36*). A total of 20 injections were performed to fractionate every single sample. Moreover, the column selectivity was tested along the fractionation procedure by monitoring the retention times of a polarity mixture. Whenever deviations were greater than 5%, the column was reactivated (*37*). The distribution of selected chemical classes of PAC along the 36 fractions is shown in Figure 2.

(c) RP-LC. NP-LC fractions, selected on the basis of their mutagenicity, were dissolved in ACN (nominal mass 20 μg) and injected, via a 20-μL loop fitted to a high-pressure valve (Rheodyne) into a 250 × 4.6 i.d. mm analytical column packed with 5-μm octadecylsilane polymeric phase (Vydac). The chromatographic system was similar to that used for the NP-LC fractionation. A binary linear gradient of ACN in water from 60% to 100% ACN at 1% ACN, holding isocratically the final composition for 2 min, was performed. The flow rate was held at 1 mL min^{-1}.

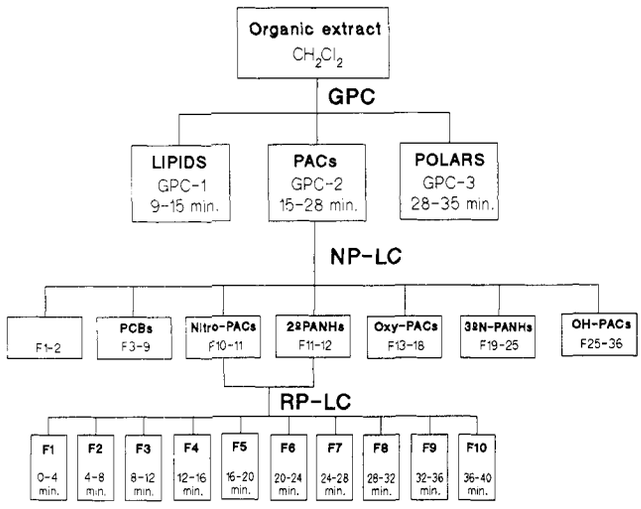

Figure 2. Fractionation scheme of sediment organic extracts for mutagenicity testing.

Fractions were automatically collected (Gilson) every 4 min, yielding a total of 10 per injection. A total of five injections were performed in order to fractionate every single sample. Subfractions obtained for mutagenicity testing were solvent transferred to DMSO and to isooctane (NP-LC fractions 1–16) or dichloromethane (NP-LC fractions 17–36) for chemical analysis. Blank extracts were obtained in parallel for both mutagenicity testing and chemical analysis.

Capillary Gas Chromatography Analysis (CGC). NP-LC and RP-LC fractions were analyzed by CGC, using a Carlo-Erba 5300 (Milan, Italy) equipped with an on-column injector and a dual-detection system. A 25 m × 0.20 mm i.d. fused-silica column coated with SE-54 (0.10-μm film thickness) was used. The column effluent was diverted to an ECD (30% of column effluent), and to FPD or NPD (70%). The 20–36 NP-LC fractions were analyzed as silyl ether derivatives (BSTFA at 70 °C for 1 h in a nitrogen atmosphere). Hydrogen was the carrier gas at 50 cm s^{-1} linear velocity. The column temperature was programmed from 60 to 300 °C at 6 °C min^{-1}. Detector temperature was held at 250–330 °C.

Capillary Gas Chromatography–Mass Spectrometry Analysis (CGC–MS). Electron impact (EI) at 70 eV and negative ion chemical ionization (NICI) CGC–MS analyses were performed in a Hewlett-Packard 5985A instrument interfaced to a 9825A data system. Helium was used as carrier gas (30 cm s^{-1}), and the capillary column was fitted directly into the ion source. Other chromatographic conditions were identical to those described in the CGC analysis. The ion source and mass analyzer were held at 180 and 120 °C, respectively. Methane was used as reagent gas at 0.8 Torr in the ion source for the NICI. The mass axis was calibrated with perfluorotributylamine using fragments at m/z 264, 414, and 633 for NICI. Data acquisition was obtained from 30 to 600 amu at 0.9 scans s^{-1}. EI mass spectra assignments were accomplished by library search using the PBM algorithm (match quality above 0.85) in the J. Wiley spectral database (~120.000 mass spectra). NICI mass spectra were also compared with reported data (*38*). Positive identifications were accomplished by coinjection with authentic standards.

Quantitation. Quantitation was performed by the external standard procedure, using analytical grade calibrants. Individual PCB congeners, organochlorinated pesticides, and polycyclic aromatic ketones (PAKs) were quantified by CGC-ECD and nitrated polycyclic aromatic compounds (nitro-PACs) by CGC-NPD. PAH quantitation was accomplished by CGC–MS in the EI ionization mode. Reference materials SRM 1647 from the National Institute of Science and Technology (Gaithersburg, MD) and SH-4 from the National Research Council (Ottawa, ON, Canada) were used for calibration and method validation, respectively.

Mutagenicity Testing. GPC, NP-LC, and RP-LC fractions were tested against the *Salmonella* microsome assay according to the plate incorporation procedure (*39*). The assay with the *Salmonella typhimurium* strain TA98, obtained from Professor B. N. Ames (University of California, Berkeley), was performed in the presence of exogenous metabolic activation (+S9). This strain was selected in this study due to its enhanced sensitivity for assaying PAC in multicomponent mixtures (*24*, *41*, *42*). Each fraction, with the tester strain and S9 mix, was preincubated for 20 min at 37 °C according to procedures previously described (*40*). In addition, RP-LC fraction 8 was also assayed against the nitroreductase-deficient strains TA98NR and TA98/1, 8DNP, which were kindly supplied by Professor H. S. Rosenkranz (Case Western Reserve University). S9 liver fraction was obtained from male Sprague–Dawley rats induced with Aroclor 1254. S9 was used at the standard dose (20 μL $plate^{-1}$). All samples and controls tested were dissolved in DMSO, using 50 μL of the resultant solution per plate. After a 48-h incubation period at 37 °C, cytotoxicity was checked by microscopic observation (60×) of the background lawn and colonies were handly counted. The TA98+S9 spontaneous reversion rates was 45 ± 5. 2-Aminofluorene in the presence of S9 mix was used as the positive control of the strain, 0.5 μg inducing 200 ± 33 revertants $plate^{-1}$.

GPC fractions were assayed at four doses in triplicate against TA98+S9, in two separate experiments. Dose–response relationships were estimated by using least-squares regression. Concurrently, NP-LC fractions from selected sampling sites (M+O, D, and F, Figure 1), were dissolved in the same volume of DMSO and assayed against the TA98+S9 tester strain in two separate experiments, performed at a single dose. A first experiment was carried out to determine whether the assayed dose was or was not toxic and, then, a confirmative experiment was performed in duplicate plates.

Results and Discussion

Chemical Fractionation and Mutagenicity Distribution of Sediment Extracts. The TOC and the extractable organic matter of the studied sediment samples (Table I) reflect the contribution of point pollution sources. The higher TOC contents were found in the stations influenced by direct discharges, namely, urban sewage sludge (F), river water (D), and treatment plant effluent (L). However, sampling sites located farther from these sources (stations G–R) still exhibited remarkable TOC levels, indicating either the importance of diffused pollution or the transport from point sources.

The analytical protocol designed for the application of the bioassay-directed strategy to perform the identification of genotoxic components in the extracts included three levels of fractionation, namely, GPC, NP-LC, and RP-LC (Figure 2). The relative mass distribution of the GPC fractions referred to the total extract is shown in Table I. GPC-1 was the most abundant fraction in all the stations. GPC-2 and GPC-3 exhibited similar relative concentrations, although in station D, located close to the Besos river mouth, GPC-2 was more abundant. The 75–90% mass recovery in this level of fractionation is consistent with an elution of aliphatic hydrocarbons earlier

Table I. Mass and Mutagenicity Distributions of the GPC Fractions of Sediment Extracts

sampling sites[a] (water depth, m)	TOC, %	% dry wt sediment[b]	% organic extract[b]	mutagenic activ[c] slope ± SE	r
Besos estuary					
C (15)	2.1				
D (28)	5.9				
GPC-1		0.16	50.0	T[d]	0.222
GPC-2		0.07	21.7	115.9 ± 0.8	0.939
GPC-3		0.01	4.3	47.4 ± 1.4	0.882
Total		0.24	76.0		
dumping site					
E (53)	6.4				
F (49)	8.4				
GPC-1		1.47	83.3	18.8 ± 2.2	0.608
GPC-2		0.06	3.4	48.6 ± 0.5	0.986
GPC-3		0.07	3.9	38.6 ± 1.1	0.917
Total		1.60	90.6		
G (54)	2.3				
H (55)	1.2				
I (55)	1.3				
J (58)	2.2				
K (62)	1.4				
coastal sites					
L (18)	4.5				
GPC-1		0.63	62.8	39.1 ± 0.9	0.944
GPC-2		0.05	5.2	131.6 ± 1.0	0.939
GPC-3		0.07	7.0	37.3 ± 0.6	0.999
Total		0.75	75.0		
M (41)	3.0				
GPC-1		nd	nd	26.0 ± 1.0	0.989
GPC-2		nd	nd	1246.6 ± 0.1	0.999
GPC-3		nd	nd	24.8 ± 1.3	0.882
N (16)	nd				
GPC-1		0.11	58.5	20.0 ± 2.0	0.803
GPC-2		0.01	4.8	1483.3 ± 0.1	0.980
GPC-3		0.02	11.7	83.3 ± 0.5	0.632
Total		0.14	75.0		
O (38)	3.8				
GPC-1		0.13	68.3	158.3 ± 0.2	0.967
GPC-2		0.01	2.9	2773.3 ± 0.2	0.966
GPC-3		0.01	4.2	153.3 ± 1.0	0.998
Total		0.15	75.4		
P (56)	2.8				
R (19)	2.4				

[a] Locations are indicated in Figure 1. [b] nd, not determined. [c] Revertants per milligram (TA98+S9) ± standard error estimate. [d] T, toxic to the tester strain.

than GPC-1 (Figure 2). Conversely, most of the mutagenicity is recovered in GPC-2 (78–98%), because size exclusion and partitioning, the predominant separation mechanisms, enable a remarkable enrichment in this fraction of synthetic chemicals (e.g., PAC) from lipidic and biogenic coextractants (*35*, *43*).

Considering the spatial variability of the mutagenicity on the basis of GPC-2 values (Table I) it is interesting to note that the values found in sampling sites M–O exceed by 1 order of magnitude those found in stations located near the pollutant sources (F and D), a fact that can be attributed to the existence of masking effects between components in the most polluted samples that give rise to a decrease of mutagenicity of the whole extract. However, these levels of mutagenicity are comparable to those reported for polluted riverine sediments (*22*), thus stressing the importance of the precise determination of mutagenic sources in this area.

In order to investigate the composition of the most mutagenic fractions (GPC-2), we have selected three different samples: two representative of the main pollution sources in the area (sampling sites F and D) and another, consisting of a composite of extracts M and O, representative of the stations exhibiting the highest mutagenicity levels. Therefore, the above-mentioned GPC-2 fractions were submitted to a sequential NP and RP-LC fractionation (Figure 2), as described in the Experimental Section.

The distribution of the mass and mutagenic activity (TA98+S9 mix) within the 36 NP-LC fractions of each sample is shown in Figure 3. The mass distribution is bimodal with a maximum at low polarity (fractions 2–4) and a smaller one at the polar end (fractions 26–28). The mass recovery along the fractionation procedure was higher than 95%, and the two maxima accounted, respectively, for 55–65% and 7–11% of the total mass. The distribution pattern of the mutagenicity does not parallel the mass distribution and depends on the sampling site (Figure 3). In this regard, a maximum was observed for the low-polarity fractions (3–7) in the estuarine sample (D), whereas another maximum of mutagenicity appeared in the polar fractions (26–28) of sampling sites M+O, accounting for

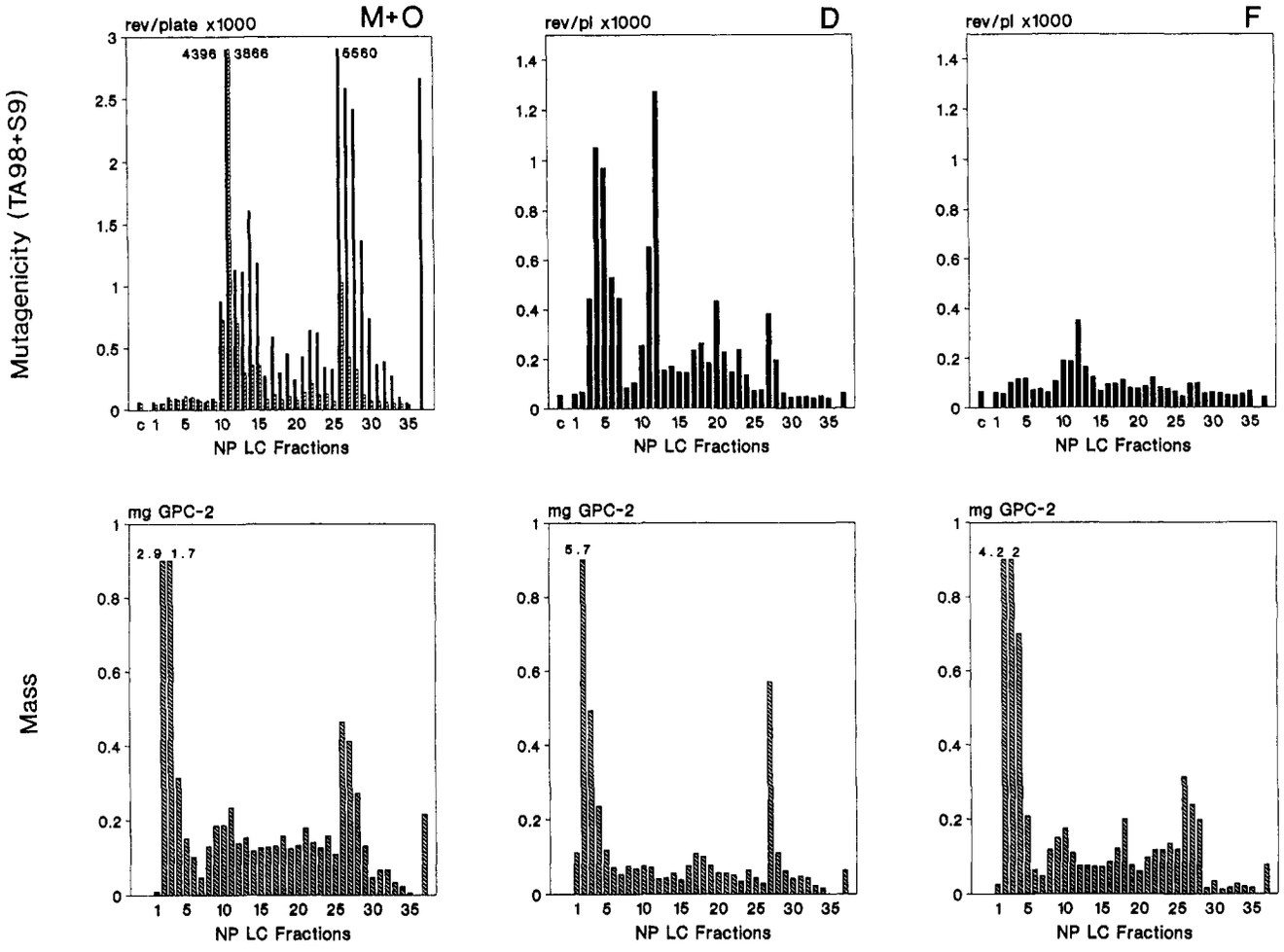

Figure 3. Mutagenicity (TA98+S9) and mass distribution among the NP-LC subfractions obtained from 10 mg of GPC-2 fractions (solid bars). In the M+O sampling site, an additional dose of 1 mg of GPC-2 fraction was also assayed (slashed bars).

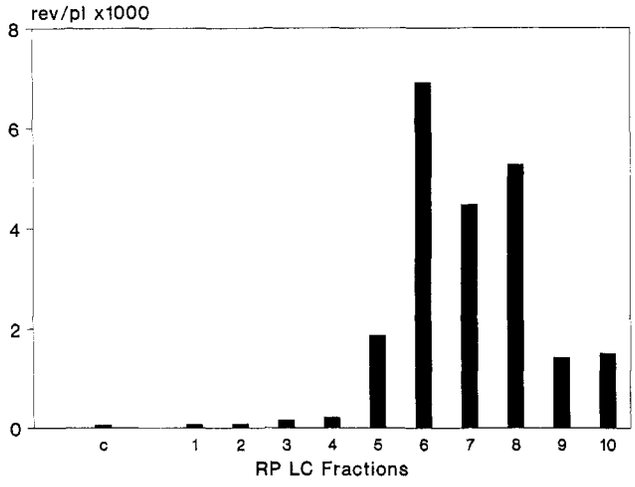

Figure 4. Mutagenicity distribution among the RP-LC subfractions obtained from the maximum of mutagenicity of the NP-LC (subfractions 11–14) isolated from sampling sites M+O.

50% of the total mutagenicity. On the other hand, the medium-polarity fractions (10–15) exhibited a significant response for all samples. The extremely high mutagenicity values found, especially in station M+O, may infer a possible response saturation or toxic effects to the tester strain. Consequently, this GPC-2 fraction was tested using 10 times more dilute doses. In this case, the maximum was also evident, accounting for 36% of the mutagenicity recovered at this level of fractionation.

Taking into account the widespread occurrence of mutagenic compounds in these intermediate-polarity fractions, a pool of fractions 11–14 was submitted to RP-LC fractionation. The mutagenic activity showed a distribution maximizing at fractions 6–8 (Figure 4), with values significantly high, considering the reduced amount of fraction assayed (total amount fractionated <1 mg). This confirms that extensive fractionation is required to concentrate the mutagenicity in representative samples, where masking, synergistic, or antagonistic effects, which make difficult the assessment of the environmental genotoxicity, have been reduced or eliminated. In the following section, a detailed study of the chemical composition of the different fractions, using CGC–MS in the EI and NICI modes, is reported.

Identification of Xenobiotics and Their Contribution to the Mutagenicity. (a) Organohalogenated Compounds (OCs). The low-polarity NP-LC subfractions (3–6) contained a diversity of OCs (Table II). Among them, penta- and hexachlorobenzenes and from penta- to decachlorobiphenyls (PCBs) exhibited a remarkable abundance, especially in the M+O and F sampling sites (644–1040 ng g^{-1} as Aroclor 1254). In sites exhibiting the higher PCB concentrations (M+O, F), chlorobrominated substituted biphenyls and brominated diphenyl ethers were identified. The former class of compounds is present in PCB formulations (*44*), the latter is widely used as flame retardants and reported in several aquatic compartments (*45*, *46*).

Several chlorinated pesticides were also identified at high concentrations in the low-polarity fractions: i.e., γ-HCH, DDDs, DDEs, and α- and β-chlordane (Tables II and III). Dichlorobenzophenone, a thermal degradation product of dicofol, dieldrin, γ-chlordene, and tetradifon

Table II. Selected Components Identified in the NP-LC Subfractions

fractn	diagnostic ions[a]	occurrence	identification[b]	mutagen.[c]
		Halogenated Compounds		
3	250, 128	M+O	pentachlorobenzene[2,4]	
3	284, 250	M+O, F	hexachlorobenzene[2,4,6]	0
3, 4	326	M+O, F, D	pentachlorobiphenyls[2,4]	
3, 4	360	M+O, F, D	hexachlorobiphenyls[2,4]	
3, 4	394, 360	M+O, F, D	heptachlorobiphenyls[2,4]	
3, 4	430, 394	M+O, F, D	octachlorobiphenyls[2,4]	
3	464, 430	M+O	nonachlorobiphenyls[2,4]	
3	498, 464	M+O	decachlorobiphenyl[2,4]	
3	374, 338, 292	M+O	chlorobromobiphenyl[2,4]	
3	424, 389, 354	M+O, D	heptachlorodibenzo-*p*-dioxins[2,4]	
3	460, 423, 390	M+O, D	octachlorodibenzo-*p*-dioxin[2,4]	0
3	506, 470, 436	M+O	chlorotetrabromobiphenyl[2,4]	
3	562, 482, 161	M+O	pentabromodiphenyl ether[2,4]	
3	540, 503	M+O	dichlorotetrabromobiphenyl[2,4]	
3	574, 540, 506	M+O	tetrabromotrichlorobiphenyl[2,4]	
4	408, 340, 237	D, F	β-chlordane[2,4,6]	0
4	410, 266, 237	D, F	α-chlordane[2,4,6]	
5, 6	255, 71	M+O, D	γ-HCH[2,4,6]	0
6	248, 71	M+O	DDTs[2,4]	
5	316	M+O, D, F	4,4′-DDE[2,4,6]	0
6	248, 35	M+O	2,4′-DDD[2,4,6]	
5, 6	318, 248, 283	M+O, D	4,4′-DDD[2,4,6]	+
9, 10	338, 303	M+O, F	γ-chlordene[2,4]	
10, 11	378, 346, 236	M+O, F	dieldrin[2,4,6]	0
11, 12	356, 320, 245	M+O, F, D	tetradifon[1,2,4,6]	
14–18	366	M+O, F, D	unknown[1–4]	
26	294, 221	M+O	carboxyhydroxyphenanthrene[2,4]	
		PAHs and S-PACs		
3	*142*	M+O	methylnaphthalene[1]	
3	*156*	M+O	dimethylnaphthalene[1]	
4	*154*	M+O, F, D	1,1′-biphenyl[1,6]	
4	*166*	M+O, F, D	9*H*-fluorene[1,6]	0
4	*184*	M+O, F, D	dibenzothiophene[1,5,6]	0
4	*178*	M+O, F, D	phenanthrene[1,6]	+?
4	*178*	M+O, F, D	anthracene[1,6]	0
4	*192*	M+O, F, D	C_1-*phenanthrenes*[1]	+?
4	*198*	M+O, F, D	C_1-dibenzothiophenes[1,5]	
4	*212*	M+O, F, D	C_2-dibenzothiophenes[1,5]	
4	*206*	M+O, F, D	C_2-phenanthrenes[1]	+?
5	*204*	M+O, F, D	2-phenylnaphthalene[1]	
4–8	*202*	M+O, F, D	fluoranthene[1,2,6]	+
4	*202*	D	acephenanthrylene[1,2]	0
4–7	*202*	M+O, F, D	pyrene[1,2,6]	0
4–7	*216*	M+O, F, D	C_1-pyrenes[1]	+?
4	*234*	M+O, F, D	benzo[*b*]naphtho[2,1-*a*]thiophene[1,5,6]	?
4–6	226	M+O, F, D	benzo[*ghi*]fluoranthene[1,2,6]	+?
5	226	F	cyclopenta[*cd*]pyrene[1,2,6]	+?
4	*248*	M+O, F, D	C_1-benzonaphthothiophene[1,5]	
6–8	*207, 129, 91*	M+O, F, D	styrene trimer[1]	
3–6	228	M+O, F, D	benzo[*c*]phenanthrene[1]	+
3–6	*228*	M+O, F, D	benz[*a*]anthracene[1,6]	+
3–6	*228*	M+O, F, D	chrysene + triphenylene[1,6]	+,+
4–6	*242*	M+O, F, D	C_1-chrysene[1]	+
4–8	252	M+O, F, D	benzo[*j*]+[*b*]fluoranthenes[1,6]	+
4–8	252	M+O, F, D	benzo[*k*]*fluoranthene*[1,6]	+
4–8	252	M+O, F, D	benzo[*a*]pyrene[1,2,6]	+
4–8	252	M+O, F, D	benzo[*e*]pyrene[1,6]	+
4–8	252	M+O, F, D	perylene[1,6]	+
6	268	M+O, F	methylcholanthrene[1,2]	+
5, 6	266	M+O, D, F	C_1-substituted m/z 252[1,2]	+?
6	280	M+O	C_2-substituted m/z 252[1,2]	+?
5, 6	276	M+O, D, F	indeno[1,2,3-*cd*]pyrene[1,2,6]	+
5, 6	278	M+O, D	dibenzanthracene[2]	+
5, 6	276	M+O, D, F	benzo[*ghi*]perylene[1,2,6]	+
6, 7	276	M+O, D, F	PAH m/z 276[1,2]	+?
6, 7	*230, 215, 202*	M+O, F, D	*o*-terphenyl[1]	
6	*230, 202*	M+O, F, D	*p*-terphenyl[1]	
6, 7	*282*	M+O, F, D	3,3′-dimethyl-1,1′-naphthalene[1]	
5, 6	290	M+O, D	C_1-substituted m/z 276[1,2]	+?
5, 6	302	M+O, D	dibenzopyrene[1,2]	+?
4	300	M+O, D, F	coronene[1,2,6]	+
9	*306, 215*	M+O, F, D	quaterphenyl isomer[1]	
		Oxy-PACs		
9	216	F	chlorobenzophenone[2]	
10–12	*250, 215, 139*	M+O, F, D	dichlorobenzophenone[1,2,4,6]	

Table II (Continued)

fractn	diagnostic ions[a]	occurrence	identification[b]	mutagen.[c]
10, 11	182	M+O	benzophenone[1,2,6]	
10	196	M+O	xanthone[2,6]	
10	*173, 145, 109*	M+O	dichlorobenzaldehyde[1]	
11, 12	*208, 180, 152*	M+O, D	anthracene-9,10-dione[1,26]	0
11, 12	*222, 165, 76*	M+O, D	C_1-substituted PAK m/z 208[1,2]	+?
10–12	258	M+O, D, F	benz[*a*]anthracene-7,12-dione[1,2,6]	+?
10–14	*180, 152, 126*	M+O, D, F	9*H*-fluoren-9-one[1,2,6]	+?
11	232	M+O	fluoranthenequinone[1,2]	
11, 12	*230, 202, 101*	M+O, F, D	7*H*-benz[*de*]anthracen-7-one[1,2,6]	+
11	280	M+O	dibenzofluorenone[2]	
10–13	194	M+O, F, D	C_1-fluoren-9-one[2]	0
10–13	208	M+O, F, D	C_2-fluoren-9-one[2]	0
11, 12	*204, 176, 135*	M+O, F, D	4*H*-cyclopenta[*def*]phenanthren-4-one[1,2,6]	+?
10–15	222	M+O, F, D	C_3-fluorenone[1,2]	+?
15–18	206	M+O, F	phenanthrenecarboxaldehyde[1,2]	
15, 16	220	M+O, F	C_1-phenanthrenecarboxaldehyde[1,2]	
15–17	220	M+O	coumarine PAH m/z 202[1,2]	
15–17	246	M+O, F	coumarine PAH m/z 228[1,2]	+?
15–18	254	M+O, D	benzopyrenone[1,2]	
17–18	234	M+O	C_2-phenanthrenecarboxaldehyde[2]	
18	218, 179	F	phenanthren-4-one[2,6]	
			Nitro-PACs	
10	247	F	1-nitropyrene[2–4,6]	++
10	247	F	nitrofluoranthene[2,4]	
10, 11	277	M+O	nitropyrenequinone[2–4]	+?
10	275, 261	M+O	nitro-*p*-terphenyl[2–4]	
10, 11	297, 267, 252	M+O	6-nitrobenzo[*a*]pyrene[2–4,6]	+
10, 11	297, 267, 252	M+O	1+2-nitrobenzopyrene[2,3]	++
11	273	M+O	6-nitrochrysene[2–4,6]	+?
26	284, 254	M+O+O	dinitrophenanthren-4-one	+?
26, 27	289	M+O, F	hydroxynitrochrysene[2–4]	+?
27	263	F	hydroxynitropyrene[2,3,6]	+?
28	275	M+O	nitrophenanthrenecarboxaldehyde[2–4]	+?
28	238, 224, 126	M+O	methylnitroacridines[2–4]	
			Aromatic Carboxylic Acids, Anhydrides, and Esters	
10, 11	*203, 173, 145*	M+O	dichloromethyl benzoate[2,4]	
14	162	M+O	C_1-phthalic anhydride[2,4]	
14, 15	176	M+O	C_2-phthalic anhydride[2,4]	
17–19	390, 148	M+O, F, D	diethylhexyl octyl phthalate[2,4]	0
18, 19	418, 260, 148	M+O, F	dinonyl phthalate[2,4]	
19, 20	198	M+O	1,8-naphthalic acid anhydride[2,4,6]	+?
19	248	M+O	benzonaphthalic anhydride[2,4]	+?
23	278, 206, 148	M+O, D	dibutyl phthalate[2,4]	
26	194, 148, 109	M+O	dimethyl terphthalate[2,4]	0
29–37	*179, 105*	M+O	benzenecarboxylic acid[2,4,6]	
36	*262, 247, 173*	M+O	dichlorobenzenecarboxylic acid[2,4]	
			Phenols	
10	210, 176	M+O	trichloroanisole[2,3]	
16, 17	*292, 221, 207*	D, F	nonylphenol TMS	0
18	252, 79	D	2,4-dibromophenol[2,4,6]	
18, 19	296, 79	M+O, F, D	dibromodimethoxybenzene[2,4,6]	
24–26	211, 139	M+O	4-nitrophenol[2,4,6]	0
26, 27	139	M+O, D	2-nitrophenol[2,4,6]	0
26, 27	207, 190, 177	M+O	dichloronitrophenol[2,4]	
27, 28	210	M+O	dihydroxyanthracene[2,4]	
			Other Nitrogen Compounds	
9	*182, 152, 105*	M+O, F, D	azobenzene[1–3]	+
25, 26	*195*	M+O, F	trichloroaniline[1–3]	0
10–12	167, 139	F	carbazole[1,3,6]	0
11, 12	*217*	D, F	benzo[*c*]carbazole[1,3]	0
12–14	*181, 148, 108*	M+O	methylbenzthiazole[1,3,5]	
19	*179, 73*	M+O	acridine[2,3,6]	
26	229	M+O	tetrachloroaniline[1–3]	
26–28	229	M+O, F, D	benzacridine[2,3]	+?
26	243	M+O, F, D	C_1-benzacridine[2,3]	+?
26, 28	253	M+O	phenanthroquinoline[2,3]	
28	203	M+O	azafluoranthenes[2,3]	
28	197, 180, 165	M+O	methylhydroxycarbazole[2,3]	
32	279	M+O	dibenzacridine[2,3]	+?

[a] It is referred to NICI MS unless stated. EI MS diagnostic ions are in italics. [b] Method: (1) EI mass spectra, (2) NICI mass spectra, (3) NPD positive response, (4) ECD positive response, (5) FPD positive response, (6) positive identification by coinjection with an authentic standard. [c] Mutagenicity summary response according to reported results: (+) mutagenic, (0) nonmutagenic, (+?) belongs to a mutagenic class or results are not conclusive (*4, 51–53, 86–89*).

Table III. Quantitative Distribution of Selected Compounds (ng g^{-1} dry wt)

compound	sampling site[a] D	M+O	F
PCBs			
IUPAC no. 52	17	68	70
IUPAC no. 101	15	126	66
IUPAC no. 138	17	246	104
IUPAC no. 153	10	218	103
IUPAC no. 180	6	204	88
4,4′-DDE	41.9	19.0	115.3
hexachlorobenzene	8.5	12.0	118.2
dieldrin	0.9	nd	12.0
tetradifon	2.4	nd	5.6
PAHs			
phenanthrene	80	27.0	38.8
anthracene	15.8	10.0	42.0
fluoranthene	274.9	81.8	44.6
pyrene	267.5	88.4	60.2
benz[*a*]anthracene	118.2	46.8	122.2
chrysene + triphenylene	141.3	56.4	83.4
benzo[*j*+*b*]fluoranthene	130.4	105.4	82.0
benzo[*a*]pyrene	118.1	nd	20.6
benzo[*a*]pyrene	85.7	50.4	36.0
perylene	28.7	nd	nd
indeno[1,2,3-*cd*]pyrene	59.6	32.4	12.0
benzo[*ghi*]perylene	45.6	8.6	17.4
coronene	3.0	1.0	nd
PAKs			
9*H*-fluoren-9-one	7.0	nd	22.5
benzo[*a*]fluoren-9-one	1.8	nd	19.6
benzo[*c*]fluoren-9-one	24.2	nd	24.2
nitro-PACs			
carbazole	nd	nd	8.1
1-nitropyrene	nd	nd	0.68
6-nitrochrysene	nd	0.52	nd
x-nitrobenzofluoranthene	nd	0.26	nd
6-nitrobenzo[*a*]pyrene	nd	0.34	nd
nitrodibenzopyrene	nd	0.33	nd

[a] nd, below detection limit 0.1 ng g^{-1} dry wt.

are the major components of the intermediate fractions (11–12) (Table II and Figure 5A).

Another class of OCs present at lower concentrations in the nonpolar fractions is the hepta- and octachlorodibenzo-*p*-dioxins, probably due to their higher persistence in the aquatic environment or higher lipophilicity (log K_{ow} = 9.1–10.5) (*47*). This pattern of distribution has been previously reported on lacustrine sediments (*48*) and it can be attributed to waste incineration processes.

Despite most of the halogenated compounds identified are carcinogenic (*49*), their contribution to the fraction mutagenicity is rather limited. In fact, only the 4,4′-DDD has been recognized as mutagenic among the organohalogenated compounds present in the low-polarity fractions.

(b) Polycyclic Aromatic Hydrocarbons (PAHs). A large variety of parent and alkylated polycyclic aromatic hydrocarbons containing from two to seven aromatic rings have been identified (Table II). The higher concentrations were found in the Besos estuary (station D, Table III), where the ratio of methylphenanthrenes to phenanthrene (MP/P), close to 0.78, indicates a predominance of pyrolytic sources. Conversely, this ratio was as high as 6.35 in the sewage disposal site (station F), pointing to a predominance of fossil sources.

Several parent and alkylated PAH-containing sulfur heterocycles (S-PACs) were also identified in the low-polarity NP-LC fractions (Table II). The predominance of alkylated dibenzothiophenes and benzonaphthothiophenes suggests a fossil origin. Furthermore, their spatial distribution covariates with other fossil fuel indicators (i.e., MP/P and UCM).

The occurrence of these PAHs may account for the mutagenicity maximum observed in fractions 3–7. Most of the four to six and some of the three condensed aromatic ring PAHs identified are, in fact, mutagenic in the presence of exogenous metabolic activation (*50–52*). In this regard, the highest levels of both mutagenicity and PAH concentration were found in the Besos river estuary, where pyrolytic sources are more evident. Consequently, the mutagenicity recovered in the low-polarity fractions could be attributable to pyrolytic sources rather than to fossil ones. In addition, some of the S-PACs identified (i.e., benzo[*b*]naphtho[2,1-*d*]thiophene) have also been recognized as mutagenic (*53*).

(c) Oxygenated-PACs (Oxy-PACs). A large variety of parent and alkylated polycyclic aromatic ketones (PAKs) and aromatic carboxaldehydes were identified as major components in the intermediate-polarity NP-LC fractions (10–14) (Table II and Figure 5). 9*H*-Fluoren-9-one and its C_1–C_3 alkylated derivatives, 4*H*-cyclopenta[*def*]phenanthren-4-one and 7*H*-benz[*de*]anthracen-7-one, are the most representative of this class of compounds. The concentrations were similar to those of the parent PAHs (Table III).

PAKs mostly originated by oxidation from reactive PAHs during combustion of fossil fuels or wood (*54–56*), or photooxidation during the atmospheric transport of soot particles (*56–58*). However, they can also be formed in the aquatic environment. Ehrhardt and Doabul (*60*) have identified some PAKs in the dissolved phase of seawater, assuming an "in situ" photolysis of PAHs of fossil origin. It is also well-known that the metabolic degradation of PAHs proceeds through the formation of oxygenated intermediates. In spite of the wide distribution of these compounds in the environment, their occurrence in marine sediments is almost undocumented (*59*).

Other classes of oxy-PACs identified in the intermediate fractions of the sewage disposal and coastal sediment extracts (stations M+O and F) were anthraldehydes (two isomers) and their mono- and dialkylated derivatives. These compounds have been previously identified in vehicular exhaust emissions (*53*, *54*) and in urban particulate matter (*56*). Furthermore, several coumarines (xanthone, 5*H*-phenanthro[4,5-*bcd*]pyran-5-one, and chrysene coumarine) and parent polycyclic aromatic quinones (anthracene-9,10-dione, fluoranthenequinone, and benz[*a*]anthracene-7,12-dione) have been identified at high concentrations in station M+O. These chemical classes have been previously reported in diesel exhaust and urban particulate matter (*56*, *61*, *62*). Their occurrence in marine sediments is reported for the first time and could be considered as tracers of inputs from urban street runoff.

A variety of alkylated and halogenated phenols have also been identified in this area (Table II). However, taking into account their apparently low contribution to sediment mutagenicity, they will be considered elsewhere (*63*).

Finally, the 1,8-naphthalenedicarboxylic acid anhydride and its benzologue, identified in coastal sediments (Table II), represent markers of diesel exhaust emission (*64*, *65*). Apparently, environmental chamber experiments have provided evidence that they are the degradation products of several PAHs in the presence of sunlight with traces of reactive atmospheric species (e.g., O_3) (*66*).

The contribution of the mutagenicity of the extracts of the different classes of oxy-PAHs identified in the intermediate fractions is rather limited but it cannot be underestimated. Benzopyrenone and aromatic carboxaldehydes are slightly direct-acting mutagens (*67*) and

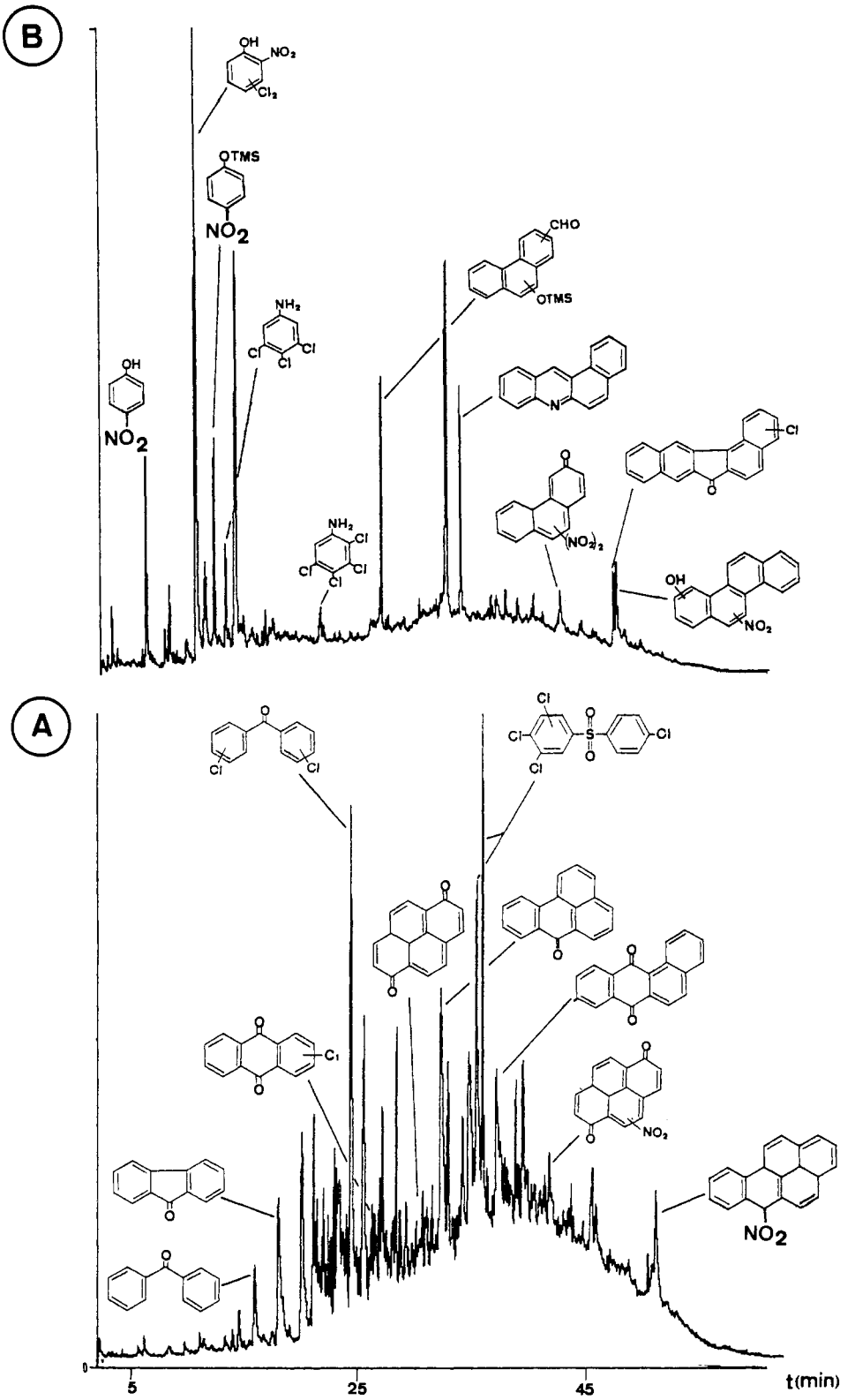

Figure 5. NICI MS total ion current of the strongly mutagenic NP-LC subfractions: (A) 11 and (B) 26 isolated from the sampling sites M+O.

some quinones have demonstrated some activity (*68*). In addition, some dicarboxylic acid anhydrides and coumarine PAH derivatives are also slightly mutagenic (*67*, *69*). The distribution of oxy-PACs in the RP-LC fractions, most of them eluting in earlier fractions than the mutagenicity maximum (Table IV and Figure 4), is further evidence of their small contribution, if any, to the mutagenicity of the corresponding fractions. Nevertheless, the high specificity of these source markers, and their apparent stability in sediments, is particularly interesting for recognition of urban inputs into coastal areas, even though their fate in the marine environment is still unknown.

(d) Nitroarenes (Nitro-PACs). These constitute a novel class of compounds identified in the intermediate-polarity fractions of coastal sediments and in the sewage disposal area (Figure 5 and Table II). In fact, 6-nitrochrysene, 6-nitrobenzo[*a*]pyrene, and 1-nitropyrene have been positively identified for the first time in marine sediments. In addition, *x*-nitrofluoranthene, *x*-nitrobenzofluoranthene, 1+3-nitrobenzo[*a*]pyrene, *x*-nitro-

Table IV. Chemical Composition of the Mutagenic RP-LC Fractions Isolated from M+O Sampling Site

compd no.[a]	fractn	diagnostic ions,[b] m/z	identification	mutagen.[c]
1	5	244	C_1-PAK m/z 230	0
2	5	258	benz[a]anthracenedione	+?
3	5, 6	277	nitropyrenequinone	+?
4	5	253	nitroanthraquinone	+?
5	6	254	pyrenequinone	
6	5	273	6-nitrochrysene	+
7	6	366	unknown	
8	7, 8	303	nitrobenzanthracenedione	+?
9	7	297, 267, 252	nitrobenzofluoranthene	+?
10	7, 8	297, 267, 252	1+3-nitrobenzo[a]pyrene	+
11	7, 8	297, 281, 267	6-nitrobenzo[a]pyrene	+
12	7	321, 305, 292	nitroindeno[1,2,3-cd]pyrene	+
13	8	336	γ-chlordene	
14	10	392	unknown	

[a] Compound number corresponds to Figure 6. [b] NICI MS diagnostic ions. [c] See Table II for key.

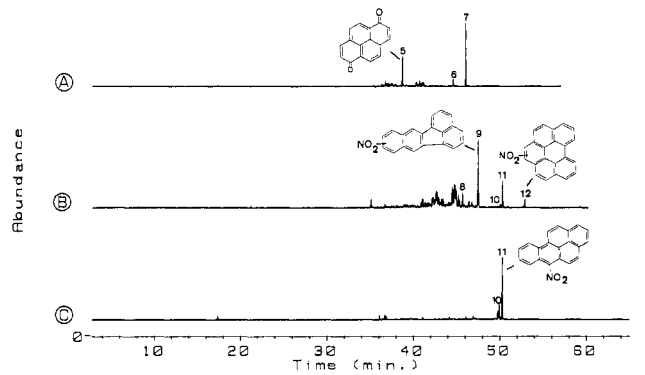

Figure 6. NICI MS total ion current of the strongly mutagenic RP-LC subfractions: (A) 6, (B) 7, and (C) 8. Compound identification is listed in Table V.

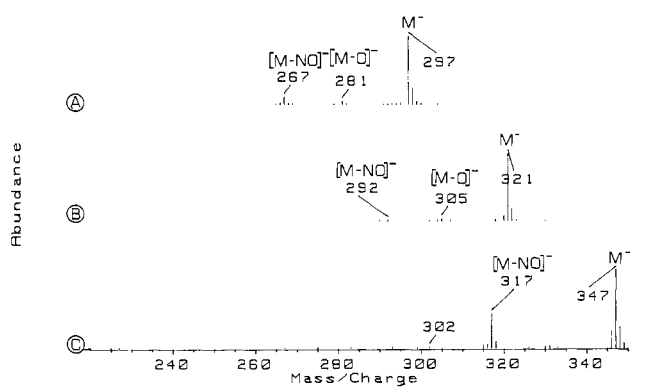

Figure 7. NICI mass spectra of the major nitro-PACs identified in the mutagenic RP-LC fractions: (A) 6-nitrobenzo[a]pyrene, (B) x-nitroindeno[1,2,3-cd]pyrene, and (C) x-nitrodibenzopyrene.

indeno[1,2,3-cd]pyrene, and x-nitrodibenzopyrenes were also tentatively identified (Figure 6). The NICI mass spectra of these compounds exhibited the molecular anion [M^-] as the base peak, and $[M - O]^-$ and $[M - NO]^-$ or $[M - NO_2]^-$ as small fragments (Figure 7), attributable to electron capture and dissociative mechanisms (*70*).

Several sources of nitro-PAC in the marine environment can be envisaged, among them, atmospheric transport of vehicular emissions or street runoff, since 1-nitropyrene, 6-nitrochrysene, x-nitroterphenyl, and 6-nitrobenzo[a]-pyrene have been previously reported in diesel and leaded gasoline exhaust emissions (*67*, *55*). In this respect, it is interesting to note that diesel exhausts are particularly enriched in 1-nitropyrene, whereas leaded gasoline contains higher concentrations of 6-nitrobenzo[a]pyrene (*71*, *72*). The similar concentrations of these components (Table III) suggest comparable inputs from leaded and diesel vehicular emissions. The other nitro-PACs identified in this study have not been previously reported in the marine environment, and the predominance of higher molecular weight components could indicate an accumulation of the most hydrophobic species in sediments. Recently, Suzuki et al. (*73*) also reported the formation of nitroarenes, namely, 1-nitropyrene, by photolytic nitration of pyrene in seawater containing nitrite, but this seems to be an unlikely source in the present area of study.

Although most of the nitro-PACs are direct-acting mutagens, some of them are more active in the presence of exogenous metabolic activation (*74*). In this regard, 1-nitropyrene, 2- and 6-nitrochrysenes, and 6-nitrobenzo-[a]pyrene are direct mutagens but their mutagenicity is enhanced in the presence of metabolic activation. Therefore, the mutagenicity recovered in fractions 7 and 8 of the RP-LC fractionation (Figure 4) could be accounted for by the identified nitrobenzopyrenes and 6-nitrochrysene. Another piece of supporting evidence for the contribution of nitro-PAC to this mutagenicity is the remarkable reduction of the activity of the fraction 8 when tested against the deficient nitroreductase strains TA98NR and TA98/1,8DNP (Table V). Other possible candidates are x-nitrobenzofluoranthene and nitrodibenzopyrenes identified in fractions 7 and 10, respectively.

The components responsible for the mutagenicity recovered in the RP-LC fraction 6 are still unknown, since the major one (compound 7 in Figure 6) has not yet been identified (Table IV). This compound [EI (m/z): M^+ 366 (66), 368 (36), 310 (21), 248 (35), 84 (100) and NICI (m/z): M^- 366 (100), 368 (40)] may contain two nitrogens and two chlorine atoms according to CGC (NPD-ECD) and mass spectral data. Although it was recognized as a major component in the intermediate-polarity NP-LC fractions, its major occurrence in the sewage disposal area, where the lowest levels of mutagenicity were found (station F, Figure 3) could indicate a rather limited contribution of this compound to the mutagenicity of RP-LC fraction 6.

Other nitro-PACs, containing additional polar substituents (hydroxy or carboxy), and nitro-substituted azaarenes have also been tentatively identified in the polar fractions (Table II and Figure 2). Several nitrohydroxy compounds and nitroquinones have been previously identified in diesel exhaust emissions and in urban atmospheric particulate matter (*29*, *75*). In addition, nitrophenolic compounds and several chlorinated derivatives were also identified as major components of the polar fractions (i.e., fraction 26, Figure 5B). These could be

Table V. Mutagenicity (Revertants Plate^{-1}) of the RP-LC Fraction 8 Isolated from M+O Sampling Site against Different Nitroreductase-Deficient Tester Strains[a]

	TA98	TA98NR	TA98/ 1,8DNP
spontaneous reversion	41	38	27
equiv to 1 mg of GPC-2[b]	776	378	76

[a] Assays were carried out in the presence of S9. [b] Assays were performed in duplicate.

secondary pollutants formed by tropospheric transformation of monoaromatic chemicals (*76*) or by hydrolytic or photolytic processes from several organophosphorus pesticides (*77*) and may account for the maximum of mutagenicity exhibited by these polar NP-LC fractions (Figure 3).

Other Nitrogen-Containing Compounds. Chlorinated anilines and azobenzene have been identified in the intermediate fractions (Table II). They are industrial synthetic intermediates, but the latter may also be the degradation product of 1,2-diphenylhydrazine (*78*), a synthetic intermediate used in dyestuff production. On the other hand, azaarenes and carbazoles are characteristic components of crude oils (*79, 80*) and coal tars (*81*). In addition, some of them have been identified in urban atmospheric particulate matter (*82*) and sediments from enclosed bays (*83*). Methylbenzthiazoles, also identified in the intermediate-polarity fractions, appear to be derived from the use of antioxidants in the manufacture of rubber tires, and consequently, they have been proposed as indicators of street runoff (*84*). Finally, in the most polar fraction (36), the well-known trialkylamines (TAMs) (*85*), ascribed to cationic surfactants, have been recognized.

Among the nitrogen-containing compounds identified, azobenzene is the only known contributor to the mutagenicity of the intermediate-polarity fractions, whereas several azaarenes and nitrohydroxy and other polar-substituted nitrated compounds could contribute to the mutagenicity recovered in the polar fractions (i.e., 26–28). In fact, the benz[*c*]acridines and dibenz[*a,j*]acridines have demonstrated high activity with TA-100 in the presence of metabolic activation (*51, 52*). Indeed, a fraction containing three to four aromatic ring azaarenes isolated from Black River sediments exhibited mutagenic activity against TA98+S9 (*22*).

Conclusions

The bioassay-directed chemical analysis of coastal sediment extracts has proved to be an adequate approach for the identification of genotoxic components in the marine environment. The application of semipreparative GPC to the organic extracts enabled recovery of most of the genotoxic compounds in a single fraction. Further separation of this fraction by NP-LC into 35 fractions exhibited a two–three modal mutagenicity distribution, with the highest level being recovered in the intermediate fractions (11–13). These were further separated by RP-LC into 10 subfractions and the mutagenicity was recovered in those where nitrated arenes (four to six aromatic rings) were present. Among them, 1-nitropyrene, 6-nitrochrysene, and 6-nitrobenzo[*a*]pyrene were positively identified for the first time in coastal sediments.

PAHs containing four to six aromatic rings were identified in the mutagenicity maximum of the low-polarity NP-LC fractions (4–6). On the other hand, polar-substituted nitroarenes and azaarenes possibly accounted for most of the mutagenicity maximum observed in the polar fractions (26–28).

The distribution of the mutagenicity in the area of study illustrates the complexity of the direct assessment of genotoxicity in coastal environments. In fact, sediments collected in the main pollutant sources, namely, the Besos estuary (station D) and the sewage disposal area (station F) exhibited lower mutagenic activity than sampling sites located farther (stations M–N). The composition of the relevant fractions indicates that pyrolytic sources (either by street runoff or atmospheric deposition) are the major contributors to the mutagenicity in coastal urban areas and nitroarenes the major compounds of concern.

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Registry No. PCB-52, 35693-99-3; PCB-101, 37680-73-2; PCB-138, 35065-28-2; PCB-153, 35065-27-1; PCB-180, 35065-29-3; *v*-HCH, 58-89-9; DDT, 50-29-3; 4,4′-DDE, 72-55-9; 2,4′-DDD, 53-19-0; 4,4′-DDD, 72-54-8; pentachlorobenzene, 608-93-5; hexachlorobenzene, 118-74-1; pentachlorobisphenyl, 25429-29-2; hexachlorobiphenyl, 26601-64-9; heptachlorobiphenyl, 28055-71-2; octachlorobiphenyl, 55722-26-4; nonachlorobiphenyl, 53742-07-7; decachlorobiphenyl, 2051-24-3; chlorobromobiphenyl, 56486-89-6; heptachlorodibenzo-*p*-dioxin, 37871-00-4; octachlorodibenzo-*p*-dioxin, 3268-87-9; chlorotetrabromobiphenyl, 138542-41-3; pentabromodiphenyl ether, 32534-81-9; dichlorotetrabromobiphenyl, 107227-63-4; tetrabromotrichlorobiphenyl, 138542-42-4; β-chlordane, 5103-74-2; α-chlordane, 5103-71-9; γ-chlordane, 56641-38-4; dieldrin, 60-57-1; tetradifon, 116-29-0; carboxyhydroxyphenanthrene, 138542-43-5; methylnaphthalene, 1321-94-4; dimethylnaphthalene, 28804-88-8; 1,1′-biphenyl, 92-52-4; 9*H*-fluorene, 86-73-7; dibenzothiophene, 132-65-0; phenanthrene, 85-01-8; anthracene, 120-12-7; C_1-phenanthrene, 31711-53-2; C_1-dibenzothiophene, 30995-64-3; 2-phenylnaphthalene, 612-94-2; fluoranthene, 206-44-0; acephenanthrylene, 201-06-9; pyrene, 129-00-0; C_1-pyrene, 27577-90-8; benzo[*b*]naphthol[2,1-*d*]-thiophene, 239-35-0; benzo[*ghi*]fluoranthene, 203-12-3; cyclopenta[*cd*]pyrene, 27208-37-3; C_1-benzonaphthothiophene, 67526-85-6; styrene trimer, 28213-80-1; benzo[*c*]phenanthrene, 195-19-7; benz[*a*]anthracene, 56-55-3; chrysene, 218-01-9; triphenylene, 217-59-4; C_1-chrysene, 41637-90-5; benzo[*k*]fluoranthene, 207-08-9; benzo[*a*]pyrene, 50-32-8; benzo[*e*]pyrene, 192-97-2; perylene, 198-55-0; methylcholanthrene, 56-49-5; indeno[1,2,3-*cd*]pyrene, 193-39-5; dibenzanthracene, 67775-07-9; benzo[*ghi*]-perylene, 191-24-2; *o*-terphenyl, 84-15-1; *p*-terphenyl, 92-94-4; 3,3′-dimethyl-1,1′-naphthalene, 34042-82-5; dibenzopyrene, 58615-36-4; coronene, 191-07-1; chlorobenzophenone, 51330-06-4; dichlorobenzophenone, 90-98-2; benzophenone, 119-61-9; xanthone, 90-47-1; dichlorobenzaldehyde, 31155-09-6; anthracene-9,10-dione, 84-65-1; benz[*a*]anthracene-7,12-dione, 2498-66-0; 9*H*-fluoren-9-one, 486-25-9; fluoranthenequinone, 39407-42-6; 7*H*-benz[*de*]-anthracen-7-one, 82-05-3; dibenzofluorenone, 83589-46-2; C_1-fluoren-9-one, 77468-39-4; 4*H*-cyclopenta[*def*]phenanthren-4-one, 5737-13-3; phenanthrenecarboxaldehyde, 77468-40-7; C_1-phenanthrenecarboxaldehyde, 125166-26-9; benzopyrenone, 87933-79-7; phenanthren-4-one, 138542-39-9; 1-nitropyrene, 5522-43-0; nitrofluoranthene, 77468-36-1; nitropyrenequinone, 80267-75-0; nitro-*p*-terphenyl, 88526-52-7; 6-nitrobenzo[*a*]pyrene, 63041-90-7; 1-nitrobenzopyrene, 70021-99-7; 2-nitrobenzopyrene, 138542-40-2; 6-nitrochrysene, 7496-02-8; dinitrophenanthren-4-one, 138570-98-6; hydroxynitrochrysene, 116212-01-2; hydroxynitropyrene, 104557-41-7; nitrophenanthrenecarboxaldehyde, 138542-44-6; methylnitroacridine, 138542-45-7; dichloromethyl benzoate, 2648-61-5; C_1-phthalic anhydride, 30140-42-2; diethylhexyl octyl phthalate, 138570-99-7; dinonyl phthalate, 84-76-4; 1,8-naphthalic acid anhydride, 81-84-5; dibutyl phthalate, 84-74-2; dimethyl terphthalate, 120-61-6; benzencarboxylic acid, 65-85-0; dichlorobenzenecarboxylic acid, 75248-87-2; trichloroanisole, 53452-80-5; 2,4-dibromophenol, 615-58-7; dibromodimethoxybenzene, 96141-26-3; 4-nitrophenol, 100-02-7; 2-nitrophenol, 88-75-5; dichloronitrophenol, 26761-59-1; dihydroxyanthracene,

70143-54-3; azobenzene, 103-33-3; trichloroaniline, 54686-91-8; carbazole, 86-74-8; benzo[c]carbazole, 34777-33-8; methylbenzthiazole, 55299-16-6; acridine, 260-94-6; tetrachloroaniline, 53014-40-7; benzacridine, 12041-95-1; C_1-benzacridine, 54116-90-4; phenanthroquinoline, 104220-33-9; azafluoroanthene, 89126-45-4; methylhydroxycarbazole, 138542-46-8; dibenzacridine, 65777-07-3.

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