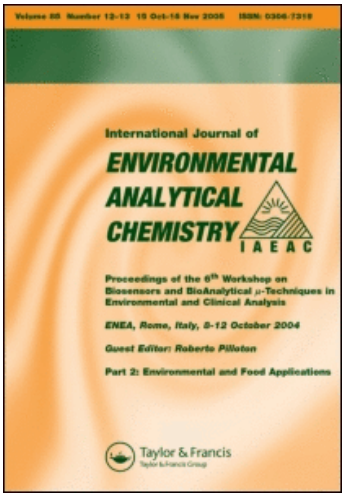

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CHROMATOGRAPHIC ASSAY OF POLYNUCLEAR AROMATIC HYDROCARBONS IN REFINED MINERAL OILS APPLIED AS DISPERSING AGENTS FOR HERBICIDES

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Representative refined mineral oil samples—crude petroleum straight-run distillates—potentially used in the manufacture of crop protection products were analyzed by isocratic and gradient high-performance liquid chromatography (HPLC) to investigate their trace polynuclear aromatic hydrocarbons (PAH) content. Liquid–liquid extraction followed by size-exclusion column chromatography has been employed to isolate the fraction containing PAHs with more than two fused rings from the oils studied. The identity of individual PAHs has been confirmed by comparing the UV and fluorimetric detector signals with those of reference standards. The level of extracted PAHs in the oil samples is discussed with respect to their physical and chemical properties.

KEY WORDS: PAHs, gradient HPLC, mineral oils, plant protection.

INTRODUCTION

The use of mineral oils as dispersing agents for herbicides containing different active ingredients, e.g. 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (Atrazine), 3,6-di-chloro-o-anisic acid (Dicamba), and 2-(2,4-dichlorophenoxy)acetic acid (2,4-D), leads to their effective adherence and coating on the weed surface. A mineral oil additive to herbicide also intensifies the selectivity of active substances and increases their penetration into plant cells. Such application of these oils allows a considerable reduction in the concentration of the active crop protection substance used with, e.g., sugar-beets, corn and different vegetables.[1] Apart from economic advantages, the procedure decreases herbicide residues in soil and groundwaters which is meaningful for suitable crop rotation. Furthermore, the concentration of harmful herbicides or their degradation products in foodstuffs is reduced.

However, mineral oils and related products contain polynuclear aromatic hydrocarbons (PAHs),[2] some of which are specified as chemical carcinogens for man or animals and are widely distributed in the human environment.[3] The presence of trace amounts of PAHs in mineral oils used in plant protection can lead to an increase of the exposure of the general population to these ubiquitous chemical pollutants.

Various analytical approaches, which involve mainly chromatographic

Table 1 Physicochemical properties of the analyzed mineral oils

Mineral oil samples	*Distillation temperature* (°C)	*Flash temperature* (°C)	*Freezing temperature* (°C)	*Viscosity (cSt)*	
				50 °C	*100 °C*
1	332–471	204	+16	58	4.70
2	403–516	220	−21	47	8.20
3	390–440	215	−18	78	5.25
4	380–400	180	−18	79	4.91
5	405–460	210	−12	60	6.20
6	370–430	190	−5	95	7.50

techniques,[4] are used for PAH determination in mineral oils used in cosmetic,[5] pharmaceutical,[6] jute[7] and food[8] industries. This paper describes the use of HPLC with two different separation or detection modes for the determination of trace levels of PAHs in various mineral oil probes.

EXPERIMENTAL

Materials

The samples of mineral oils studied were obtain from the Institute of Organic Industry (Warsaw, Poland), the Institute of Heavy Organic Synthesis (Kędzierzyn, Poland) and the Chemical Factory Organic-Azot (Jaworzno, Poland). The characteristic values of these oils were obtained according to standard methods[10] and are given in Table 1.

The solid PAH standards, i.e. anthracene, phenanthrene, pyrene, fluoranthene, chrysene, benz(a)anthracene, benzo(a)pyrene, benzo(e)pyrene, perylene, benzo(b)-fluoranthene, benzo(j)fluoranthene, indeno(1,2,3-cd)fluoranthene, pentacene, dibenz-(a,h)anthracene, and coronene, were obtained from the Biochemisches Institut für Umweltcarcinogene (Ahrensburg, FRG) or Fluka AG (Buchs, Switzerland).

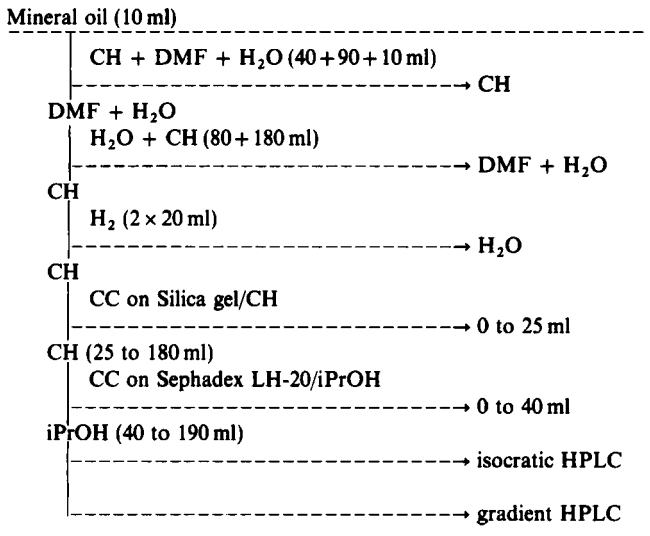
Standard solutions containing 40–140 ng/μl of the individual PAH or mixtures of them were prepared in suprapure cyclohexane (E. Merck, Darmstadt, FRG). Pentacene (0.25 mg/ml in cyclohexane) was used as an internal standard.

Spectrograde acetonitrile, methanol and isopropanol as supplied by E. Merck, heptane and dimethylformamide from Reachim (USSR) or POCh (Gliwice, Poland), redistilled before use, were applied as the mobile phase components.

Silica gel (60/120 mesh) from BDH (Poole, UK) and Sephadex LH20 (25–100 μm) supplied by Pharmacia Fine Chemicals AB (Uppsala, Sweden) were used as the supports in column chromatography (CC) experiments. All glassware was cleaned with potassium permanganate in sulfuric acid solution according to Castegnaro *et al.*[11]

Instrumentation

Isocratic HPLC elution was performed using a Liquochrom 2010 liquid chromato-



CH = Cyclohexane, DMF = Dimethylformamide, iPrOH = Isopropanol, CC = Column chromatography.

Figure 1 Scheme of PAHs enrichment procedure.

graph (Labor MIM, Budapest, Hungary) equipped with a fixed loop (20 μl) injector, multiwavelength UV detector, Radelkis OH-814/1 strip-chart recorder and Cobrabid KB5503 integrator. Stainless steel columns (25 × 0.46 mm I.D.) filled with Chromsil silica gel[9] were used in this study.

Gradient HPLC elution was carried out with a LDC gradient modular liquid chromatograph (Laboratory Data Control, Stone, UK) equipped with a single wavelength UV detector and filter fluorimetric detector, dual channel TZ4200 (Laboratorni Pristroje, Prague, Czechoslovakia) strip-chart recorder. A stainless-steel column (25 × 0.46 cm I.D.) packed with Li-Chrosorb RP-18 (($d_p = 10\ \mu m$) (E. Merck) was used.

The column chromatographic investigations were carried out with all-glass columns sealed in a glass jacket, supplied by VEB Werk für Technisches Glas, Ilmenau, GDR, a Elmed-304 peristaltic pump and Premed 206N fraction collector. These experiments were performed at (25 ± 1) °C using a U-10 water thermostat. A rotary evaporator Unipan 350 was used in all experiments.

Procedure

Extraction and clean-up of PAHs from oils

The enrichment of PAHs from analyzed mineral oils was carried out by the method of Grimmer and Bohnke[12] with some modification. The scheme is presented in Figure 1. To a 250 ml separatory funnel, 10 ml of the mineral oil, 40 ml of cyclohexane, and 100 ml of DMF-water (90:10) were added. The contents were vigorously shaken and allowed to separate into two layers. The upper layer (cyclohexane) was discarded and the lower layer (DMF-water) was diluted with

80 ml of water followed by extraction with 180 ml of cyclohexane in a 500 ml separatory funnel. The lower DMF-water layer was discarded and the upper cyclohexane layer was washed twice with 20 ml of water and dried over anhydrous Na_2SO_4. The cyclohexane was evaporated to about 0.5 ml with a rotary evaporator under an argon atmosphere (flow rate 20 ml/min) at 35 °C and the PAH fraction, so obtained, was weighed and used for further separation.

Column chromatography

The PAH-rich fraction was further purified by column chromatography using silica gel as absorbent (60/120 mesh, 75 g in a 30 × 1.5 cm I.D. glass column) activated at 130 °C for 8 h and deactivated with 2 wt.% of water. The column was eluted with 180 ml cyclohexane at a flow rate 2 ml/min. The first 25 ml were discarded and the rest (155 ml containing all the PAHs with two or more fused rings) was collected in a round-bottomed flask. The cyclohexane was evaporated from the latter fraction almost to dryness with a rotary evaporator, under conditions mentioned earlier, and the residue was dissolved in 1 ml isopropanol.

Isolation of PAHs containing more than two fused rings was achieved on a 20 × 1 cm I.D. glass column packed with 10 g of Sephadex LH20 and equilibrated with 50 ml isopropanol for 24 h. The residue in isopropanol obtained from the silica gel column was quantitatively transferred to this column and the flask was rinsed twice with 1 ml of isopropanol. The rinsings were also added to the column. A first elution was carried out with 40 ml isopropanol, at a flow rate 1 ml/min. This fraction was discarded; then, elution was carried out with 150 ml of the same solvent. The latter fraction was concentrated to about 1 ml using a rotary evaporator under the conditions specified above, and 1 ml of acetone was added. The concentrate so obtained was transferred quantitatively to a 10 ml concentration tube. The flask was rinsed twice with 1 ml of acetone and the rinsings were added to the tube. The solvent was evaporated almost to dryness and the residue containing PAHs was weighed and dissolved in 100 μl of DMF. It was stored in a dark flask for HPLC analysis.

HPLC analysis

The isocratic HPLC conditions were as follows: mobile phase, heptane (dried and degassed); flow rate, 0.75 ml/min; column temperature, 25 °C; injection volume, 20 μl; detection wavelength, 254 nm; detector sensitivity, 0.05 a.u.f.s; chart speed, 0.25 mm/sec.

The gradient HPLC conditions were as follows: three-component mobile phase (methanol–water–acetonitrile) with composition changing according to the following programme.

Calculation methods

For calculating the PAH concentrations, the chromatograms recorded by gradient HPLC with flourescence detection were used. A standard solution with known PAH concentration was injected just before sample analysis. If V_I (ml) is the volume of the sample extract injected and h_1 (cm) is the peak height of the ith PAH in the sample, its concentration C_i (μg/kg) in the original mineral oil sample is given by

Time (min)	Mobile phase composition[a]		
	%MeOH	$\%H_2O$	%AcCN
0	75	25	0
12	85	15	0
24	85	15	0
32	0	0	100
60	0	0	100

[a]Flow rate, 1.5 ml/min; column temperature, 25°C; injection volume, 20 μl; detection wavelengths. 254 nm (UV III Monitor), 310 nm (excitation) and 440 nm (emission) (Fluoro Monitor III); slit width for both monochromators, 8 nm; detector sensitivity, 0.015 and 5 a.u.f.s for absorbance and fluorescence detection, respectively; chart speed, 0.05 mm/sec.

$$C_i = V_E * h_i * m_s / V_I * h_s * m \quad (1)$$

where h_s(cm) is the peak height of the PAH standard with identical retention time as the ith PAH; m_s(g) is the weight of this PAH injected with the standard solution; m(kg) is the weight of mineral oil used in the extraction procedure; and V_E(ml) is the total volume of the PAH extract analysed by HPLC.

RESULTS AND DISCUSSION

In Table 1 the physicochemical data of the mineral oils studied are given. All these mineral oils were produced during crude petroleum straight-run distillation followed by selective furfurol refining, dewaxing with solvent method and refining with hydrogen. These oils differ mainly in distillation temperature and viscosity.

Representative examples of isocratic adsorption HPLC and gradient reversed-phase HPLC of PAHs isolated from the mineral oils specified as No. 2 and No. 3 in Table 1, are presented in Figures 2 and 3, respectively. As can be seen, the use of adsorption HPLC on the silica gel column only allows group separation of the extracted PAH oils and partial separation of PAHs with high molecular weight. The poor separation efficiency of this method confirms earlier conclusions[4,13,22] that the procedure can be successfully employed only for clean-up of PAH extracts from traces of aliphatic, naphthenic and polar-aromatic compounds or as pre-fractionation technique for the isolation of specific groups of PAHs prior to final chromatographic (GC or HPLC) analysis. For this reason all investigated PAH-rich extracts were analysed by reversed-phase HPLC.

The changing mobile phase composition in linear gradient RP–HPLC leads to good resolution between the PAH standards and most PAHs present in the oil samples studied (see Figure 3). However, in our experiments, even a rapid change of the mobile phase composition from methanol–water (85:15) to acetonitrile (see **Experimental**) does not effectively separate benzo(a)pyrene and benzo(b)fluoranthene which are included[14] in a PAH isomer group—i.e. benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, benzo(e)pyrene and perylene—frequently regarded as extremely difficult to separate by HPLC.

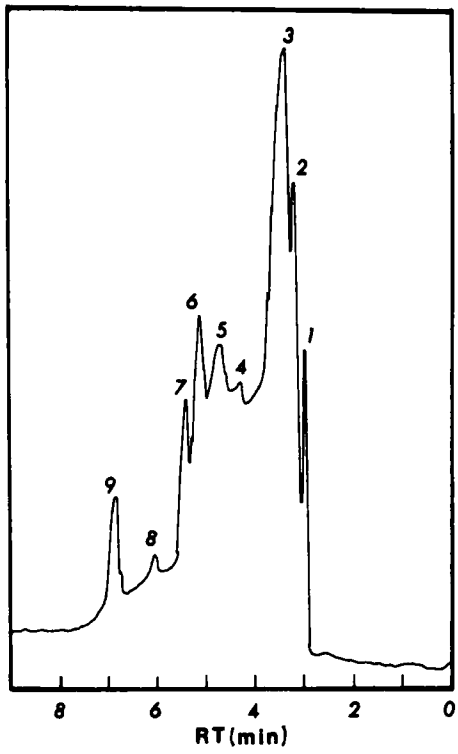


Figure 2 Isocratic adsorption HPLC of PAHs isolated from mineral oil No. 2 (conditions, see **Experimental** part). Peaks: 1, DMF; 2, Fluoranthene; 3, Pyrene; 4, Chrysene; 5, Benzo(a)anthracene; 6, Benzo(e)pyrene + benzo(b)fluoranthene; 7, Benzo(a)pyrene; 8, Perylene; 9, Dibenz(a,h)anthracene.

Eisenbeiss *et al.*[15] and Hagenmaier *et al.*[16] reported that a LiChrosorb RP-18 column resolves benzo(a)fluoranthene. benzo(k)fluoranthene and benzo(a)pyrene almost completely; this is confirmed in our study. The poor column selectivity for the benzo(e)pyrene/benzo(b)fluoranthene isomer pair may be related to packing material parameters such as the pore size diameter or octadecylsilane phase density, or to solute–solvent interactions under the present gradient conditions.[17–20]

The identification of the PAHs was performed by comparison of the retention times, UV and fluorimetric detector signals with those of reference substances. None of the HPLC chromatograms exhibited a hump due to matrix components, which testifies in favour of the clean-up procedure. The monitoring of the column effluent under compromise excitation/emission wavelength conditions (310/440 nm) and with standard UV detection (254 nm) yields chromatograms that varied widely and greatly simplified comparison of the investigated samples. If only UV detection was used, the extremely low concentration level of several PAHs in the oil studied caused the disappearance of various peaks and, therefore, quantification problems. The application of fluorescence detection resulted in improved selectivity

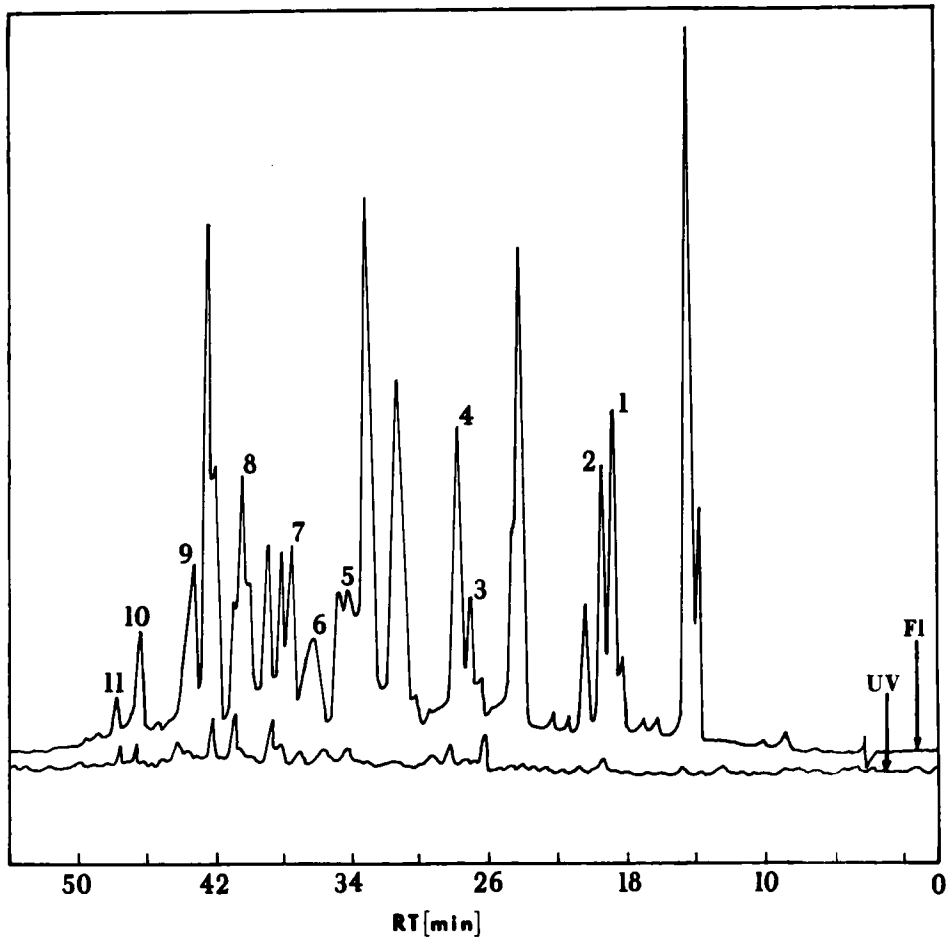


Figure 3 Gradient reversed-phase HPLC of PAHs isolated from mineral oil No. 3 (conditions, see **Experimental** part). Peaks: 1, Fluoranthene; 2, Pyrene; 3, Benzo(a)anthracene; 4, Chrysene; 5, Benzo(e)pyrene + benzo(b)fluoranthene; 6, Perylene; 7, Benzo(j)fluoranthene; 8, Benzo(a)pyrene; 9, Dibenz(a,h)anthracene; 10, Indeno(1,2,3-cd)pyrene; 11, Coronene.

and sensitivity of PAH detection, and was applied for their quantitation. Each mineral oil sample was analysed in triplicate and the concentration of each PAH calculated; the RSD was less than 18%.

The results are summarized in Table 2. It is evident that most of the 15 standard PAHs are found to be present in the samples studied. The quantity of the identified PAHs ranges from 30 to 1130 μg/kg. The total content of PAHs in these oils increases with their distillation temperature (compare Table 1), as was reported earlier[13] for mineral oils not containing olefinic hydrocarbons. The total concentration of the identified PAHs is several times lower than in lubricating oils (average concentration, 5.1 mg/kg)[21,22] and higher than in white paraffinic oils (10.6 μg/kg).[5] When comparing the PAH profiles of the investigated oils, one can conclude that the samples differ slightly in the content of pyrene, benzo(e)pyrene/

Table 2 Concentration of PAHs in mineral oils potentially used as dispersing agents for herbicides (μg/kg)

No.	*Compopund identified*	*Mineral oil number*					
		1	*2*	*3*	*4*	*5*	*6*
1.	Anthracene	—[a]	—	—	120	—	—
2.	Phenanthrene	60	—	—	60	—	—
3.	Fluoranthene	30	180	95	—	130	70
4.	Pyrene	130	400	130	170	210	190
5.	Chrysene	—	150	30	30	30	—
6.	Benzo(a)anthracene	210	920	330	—	450	380
7.	Benzo(e)pyrene + benzo(b)fluoranthene	120	1130	110	80	110	170
8.	Benzo(a)pyrene	—	65	32	—	—	—
9.	Benzo(j)fluoranthene	40	—	50	70	230	210
10.	Perylene	40	80	60	170	50	70
11.	Dibenz(a,h)anthracene	90	210	30	—	40	30
12.	Indeno(1,2,3-cd)pyrene	210	—	40	120	115	60
13.	Coronene	180	—	30	60	—	30
	Totals	1110	3135	1052	880	1365	1210

[a]Not detected with fluorescent detector.

benzo(b)fluoranthene and perylene. All investigated oils contained these PAHs. Benzo(a)pyrene, reported to be a potent carcinogen,[3] was detected in high concentration in only two oil samples (Nos. 2 and 3). The observed benzo(a)pyrene content (32–65 μg/kg) is lower than in lubricating oils (average, 62 μg/kg),[21,22] but higher than in white paraffinic oil (maximum, 8.6 μg/kg).[5]

Our results agree with earlier suggestions[21] that petroleum mineral oils mainly contain one of several isomeric PAHs. For example, for the pericondensed PAHs, in the case of our study perylene or benzo(e)pyrene predominates compared to benzo(a)pyrene (see Table 2). In most investigated samples the concentration ratio of the four-fused-ring isomers: benzo(a)anthracene and pyrene is near 2:1. For another pair of isomers, i.e. dibenzo(a,c)anthracene and dibenzo(a,h)anthracene, only the former was detected in petroleum mineral oils by Grimmer *et al.*,[21] but only the latter one in the oils studied by us. Some PAHs detected in the mineral oils investigated, e.g. phenanthrene, fluoranthene, pyrene, chrysene, benzo(a)pyrene, are present in comparable concentration in soil,[23,24] ground water[25] or on sugar-beet leaf surface.[26]

Our study shows that refined mineral oils used in the plant protection industry contain different quantities of carcinogenic PAHs. Their use in agriculture and food production can lead to enhanced human risk, especially when the occurrence of PAHs together with traces of herbicides on the plant surface results in synergistic effects.

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