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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC IDENTIFICATION OF GEOCHEMICALLY SIGNIFICANT ISOALKANE HYDROCARBONS

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SUMMARY

Computerized gas chromatography–mass spectrometry has been used for fingerprinting C₁₅–C₄₀ methyl- and polymethylalkanes in geological extracts. With proper selection of the key ions, three different series of isoprenoid hydrocarbons, exhibiting *iso*- and *anteiso*- head-to-tail and tail-to-tail structures can be observed. The regularities displayed by these series in the fragmentograms have been rationalized on the basis of the chromatographic behaviour of their components. In this respect, the possibility of predicting retention indices either by retention increments or by empirical relationships is discussed. The elution order of the isoalkanes identified is correctly established by the first-order molecular connectivity (χ), and a retention relationship giving mean absolute errors of 1.1 and 2.7 index units for Apiezon L and Dextsil, respectively, has been obtained for the C₁₅–C₄₀ regular isoprenoid series.

INTRODUCTION

Isoalkane hydrocarbons are widely distributed in geological materials such as sediments and petroleum because they are already present as such in living organisms or because they may constitute the final step in the diagenesis and maturation of biolipids. In addition, they are stable enough to survive over long geological periods. Consequently, the analysis of these hydrocarbons, and especially the comparison of their carbon skeletons with those of contemporary biolipids, can furnish much information about the origin, depositional conditions and geochemical evolution of sedimentary organic matter.

In this class of hydrocarbons, homologous series of *iso*- and *anteiso*-alkanes (I) and acyclic isoprenoids with head-to-head (II), head-to-tail (III) and tail-to-tail linkages (IV) are of special significance because they have been recognized as the remains of bacterial activity in sediments¹ and the relative distribution of certain homologues as an indication of the paleoenvironmental conditions of deposition².

However, geological extracts generally afford very complex isomeric mixtures whose identification is far from complete, thus making difficult the direct character-

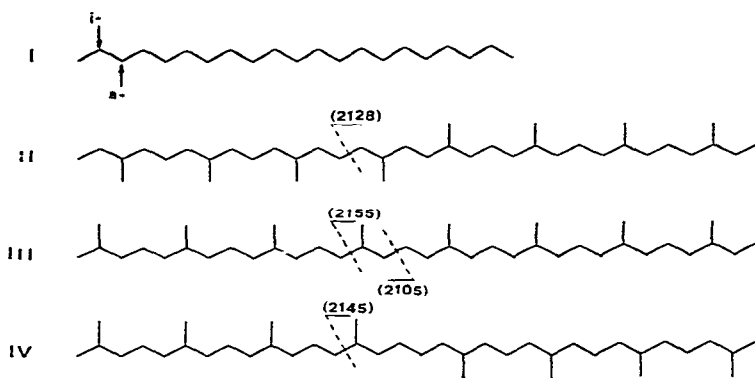


Fig. 1. Isoalkane hydrocarbon types occurring in geological samples. Dotted lines indicate a series of C_{24} isomers with their Kováts retention indices in parentheses.

ization of these components. Identifications made from gas chromatograms or mass spectra are not always unequivocal for isomeric isoalkanes. There are several examples in the literature of isoalkanes eluted separately which display very similar mass spectra³ and, also, of co-eluting hydrocarbons that exhibit very different mass spectra⁴. Therefore, the use of multiple ion monitoring in gas chromatography-mass spectrometry (GC-MS) seems to be a suitable approach for the characterization of such hydrocarbons. Moreover, bearing in mind that many of these compounds occur as a part of homologous series, the regularities observed in the mass fragmentographic profiles may be of diagnostic value, provided that the chromatographic behaviour of the suspected compounds is known or may be predicted.

It is the main purpose of this paper to give some insight into the analytical methodology that can be used for such identifications, which, in turn, can contribute to a better knowledge of these fractions and to the discovery of new series of biological markers. Owing to its particular geochemical interest, the paper will be focused on the long-chain isoalkane range ($>C_{20}$).

EXPERIMENTAL

Concentrates of isoalkane hydrocarbons were obtained from sediment extracts and deasphalted crude oils (in 40 volumes of *n*-pentane) by chromatography in a silica gel column (50×1 cm I.D.) using an adsorbent to sample ratio of 100:1. Then, saturated hydrocarbons were eluted with *n*-pentane and isoalkanes isolated by conventional procedures. Typically, the saturated fraction was treated with molecular sieve 5 Å and then subjected to adduct formation three times each in methanolic urea at 0°C and methanolic thiourea. The adducts, after decomposition in hot water, afforded fractions enriched in methyl- and polymethylalkanes, respectively.

Analysis by GC of pure compounds and purified extracts^{5,6} was performed on a Perkin-Elmer 900 instrument, equipped with a flame-ionization detector using helium as the carrier gas, with $2 \text{ m} \times 0.2$ cm I.D. columns packed with 3% Dexsil on Gas-Chrom Q (100–120 mesh) programmed from 150 to 300°C at 6°C/min, and $50 \text{ m} \times 0.25$ mm I.D. open-tubular capillary columns coated with Apiezon L, programmed from 120 to 280°C at 6°C/min. Retention indices were calculated by means

of an HP 9830 desk calculator from retention times obtained with a digital integrator (Autolab 6300) in the range 200–270°C. The operating conditions were optimized as reported previously⁷ and the mean absolute errors for the whole C₁₀–C₄₀ series were found to be 1.4 and 0.9 index units for packed and capillary columns, respectively. As this error included the variation of retention index with temperature in the range studied, a mean index value is given for each compound (see Table I).

Fingerprinting of isoalkane hydrocarbons was carried out on the saturated fractions by computerized GC-MS using an LKB 9000 S-PDP 11E10 system. The gas chromatograph was operated with glass columns, either packed with 1% Dexsil on Gas-Chrom Q (100–120 mesh) (2 m × 0.2 cm I.D.) and temperature programmed from 200 to 300°C at 4°C/min or wall coated with SE-30 (25 m × 0.25 mm I.D.) and temperature programmed from 100 to 290°C at 4°C/min, using splitless injection. The jet separator was maintained at 290°C.

RESULTS AND DISCUSSION

It is well known that isoalkane hydrocarbons exhibit characteristic fragment ions at m/e C_nH_{2n+1}, corresponding to the cleavage of bonds adjacent to tertiary carbon atoms. When these ions are used in the mass fragmentographic analysis of geological extracts, relatively constantly recurring patterns of peaks appear, as is shown in Figs. 2 and 3. Then, suitable selection and grouping of these key ions may

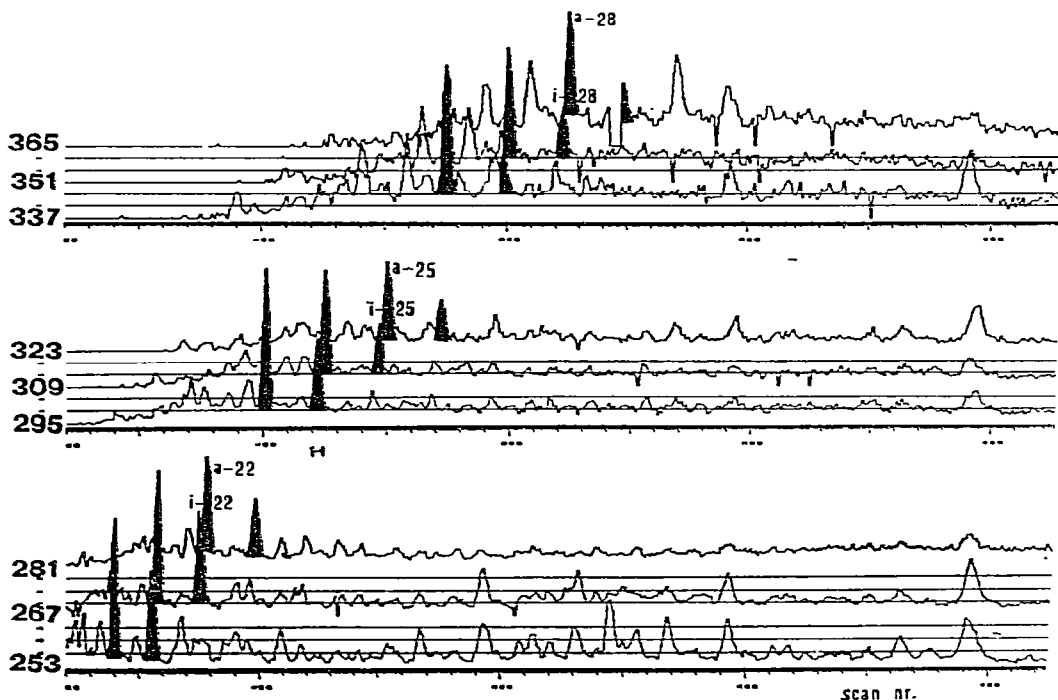


Fig. 2. Computer-reconstructed fragmentograms of methylalkanes (I) in an oil sample (branched + cyclic fraction).

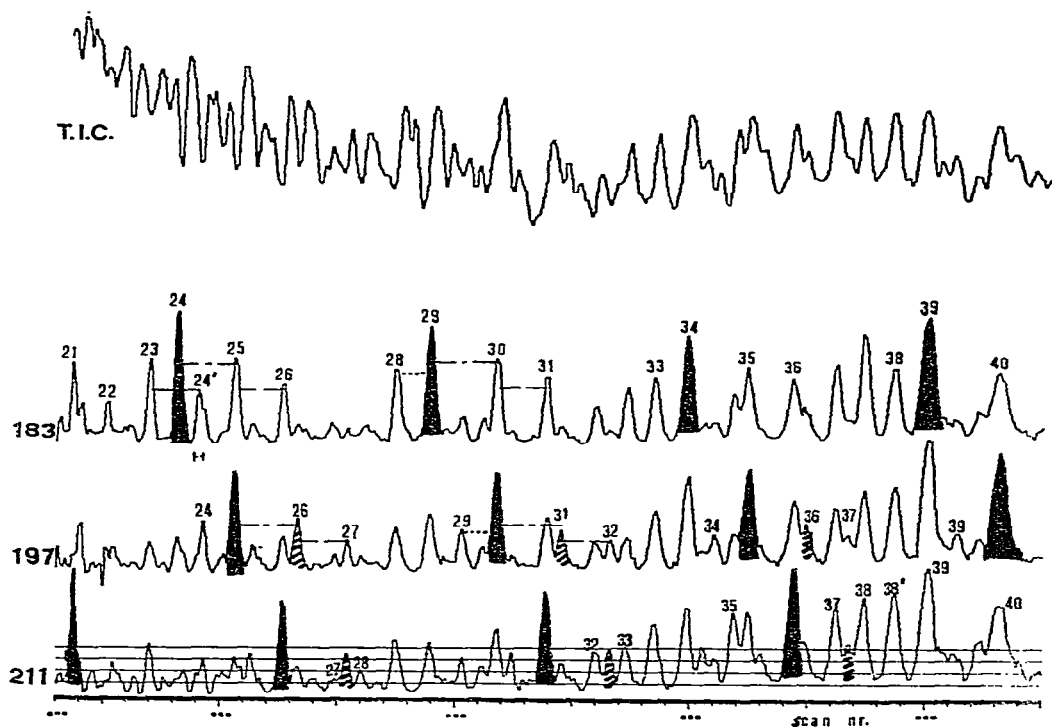


Fig. 3. As Fig. 2, for isoprenoid alkanes. The number of carbon atoms of the homologous series III, V and IV are indicated on the m/e 183, 197 and 211 profiles, respectively.

allow the characterization of homologous series, thus overcoming the possible interference between series exhibiting common diagnostic ions.

Methylalkanes (I) produce only one major fragment of the series C_nH_{2n+1} . According to the position of the single methyl branching, the same fragment ion can be exhibited by several alkanes, the most abundant being those of *iso*- and *anteiso*-series (see Fig. 2). On the other hand, regular isoprenoid alkanes (III) display intense peaks at m/e $113 + 70N$ and $113 + 14n$, where N and n depend, respectively, on the number of isoprenoid units and the number of carbon atoms in the molecule (e.g., C_{10} : $N = 0$, $n = 1$). Finally, tail-to-tail isoprenoids should present prominent peaks at m/e 113, 183, 239, 267 or 113, 183, 253, 309, 337, depending on which is the precursor, namely squalane (C_{30}) or lycopane (C_{40}) (IV), whilst head-to-head series (II) should present peaks at m/e 127, 197, 267, 323 or 113, 183, 253, 309, 379, according to the pristane or phytane molecular moieties.

In this way it is possible to discriminate the above series of isoalkanes, as is shown in Figs. 2 and 3. In addition, careful examination of Fig. 3 revealed the presence of another series of peaks giving rise to higher responses in the $127 + 70N$ and $127 + 14n$ profiles, as can be seen partially in more detail in Fig. 4.

Although much work is in progress on the unequivocal identification of this series, various evidence points to a regular *anteisoprenoid* structure (V, Fig. 4). Such an assumption is supported by the mass spectra of individual peaks and their positions in the chromatogram, with retention displacements paralleling those of the *iso* series

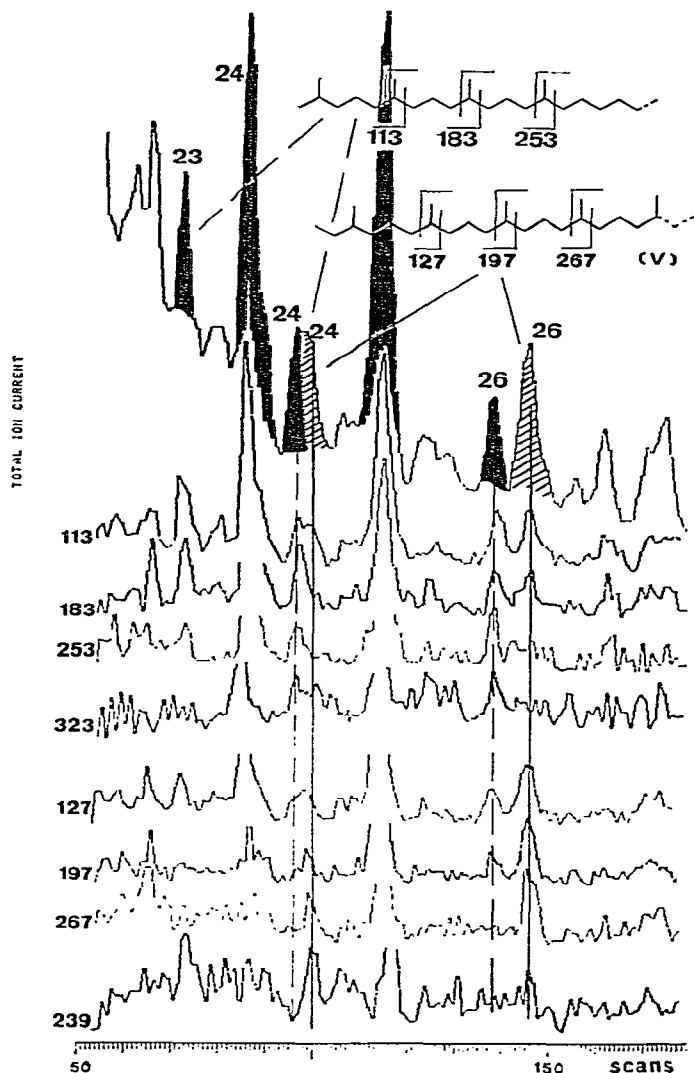


Fig. 4. Extended mass fragmentographic characterization of the C_{24} - C_{26} range shown in Fig. 3.

(III) for the elongation of the chain or the transfer of methyl groups to the *anteiso*-position (i - C_{24} \rightarrow a - C_{24}) (see the dotted lines in Figs. 2 and 3).

In order to substantiate these correlations, retention increments on Apiezon L and Dexsil were calculated for the whole C_{10} - C_{40} isoprenoid series (Table I) and further applied to the determination of retention indices of other series, in a similar manner to that described by Shlyakhov *et al.*⁸ for the lower C_{20} homologues. The results obtained for the regular *anteiso*-series (3, 7, 11, 15, . . . polymethylalkanes) showed fair agreement with those determined experimentally, as reported elsewhere⁶.

In an additional application, the calculated retention indices for the different C_{24} isomers, originating from the higher homologues (see Figs. 1 and 4), provide confirmation of the proposed structures for those isomers.

TABLE I

KOVÁTS RETENTION INDICES OF C₁₀-C₄₀ ISOPRENOID HYDROCARBONS

Retention increments corresponding to methyl and isoprenoid unit chain elongations are indicated by squares and circles, respectively.

Apiezon L	Carbon number										
<i>n</i>	10 + <i>n</i>	15 + <i>n</i>	20 + <i>n</i>	25 + <i>n</i>	30 + <i>n</i>	35 + <i>n</i>					
0	9 3 1 [86]	(43 ⁺) [84]	1 3 6 5 [84]	(42 ⁺) [83]	1 7 8 9 [85]	(41 ⁺) [85]	2 2 0 3 [85]	(41 ⁺) [84]	2 6 2 7 [84]	(41 ⁺) [85]	3 0 4 0 [85]
1	1 0 1 7 [92]	(43 ⁺) [89]	1 4 4 9 [89]	(42 ⁺) [88]	1 8 7 2 [88]	(42 ⁺) [88]	2 2 9 3 [88]	(41 ⁺) [88]	2 7 1 1 [88]	(41 ⁺) [88]	3 1 2 5 [88]
2	1 1 0 9 [96]	(42 ⁺) [94]	1 5 3 8 [94]	(42 ⁺) [92]	1 9 6 0 [92]	-	-	-	-	-	-
3	1 2 0 5 [56]	(42 ⁺) [53]	1 6 3 2 [53]	(42 ⁺) [53]	2 0 5 2 [53]	(41 ⁺) [53]	2 4 7 0 [53]	(41 ⁺) [52]	2 8 8 5 [52]	(41 ⁺) [52]	3 2 9 8 [52]
4	1 2 6 1 [104]	(42 ⁺) [104]	1 6 8 5 [104]	(42 ⁺) [104]	2 1 0 5 [104]	(41 ⁺) [104]	2 5 2 3 [104]	(41 ⁺) [104]	2 9 3 7 [104]	(41 ⁺) [104]	3 3 5 0 [104]
5	1 3 6 5 [104]	(42 ⁺) [104]	1 7 8 9 [104]	(41 ⁺) [104]	2 2 0 8 [104]	(41 ⁺) [104]	2 6 2 7 [104]	(41 ⁺) [104]	3 0 4 0 [104]	(41 ⁺) [104]	3 4 5 4 [104]

A general feature displayed by isoalkanes is that their retention times decrease when methyl branches migrate towards the ends of the chain. Hence, for example, the elution order of the above C₄₀ hydrocarbons should be IV < III ≈ II, and this trend can be highly useful for the characterization of these fractions if it can be adequately established.

Bearing in mind that topological parameters are especially suitable for the characterization of molecular branching, we try to establish an empirical relationship that would be able to predict the chromatographic behaviour of such hydrocarbons. In this respect, it has been shown that retention indices are strongly correlated with the first-order molecular connectivity (χ)⁹, although until now methods have been developed only for low-molecular-weight isoalkanes¹⁰. Thus, the following retention-connectivity relationships were established for the isoprenoid series:

$$\text{Apiezon L: RI} = 99.8 + 180.7\chi - 0.2\chi^2 + 52.8(\Delta\chi - 0.5); |\varepsilon| = 1.1$$

index units

$$\text{Dexsil: RI} = 92.2 + 183.7\chi - 0.3\chi^2 + 42.3(\Delta\chi - 0.5); |\varepsilon| = 2.7$$

index units

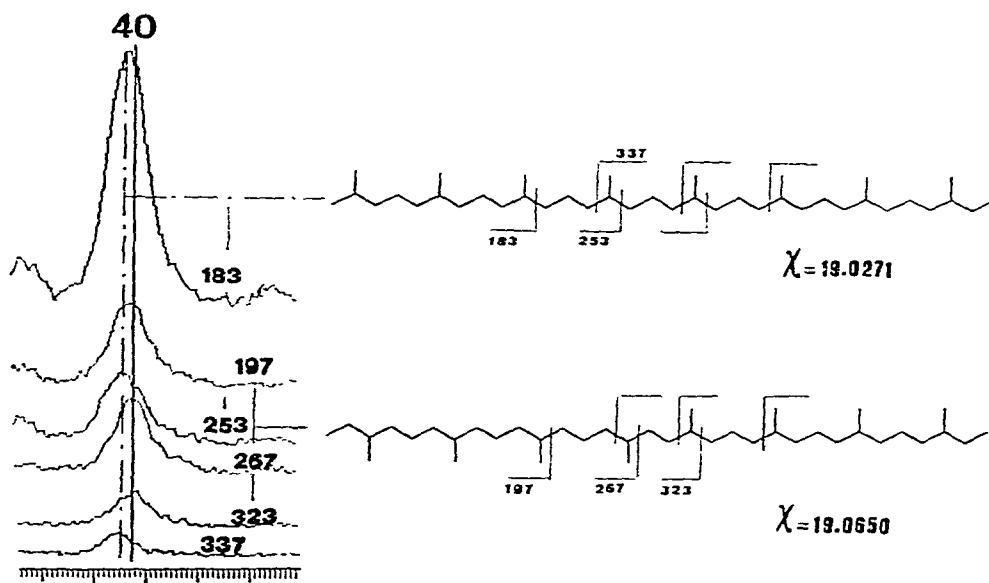
where $\Delta\chi$ represents the difference between the isoalkane and its preceding homologue. This factor was introduced to improve the correlation for the deviations displayed by the more branched isoalkanes, namely those of the C_{14+5*n*} series.

The results obtained (Table II) afford striking evidence for the adequacy of this approach, which will be extended to other series.

TABLE II

EXPERIMENTAL AND CALCULATED RETENTION INDICES FOR THE REGULAR ISOPRENOID SERIES, USING THE MOLECULAR CONNECTIVITY (χ) RELATIONSHIP

Carbon No.	χ	Apiezon L			Dexsil		
		RI_{exp}	RI_{calc}	ϵ	RI_{exp}	RI_{calc}	ϵ
14	6.5198	1261	1260.1	-0.9	1270	1269.8	-0.2
15	7.0578	1365	1365.1	0.1	1375	1373.9	-1.1
16	7.5578	1449	1451.7	2.7	1457	1461.8	4.8
17	8.0578	1538	1540.2	2.2	1550	1551.1	1.1
18	8.5578	1632	1628.5	-3.5	1644	1640.2	-3.8
19	8.9137	1685	1683.7	-1.3	1700	1697.4	-2.6
20	9.4516	1789	1788.2	-0.8	1804	1800.7	-3.3
21	9.9516	1872	1874.2	2.2	1884	1887.8	3.8
22	10.4516	1960	1962.1	2.1	1975	1976.3	1.3
23	10.9516	2052	2049.9	-2.1	2068	2064.7	-3.3
24	11.3076	2105	2104.7	-0.3	2122	2121.4	-0.6
25	11.8455	2208	2208.5	0.5	2225	2223.9	-1.1
26	12.3455	2293	2294.0	1.0	2304	2310.2	6.2
27	12.8455	—	2388.0	—	—	2402.4	—
28	13.3455	2470	2468.5	-1.5	2490	2485.5	-4.5
29	13.7014	2523	2522.9	-0.1	2543	2541.6	-1.4
30	14.2394	2627	2626.1	-0.9	2645	2643.3	-1.7
31	14.7394	2711	2711.0	0.0	2722	2728.8	6.8
32	15.2394	—	2807.1	—	—	2823.0	—
32	15.2394	—	2807.1	—	—	2823.0	—
33	15.7394	2885	2884.4	-0.6	2906	2902.5	-3.5
34	16.0953	2937	2938.3	1.3	2957	2958.1	1.1
35	16.6332	3040	3040.9	0.9	3060	3058.9	-1.1
36	17.1332	3125	3125.2	0.2	3137	3143.7	6.7
37	17.6332	—	3223.9	—	—	3238.1	—
38	18.1332	3298	3297.4	-0.6	3319	3315.9	-3.1
39	18.4891	3350	3351.0	1.0	3370	3370.9	0.9
40	19.0271	3454	3453.0	-1.0	3473	3470.9	-2.1

Fig. 5. As Fig. 4, for the C_{40} peak.

It is worth mentioning that molecular connectivity has been found satisfactory for the prediction of the elution order of all of the isoalkanes considered.

Fig. 5 shows an example for C₄₀ isomers, which can be only partially resolved by mass fragmentography. Consequently, mass fragmentograms can be advantageously exploited with such information. In this manner, these profiles can be used as complementary tools in oil-oil, oil-source rock and oil-pollutant source correlations, and this will be considered elsewhere⁶.

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