



Relative hopane content confirming the mineral origin of hydrocarbons contaminating foods and human milk

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Hopanes, triterpenoid hydrocarbons formed under geological conditions, were analysed to confirm the mineral origin of the unresolved complex mixtures of hydrocarbons observed in the gas chromatography with flame ionization detection chromatograms of human milk and certain foodstuffs. The 'relative hopane content' (RHC) is introduced, i.e. it is the area ratio of the sum of the hopanes and the paraffins in the same segment of the chromatogram. The RHC in various mineral oil products (motor oils, hydraulic oils, lubricating oils, Vaseline) was 3.4%, with a relative standard deviation of 19%. The RHC determined in samples of vegetable oils, mussels and clams as well as of human milk containing an unresolved complex mixture of hydrocarbons was in the same range, confirming that these samples were contaminated by mineral oil material.

Keywords: mineral oil contamination of foods and human milk, unresolved complex mixture (UCM) of paraffins, hopanes in foods and human milk, relative hopane content (RHC)

Introduction

Numerous papers have reported the widespread contamination of foodstuffs by what are assumed to be

mineral oil products. Grob *et al.* described mineral oil material from jute and sisal bags in hazelnuts, almonds, cocoa beans, rice and coffee (Grob *et al.* 1991a, b, 1992, 1993), from various other packaging materials in foods like salami, cheese and candies (Grob *et al.* 1991c), and from the lubricants in can coatings (Grob *et al.* 1997). Migration into food from polystyrene containers, waxed paper and paperboard, cans, and other packaging materials was described by Castle *et al.* (1991, 1993a, b, 1994) and Jickells *et al.* (1994a, b). Mineral paraffins used as diluents in printing inks applied to cardboard boxes were shown to be transferred to packed foods even if these were in a paper or a plastic bag (Droz and Grob 1997). Paraffin oils have been widely used as release agents and have been found in particularly high concentrations in bakery ware and candies (Grob *et al.* 1991d). Animal body fat and eggs contained mineral oil material from feed prepared with used edible oils (e.g. frying oil) contaminated by engine oil and other technical oils (Grob *et al.* 2001). In vegetable oils, frequently unresolved complex mixtures (UCMs) of paraffins were detected; concentrations sometimes reached 1000 mg kg⁻¹ (Grob *et al.* 1994, Wagner *et al.* 2001a, b, Moret *et al.* 2003). There are several sources, but lubricating oil and incompletely combusted diesel and heating oil taken up from ambient air during plant growth seem to be a relevant contribution (Neukom *et al.* 2002). The mean dietary intake of mineral paraffins by adults was estimated as 0.47 mg kg⁻¹ body weight (bw) day⁻¹, that of pre-school children as 0.98 mg kg⁻¹ bw (Food Chemical Risk Analysis 2001).

In 33 samples of human milk, a UCM of paraffins corresponding to an average of 95 mg kg⁻¹ fat was determined (Noti *et al.* 2003), suggesting the presence of mineral oil material from foods and/or cosmetics in human body fat. The exposure of babies to these paraffins can be in the region of the no observable adverse effect level (NOAEL) for Fisher 344 rats (Scientific Committee on Food [SCF] 1995). This is

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of particular concern since the UCM is commonly centred on the C₂₃–C₂₅ paraffins, which is far below the range of the molecular masses considered acceptable by the SCF.

Much data are available on UCMs of paraffins in the particulate matter of the air (e.g. Fraser *et al.* 1999, Tobias *et al.* 2001), soil and sediments (Wang *et al.* 1999, Faure and Landais 2000) and water (Stagg *et al.* 1995).

A number of not ultimately conclusive arguments were used to assign the UCMs of paraffins in foods or human milk to mineral oil material, the most convincing being that in the majority of the cases, they could indeed be traced to contamination by a mineral oil product. Other arguments were that:

- living organisms were unable to produce such highly isomerized paraffins;
- samples of the same type free of these hydrocarbons were found;
- the shape and width of the ‘hump’ reflected distillative fractionation; and
- the shape of the hump varied among samples of the same type, whereas the degradation of a natural material should result in a constant composition (e.g. Wagner *et al.* 2001a).

n-Alkanes of mineral origin are characterized by balanced even and odd numbered species and are easily distinguishable from the plant *n*-alkanes consisting of strongly predominant odd numbered species. The ratio of the odd/even numbered *n*-alkanes was termed the ‘carbon preference index (CPI)’ and is > 1 for *n*-alkanes of plant origin and close to 1 for mineral *n*-alkanes. However, often the UCM is not accompanied by *n*-alkanes, either because the contaminating mineral oil product was deparaffinated (*n*-alkanes removed during the raffination process for, for example, lubricating and hydraulic oils or most release agents) or because of weathering and biodegradation (Pollard *et al.* 1999, Wang *et al.* 1999).

Foods can contain UCMs of hydrocarbons not originating from mineral oil, such as isomerized squalene or dehydrated sterols (sterenes) resulting from raffination of edible oils. However, these hydrocarbons are unsaturated. Selective sample preparation using on-line high-performance liquid chromatography (HPLC)-gas chromatography (GC) (Grob *et al.* 1991a) or pre separation on aluminium oxide after bromination of double bonds (Wagner *et al.* 2001a) enable the removal of these possibly interfering materials from the saturated hydrocarbons.

Marker compounds

A further source of evidence for the mineral origin of a UCM of paraffins is the presence of marker compounds, such as isoprenoids (pristane and phytane) and the diagenesis products of hopanoids (e.g. hopanes) and sterols (e.g. steranes). Geochemistry understands ‘diagenesis’ as the biological or chemical transformation of organic matter at low temperature in sedimentary environments. Since the hydrocarbons represented by the UCM in the samples of interest were mostly of a molecular weight higher than pristane and phytane, the hopanes seemed most suitable. The hopanoids are a family of triterpenoid hydrocarbons with a pentacyclic skeleton comprising four cyclohexanes and one cyclopentane. They comprise over 100 naturally occurring members and are subdivided into geo- and biohopanoids. The biohopanoids are naturally present in many plants, ferns, mosses, fungi and bacteria (where they play an important role in the cell membrane), whereas the geohopanoids are their diagenetic products. Hopanols, hopanoic acids, hopenes and hopanes are the most important classes of geohopanoids.

The diagenesis of the biohopanoids was studied and discussed by Peters and Moldowan (1994), Innes *et al.* (1997), Bennet and Abbott (1999), Tritz *et al.* (1999) and Farrimond *et al.* (2000, 2002, 2003). Hopanes are formed during a process requiring geological times and probably the elevated temperatures reached during deep sediment burial. The functional groups and some or all carbon atoms of the side chain are lost. In modern sediments, the amounts of hopanes are small and masked by hopanes derived from contamination by fossil fuels, which confirms that the hopanes are an old material suitable for the confirmation of a mineral origin.

Sterols, with a tetracyclic skeleton of three cyclohexanes and one cyclopentane, undergo analogous processes. Their diagenesis converts them to sterenes and steranes, which are biodegraded earlier than the hopanes (Wang *et al.* 2001).

Besides the steranes, the hopanes are the major biomarkers used for the characterization of petroleum. First, their carbon skeletons are related to their biogenic precursors and, therefore, to the source of the petroleum (Zakaria *et al.* 2000, 2001). Second, the configurations at the positions C-17 and C-21 enable one to determine the maturity of an oil (Oros and Simoneit 2000). Finally, advanced biodegradation

can be detected through the composition of the hopanes, since the various components degrade at different rates (Prince *et al.* 1994, Oiltracers 1999, Wang *et al.* 2001).

The composition of the hopanes was analysed to determine the emission sources of organic compounds found in atmospheric particles, such as those from incompletely combusted fossil fuels in the exhaust of vehicles and in re-suspended street dust (Lang *et al.* 2002, Simoneit 2002 and references therein).

Only a few authors described the use of the hopanes and steranes for the demonstration of the presence of a mineral oil contamination. Bryselbout *et al.* (1998) used the ratio of the peak areas of two individual hopanes (18 α (H)-22,29,30-trisnorhopane, Ts, and 17 α (H),21 β (H)-hopane, 30 $\alpha\beta$) to the C₂₉ *n*-alkane (commonly the predominant *n*-alkane in plant material): a high ratio was considered indicative of the presence of mineral oil material of a molecular mass range corresponding to that of the hopanes. In fact, this ratio was significantly higher for hydrocarbons from a pine tree near a highway than for those from a pine tree in a suburb. However, this ratio is not suitable for determining the amount of mineral oil material in an extract from plant material, because the *n*-C₂₉ content varies within a wide range. Faure and Landais (2000) analysed the biomarkers to distinguish 'anthropogenic' from 'natural' organic inputs in lacustrine sediments.

The scope of the work reported in this paper was the confirmation of the mineral origin of paraffinic UCMs in samples for which this was not conclusive so far. Such a method might also be a valuable tool in case of a dispute at court about foods claimed to be contaminated by mineral oil material. The detection of hopanes might serve as evidence for the presence of mineral hydrocarbons, but it is insufficient to demonstrate that all (or at least most) material represented by a UCM is of mineral origin. For this reason, a more quantitative analysis is needed.

Materials and methods

Frozen samples of fish and olive pomace were ground and lyophilized. A total of 15 g were sonicated with 30 ml hexane for 2 h. After filtration, the solvent was eliminated using a rotary evaporator. The fat from

human milk samples was extracted as described by Noti *et al.* (2003). Milk samples were refluxed during 15 min with concentrated hydrochloric acid and extracted with pentane.

The hydrocarbons were isolated from vegetable oils or extracts by a dual-column normal-phase HPLC system as used for on-line liquid chromatography-liquid chromatography (LC-LC)-GC with flame ionization detection (GC-FID) (Wagner *et al.* 2001a): 25 cm $\times$ 2 mm i.d. columns packed with silica gel, using redistilled pentane as mobile phase at 300 μ l min⁻¹ and dichloromethane to backflush the first column. Of the following solutions in pentane, 100 μ l were injected: milk extracts and vegetable oil, 20%, olive pomace oil, 10%, fish fat 5%. Instead of transferring the relevant LC fractions (300 μ l volume) to GC, they were collected in a vial on the carousel of the autosampler. The solvent was allowed to evaporate and the residue re-dissolved in 200 μ l hexane.

The analysis of the hopanes was performed by GC-MS on an UltraTrace GC instrument equipped for large-volume on-column injection coupled to a MD800 mass spectrometer (ThermoFinnigan, Milan, Italy). A total of 80 μ l sample (LC fraction) was injected on-column by an autosampler AS800 at 3 μ l s⁻¹, applying concurrent solvent evaporation (Biedermann *et al.* 1998). A 20 cm $\times$ 0.53 mm i.d. precolumn coated in the laboratory with a 0.1 μ m film of PS-255, a dimethyl polysiloxane from Fluka (Buchs, Switzerland), was connected via a metal T-piece to a 25 cm $\times$ 0.5 mm i.d. solvent vapour exit (SVE) and a 15 m $\times$ 0.25 mm i.d. separation column coated with a 0.25- μ m film of DB-5MS (J&W Scientific, Folsom, CA, USA). The inlet pressure was 50 kPa (helium). The SVE was closed (switched to a 10 cm $\times$ 50 μ m i.d. fused silica resistance) at the end of the transfer. The column temperature was programmed from 80°C (1 min) at 25 min⁻¹ to 230°C and then at 8 min⁻¹ to 320°C.

The GC-MS system was controlled and data acquired by Excalibur software from ThermoFinnigan. The MS ionization was by electron impact; the MS detector was mostly used in selected ion monitoring (SIM) mode at *m/z* 81 and 191. The area of the hopane section of the UCM was determined by transferring the baseline from a blank chromatogram (only solvent being injected) run shortly before and subtracting the areas of the peaks on top of the UCM.

Results and discussion

Relative hopane content (RHC)

Concept of the relative hopane content (RHC). Figure 1 shows the section of a SIM (m/z 191) GC-MS chromatogram of the hopanes used for determining the RHC. The sample, a motor oil, was directly analysed by GC-MS. Peaks were identified based on published chromatograms.

First, experiments aimed at finding a way to relate the quantity of hopanes observed in a mineral oil material to the UCM formed by the paraffins. Since the mineral oil materials in foods mostly do not contain *n*-alkanes, either because of deparaffination during production or because of biodegradation at a later stage, a relationship between the hopanes and the 'hump' consisting of branched and cyclic paraffins was searched.

As shown in figure 2, the molecular weight distribution of mineral oil hydrocarbons or UCMs varies

within a wide range. For this reason, it is not possible to relate the amount of hopanes to the total of the hydrocarbons. Assuming that GC on a non-polar separation column reflects volatility (simulated distillation), the hopanes can be related to the paraffins in the segment of the hump of the same range of GC retention times. During distillative fractionation of mineral oil, paraffins and hopanes of the same volatility/GC retention time are assumed to behave in the same way. Figure 2 also shows that the hopanes are suitable to confirm the mineral origin only for products or UCMs reaching into the volatility range of the hopanes, i.e. not for cuts with a boiling point below some 340°C, such as UCM-3.

Determination of the RHC

The hopanes were detected by the fragment m/z 191 and summed up for the compounds ranging from Ts to 35 $\alpha\beta$ R (figure 1). The area of the UCM segment covering the hopanes, in terms of Kovacs indices ranging from 2860 to 3650, was determined by the

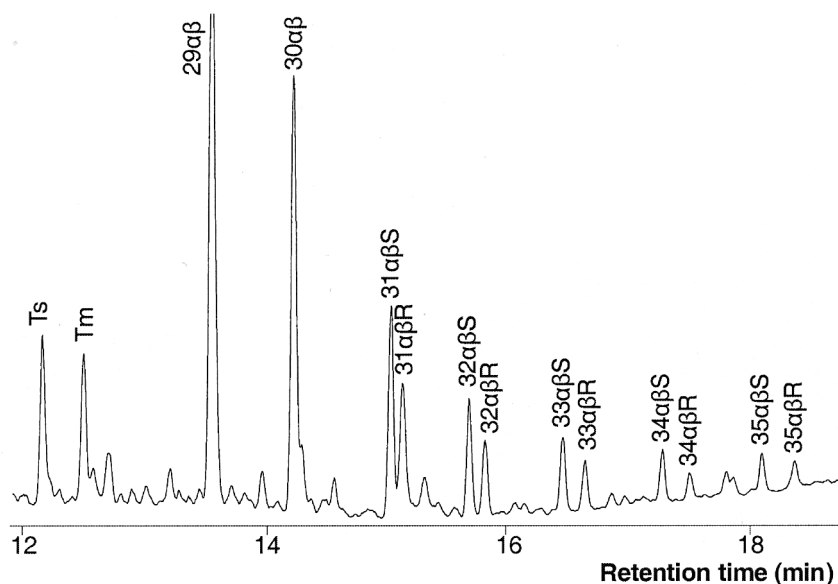


Figure 1. Hopanes used for determining the relative hopane content (RHC): Ts, 18 α (H)-22,29,30-trisnorhopane; Tm, 17 α (H)-22,29,30-trisnorhopane; 29 $\alpha\beta$, 17 α (H),21 β (H)-30-norhopane; 30 $\alpha\beta$, 17 α (H),21 β (H)-hopane; 31 $\alpha\beta$ S, 17 α (H),21 β (H),22S-homohopane; 31 $\alpha\beta$ R, 17 α (H),21 β (H),22R-homohopane; 32 $\alpha\beta$ S, 17 α (H),21 β (H),22S-bishomohopane; 32 $\alpha\beta$ R, 17 α (H),21 β (H),22R-bishomohopane; 33 $\alpha\beta$ S, 17 α (H),21 β (H),22S-trishomohopane; 33 $\alpha\beta$ R, 17 α (H),21 β (H),22R-trishomohopane; 34 $\alpha\beta$ S, 17 α (H),21 β (H),22S-tetrakishomohopane; 34 $\alpha\beta$ R, 17 α (H),21 β (H),22R-tetrakishomohopane; 35 $\alpha\beta$ S, 17 α (H),21 β (H),22S-pentakishomohopane; and 35 $\alpha\beta$ R, 17 α (H),21 β (H),22R-pentakishomohopane.

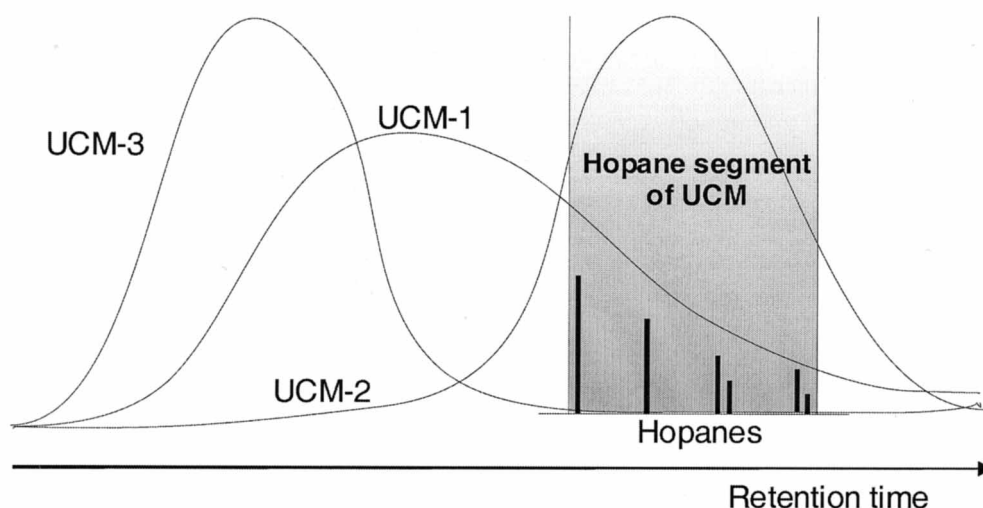


Figure 2. Schema to explain the relative hopane content (RHC) in a gas chromatogram: the sum of the peak areas of the hopanes is related to the segment of the unresolved complex mixture (UCM) of the same range of gas chromatography retention time.

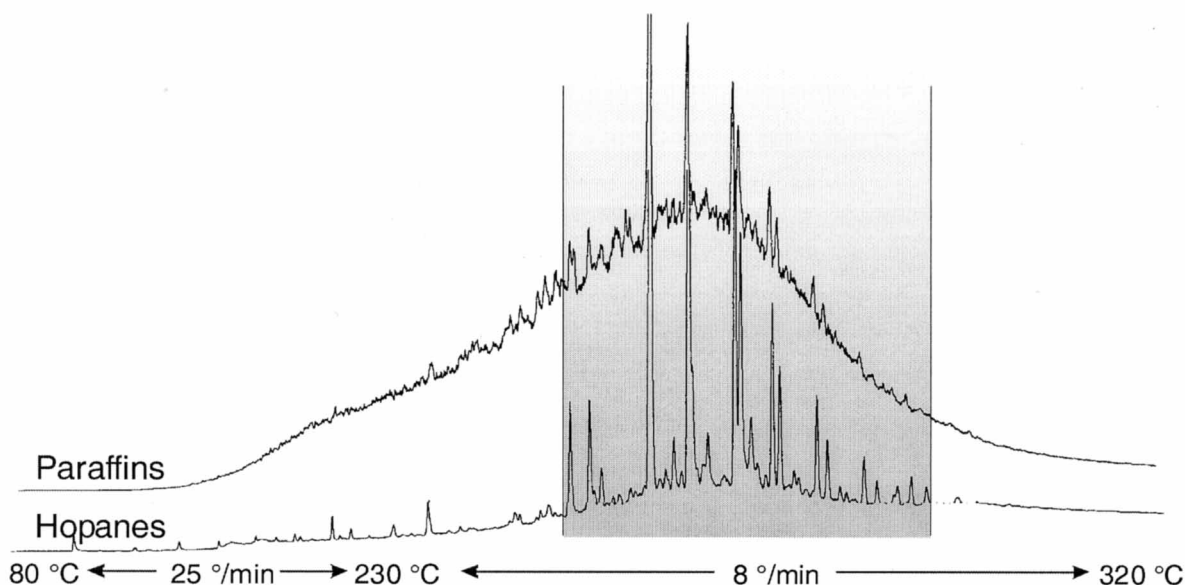


Figure 3. Hydrocarbons (m/z 81) and hopanes (m/z 191) from a motor oil with the window (shaded) analysed for the determination of the relative hopane content.

fragment m/z 81. The relative hopane content was expressed as the per cent of the hopane areas related to the area of the hopane segment of the UCM. Figure 3 shows the GC-MS chromatograms of the hydrocarbons and the hopane fraction from a motor oil of a tractor moving olive pomace around the storage area.

Calibration of the RHC. The RHC was calibrated using a range of mineral oil products possibly encountered in foodstuffs. Table 1 shows results calculated for the hopane segment of the UCM determined in three ways: by MS/total ion current (TIC), by m/z 81 recorded in full-scan or by m/z 81 recorded by SIM. For the batching oil used for the manufacturing of

Table 1. Relative hopane content in various mineral oil products. The volatility of mineral oil products is characterized by the *n*-alkane eluted at the maximum of the hump and by the percentage of the hopane segment on the total area of the hump.

Products	Relative hopane content (%)			Maximum <i>n</i> -alkane	Hopane section of the unresolved complex mixture (%)
	Full-scan, total-ion current	Full-scan <i>m/z</i> 81	SIM mode <i>m/z</i> 81		
Motor oil	0.12	3.1	2.3	C ₂₇	33
	0.19	6.5	2.4	C ₂₈	52
	0.13	3.8	4.4	C ₃₀	58
	0.17	5.0	3.3	C ₂₈	45
	0.13	4.1	3.1	C ₂₆	26
	0.15	4.2	3.1	C ₂₇	31
	0.10	3.2	2.4	C _{27–28}	36
	0.14	4.5	4.2	C _{27–28}	35
	0.12	4.1	3.5	C _{24–25}	24
	0.13	3.7	3.1	C ₂₇	40
	0.12	4.0	2.9	C _{26–27}	35
	0.18	5.9	5.0	C ₃₁	69
Hydraulic oil	0.19	4.8	3.3	C ₂₈	41
	0.20	2.5	3.0	C ₂₇	24
	0.10	3.0	4.0	C ₂₃	10
	0.13	4.3	3.7	C _{27–28}	41
	0.13	4.4	3.3	C _{27–28}	43
	0.14	4.2	3.0	C ₂₈	46
	0.16	4.8	3.4	C _{26–27}	34
	0.15	4.7	3.1	C _{27–28}	41
Lubricating oil	0.15	4.4	3.9	C _{23–24}	17
	0.15	4.3	3.9	C ₂₄ + C ₃₆	34
Vaseline	0.08	2.7	1.9	C ₂₈	41
Batching oil	0.05	2.9	2.0	C ₂₀	7
Means	0.14	4.24	3.38		37
Relative standard deviation (%)	19	21	19		34

jute bags and the Vaseline (the only samples containing *n*-alkanes), the paraffins were determined without the *n*-alkanes.

RHC depends on the mode of determining the area of the UCM segment, since no response factor was applied. As shown by the relative standard deviations (RSD), the variation of the results was similar for all three methods. MS detection in SIM mode was preferred to the alternatives because of the resulting high sensitivity.

Owing to lack of standards, RHC was not corrected by calibrated response factors, i.e. it represents an area rather than a concentration ratio. This means that the RHC needs to be re-calibrated by the analysis of (easily available) mineral oil products.

Validation of the RHC. The last column of table 1 lists the percentage of the hopane segment on the total UCM. This percentage is highest when the

maximum of the hump falls into the volatility range of the hopanes (e.g. UCM-2 in figure 1). There is a fair correlation between these two parameters (figure 4) despite the variation of the molecular mass distributions (width/shape of the hump). Figure 4 also shows that there is no correlation between the composition of the hump and the RHC, which proves that the proposed value is fairly constant for all mineral oil products reaching into the volatility range of the hopanes. This validates the RHC as a parameter to characterize a UCM as a mineral oil material.

The confirmation of the mineral origin of a UCM detected as a contaminant is restricted to materials reaching into the volatility range of the hopanes (UCM-1 and UCM-2 in figure 2). As table 1 shows, it is sufficient that a small part of the hump (some 10%) is in the relevant segment, i.e. that the hump tails into the hopane range. Usually a hump centred on *n*-alkanes around C₂₃ can still be correctly

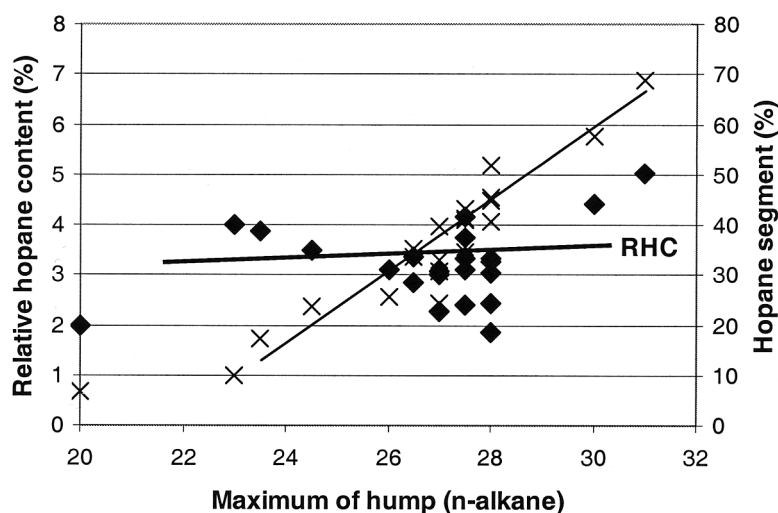


Figure 4. Relative hopane content (RHC) of the mineral oil products used for calibration (♦) against the characterization of the products by the *n*-alkane eluted at the maximum of the hump and the percentage of the hopane segment related to the total area of the hump (×).

characterized. The mixture centred on *n*-C₂₀ (the batching oil for jute bags) provided a low RHC, probably determined by the high uncertainty of integrating the hopane segment of the UCM). This gives the method a rather broad field of application covering most of the cases of interest for foods.

The RHC verifies the mineral origin only for the hopane segment of the UCM. There must be evidence that the material outside the hopane region belongs to that overlapping the hopanes, i.e. that the hump represents a homogenous material. If the contamination consists of two UCMs, one with a large portion within the hopane segment, the other largely outside, the evidence is restricted to the former.

Table 1 shows a RSD of the RHC of about 20%, which is indicative of the uncertainty under the rather favourable chromatographic conditions of a pure mineral oil product. This variability may result from different origins of the mineral oil or different biodegradation (the hopanes are degraded more slowly than most other hydrocarbons). The accuracy of integrating the area of the hopane segment of the unresolved hydrocarbons easily contributes substantial to the uncertainty. Finally, the RHC varies within the hopane window analysed: the predominant hopanes are in the early eluted part, i.e. the RHC tends to be higher in the early than in the late part of the window. This causes the RHC somewhat to depend on the distribution of the mineral oil material

within the hopane window: a mineral oil product predominantly in the first part of the window (e.g. a hump of a more volatile product tailing into the window like UCM-1 in figure 2) has a higher RHC than another one primarily in the rear part (material boiling higher than the hopanes). In conclusion, the RHC is adequate to determine whether most of a UCM of hydrocarbons is of mineral origin, but not for a reliable determination of a minor amount of hydrocarbons of non-mineral origin in a UCM largely of mineral origin.

In addition, the steranes could be used to demonstrate the mineral origin of a UCM. The hopanes were preferred since the biodegradation of the steranes is faster, i.e. the relative sterane content is expected to be less stable, and the steranes form a complex peak pattern rendering their GC determination more difficult. The steranes cover a similar volatility range as the hopanes, i.e. a combined use of hopanes and steranes does not broaden the field of application.

RHC in foods and human milk containing UCMs of paraffins

Samples of food and human milk containing a UCM of paraffinic material were re-analysed for the RHC. As for the calibration described above, the *n*-alkanes were disregarded for the determination of the hopane

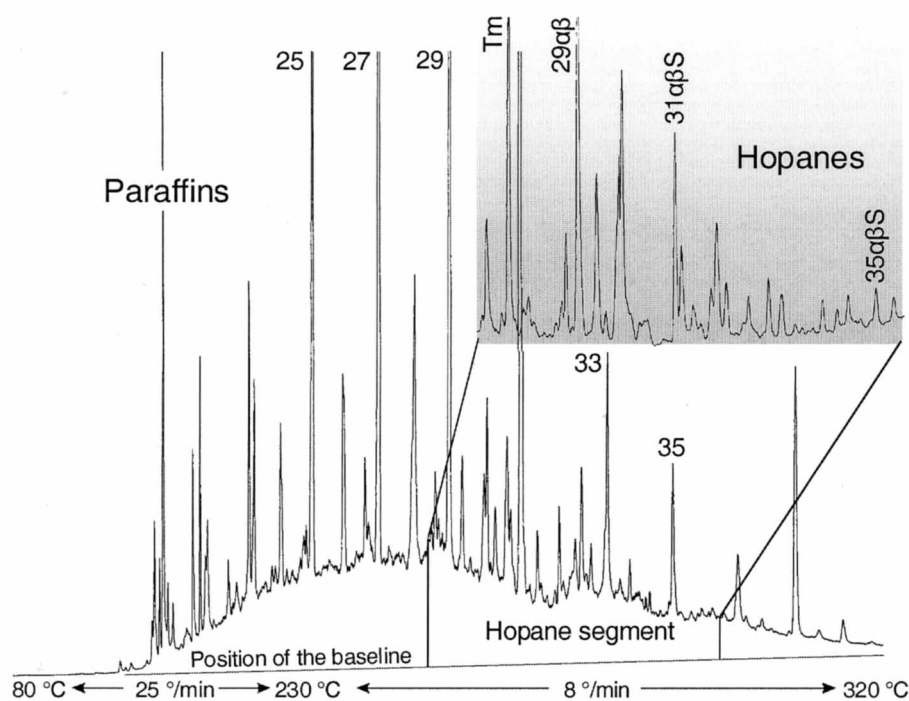


Figure 5. LC-GC-MS chromatograms of the paraffins (m/z 81) and hopanes (m/z 191) from a refined grape seed oil with an unresolved complex mixture corresponding to 35 mg kg^{-1} paraffins. The major n -alkanes are labelled by the number of carbon atoms.

segment of the UCM. These n -alkanes are anyway predominantly of plant origin.

Vegetable oils. As mentioned in the Introduction, vegetable oils often contain a UCM representing $10\text{--}30 \text{ mg kg}^{-1}$ paraffins beside the natural hydrocarbons (largely n -alkanes) from the waxes. In certain types of oils (such as grape seed, olive pomace, hazelnut, walnut and wheat germ oil), concentrations frequently reach 100 mg kg^{-1} . Extreme contents exceeded 1000 mg kg^{-1} , e.g. in safflower oil (Grob *et al.* 1994). In some instances, the contamination could be traced back to hydraulic oil from the press or paraffin oil used for cleaning purposes. In others, motor or lubricating oil from tractors used for moving olive pomace or grape seeds were the probable major sources of the contamination (Moret *et al.* 2003). However, there remained many samples, particularly oils containing a moderate amount of UCM-forming paraffins, for which the source of the UCM of paraffins could not be verified and doubts were expressed that the UCM really represented a contamination by a mineral oil material.

Figure 5 shows chromatograms from a grape seed oil containing a UCM comprising 35 mg kg^{-1} branched or cyclic paraffins. The normally shaped peaks above the hump, largely consisting of the $\text{C}_{25}\text{--}\text{C}_{35}$ n -alkanes with an odd number of carbon atoms, were not considered. The area of the hopane segment of the hump was integrated as shown, using the baseline from a blank chromatogram. The chromatogram of the hopanes is inserted and shows all the peaks observed in figure 1. The peaks recognized as hopanes by their retention time were summed up. The resulting RHC was 2.9% and, hence, in the range of the values determined for the mineral oil products (table 1). From this, it is concluded that the hopane segment of the hump represents mineral hydrocarbons. The shape of the hump suggests that the whole UCM is of the same type, i.e. that the whole amount of branched or cyclic paraffins is from a contamination by mineral oil material.

Table 2 shows analogous data from other edible oils of interest. The UCM ranged from 35 to 150 mg kg^{-1} (the almond oil showing two clearly distinguishable

humps). The RHC ranged from 2.0 to 3.7% and was, therefore, again slightly below the mean determined for the mineral oil products (table 1). In many cases, the deviation might be due to excessively generous integration of the hopane segment of the hump (actual baseline higher than in the previous blank runs). It is concluded that in all these samples, the UCM at least largely represents a contamination by mineral oil material.

Mussels and clams. Fish and other seafoods often contain rather high concentrations of branched and cyclic paraffins suspected to be of mineral origin (Solé *et al.* 1996, 2001, Grob *et al.* 1997, Moret *et al.* 1997).

Table 2. Branched and cyclic paraffins (unresolved complex mixture, UCM) and relative hopane content (RHC) in vegetable oils.

Vegetable oils	UCM (mg kg ⁻¹)	RHC (%)
Grape seed oil refined 1	35	2.9
Grape seed oil refined 2	40	2.9
Almond oil	25 + 20	2.0
Olive pomace oil refined 1	150	3.7
Olive pomace oil refined 2	150	3.6
Olive pomace oil refined 3	150	3.6
Olive pomace oil raw 1	60	2.3
Olive pomace oil raw 2	25	2.3

The chromatograms in figure 6 are from mussels collected at Lignano/Friuli (Italy). They contained 7000 mg kg⁻¹ paraffins referring to the fat, with a maximum at a retention time corresponding to that of the *n*-alkane C₂₂. The hopane segment is in the tail of the hump. The RHC was 1.7% (table 3), i.e. below the calibrated value and could support that only half of the UCM is of mineral origin. Note that there was substantial uncertainty about the integration of the hopane segment: the assumption of a higher baseline would have increased the value. There is, however, also the possibility that the uptake by the mussels is selective, discriminating against the hopanes. Another sample of mussels and two samples of clams provided similar results, whereby the hopane segment of the clams was easier to integrate and, indeed, the RHC was 3.1%.

Table 3. Branched and cyclic paraffins (unresolved complex mixture, UCM) and relative hopane content (RHC) in mussels and clams. The concentration of UCM of paraffins refers to the fat.

Seafoods	UCM (mg kg ⁻¹)	RHC (%)
Mussels 1	2000	1.5
Mussels 2	7000	1.7
Clams 1	1000	3.1
Clams 2		3.1

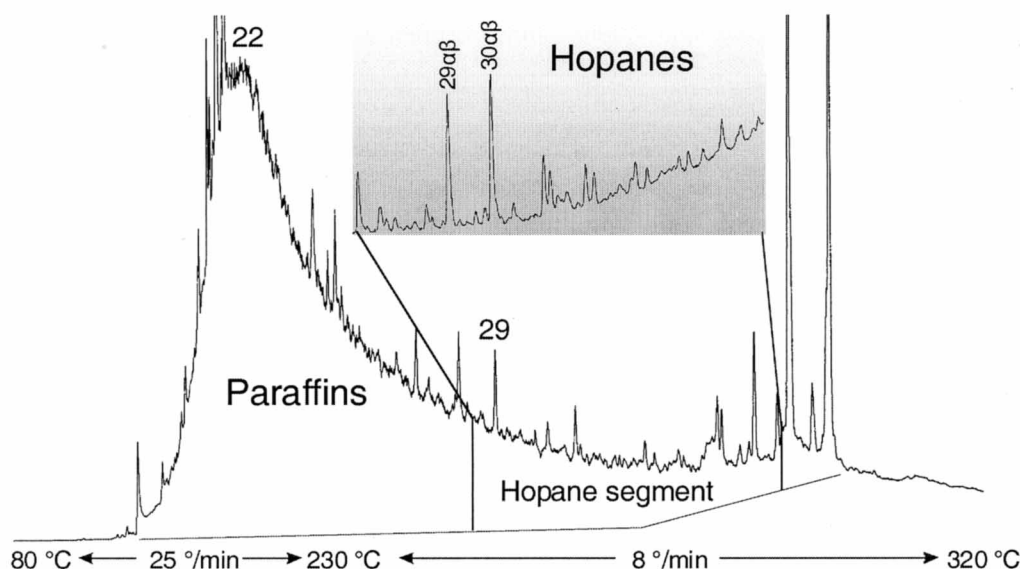


Figure 6. Paraffins and hopanes extracted from mussels.

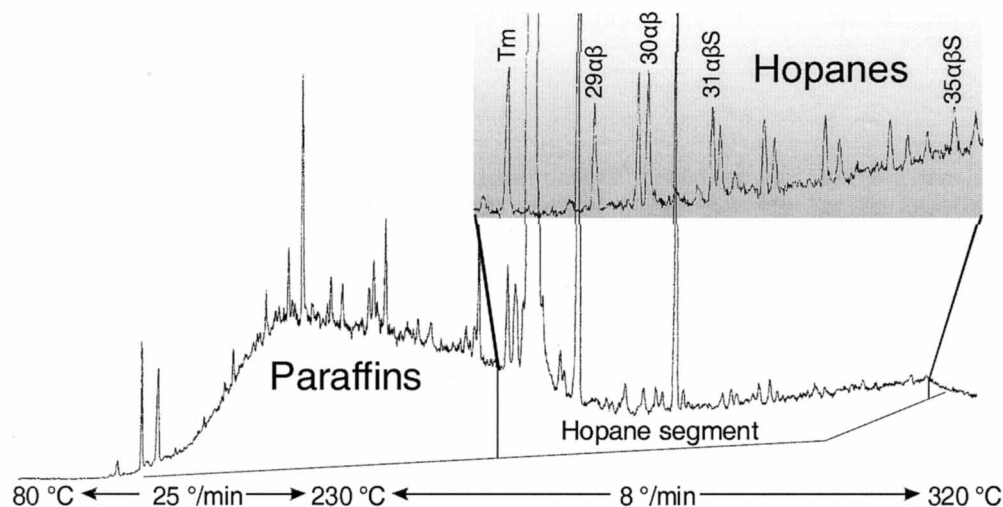


Figure 7. Unresolved complex mixture of paraffins and hopanes in human milk.

The molecular weight distribution shown in figure 6 corresponds to the fact that the uptake of mineral oil material by living organisms rapidly decreases with increasing molecular weight (European Agency for the Evaluation of Medicinal Products 1995), i.e. that from a broader range mineral oil material the components of smaller molecular mass are preferentially resorbed (the even more volatile compounds are evaporated).

Human milk. Recently, it was shown that in the initial phase of breast-feeding human milk typically contains 20–50 mg kg⁻¹ of a UCM of paraffins referring to the fat; concentrations decrease during continuing breast feeding. Extreme concentrations exceeded 1000 mg kg⁻¹, probably caused by the application of salves containing or consisting of paraffin oil (Vaseline) to the breast and transfer through the skin; the mean concentration was 95 mg kg⁻¹ fat (Noti *et al.* 2003). Initially, human milk was analysed to estimate the contamination of the human body fat with mineral oil material.

The UCM found in human milk is typically centred on the *n*-alkanes C₂₂–C₂₅. The sample shown in figure 7 is from day 4 of breast feeding and from a mother having her first child; the UCM corresponded to 35 mg kg⁻¹ paraffins in the fat. Since most paraffin oils encountered in foodstuffs and cosmetics have a higher average molecular weight, this again reflects that the uptake rapidly decreases with increasing

Table 4. Branched and cyclic paraffins (unresolved complex mixture, UCM) and relative hopane content (RHC) in samples of human milk. Concentrations refer to the fat.

Human milk	UCM (mg kg ⁻¹ fat)	RHC (%)
Milk 1	35	3.0
Milk 2	65	2.9
Milk 3	30	3.4
Milk 4	50	2.2
Milk 5	25	2.8

molecular mass (size). The mineral oil cuts centred at C₂₇ to C₂₉, used for lubricating oils and mostly predominating in foods, are just in the tail of the UCM.

The chromatogram of the hopanes contains all the compounds expected. The RHCs for the samples analysed (table 4) were around 3, which is in agreement with those expected for mineral oil products.

Conclusions

Hopanes were analysed in mineral oil products as well as in samples of food and human milk containing a UCM of paraffins suspected to be of mineral origin. The presence of the whole range of hopanes in these

food and milk samples adds evidence that the UCMs represent a contamination by mineral oil material. The sheer presence of hopanes is, however, insufficient to demonstrate that most or all material represented by the UCM is of mineral origin. For a more quantitative determination, the RHC was defined as the area ratio of the hopanes (detected by MS using m/z 191) and the paraffins in the corresponding segment of the UCM (m/z 81; comprising the region from Kovacs Index 2860–3650). The n -alkanes were not considered since from many mineral oil products they are removed during raffination or degraded by microorganisms. This RHC was shown to be fairly constant among mineral oil products of different types and origin, and to correspond reasonably to those found in food and human milk samples containing a UCM of paraffins.

The RHC provides a quantitative confirmation of the mineral origin for mineral oil materials of all types of compositions as long as they reach into the volatility range of the hopanes. Strictly speaking, it enables a statement only for the hopane segment of the UCM, but can be extended to the whole material with high probability if the paraffins are homogeneous in their molecular weight distribution.

The method was applied to three types of samples for which the contamination by mineral oil material could not conclusively be demonstrated by their history. For certain vegetable oils, such as olive pomace and grape seed oil, the RHC provided evidence against the hypothesis that some plant material could be degraded to branched and/or cyclic hydrocarbons during the often long storage of the pomace or grape seeds. For seafood, in particularly mussels and clams, it is proof against the hypothesis that the degradation of algae would produce a material similar to mineral paraffins. Via human milk it added evidence that our body fat is contaminated by mineral oil material at concentrations in the range of tens of mg kg^{-1} .

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