

Highlight Article

How “white” was the mineral oil in the contaminated Ukrainian sunflower oils?

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1 Introduction

During winter/spring 2007/2008, in the Ukraine nearly 100,000 t of sunflower oil were contaminated with mineral oil at concentrations often above 1000 mg/kg. The problem became public in April 2008 through notification by the European alert system (RASFF). The European Food Safety Authority (EFSA) advised the EU Commission about the risk to human health on April 28, 2008, with updates on May 21 and May 27 [1]. The EFSA concluded that “exposure to such oil, although undesirable, would not be a public health concern”.

On April 30, the Commission requested the national food authorities to remove the contaminated Ukrainian oil from sale, including foods containing more than 10% possibly contaminated oil as an ingredient, unless it could be shown that the oil contained less than 300 mg/kg mineral oil. In June, a legal limit of 50 mg/kg was specified for mineral paraffins in crude as well as refined Ukrainian sunflower oil. No oil exceeding this limit should be used for the preparation of foods, which means that in complex foods the limit refers to the sunflower oil [2]. The 50 mg/kg are clearly above the normal contents of mineral oil in sunflower oil, but conflicts might arise from other edible oils or foods known to occasionally or even regularly contain higher concentrations [3].

The rather high molecular mass (see also below) and the almost complete absence of *n*-alkanes suggested that the contaminant consisted of a base oil for the manufacture of lubricants or hydraulic oils. It is widely assumed that it was added

as a fraud, since in the Ukraine such oil was cheaper than sunflower oil, but there is no official confirmation.

The open collaboration of the companies involved in the production and trade of edible oils with the competent European authorities enabled a rapid settlement of the crisis. The work presented here is not intended to influence this case but to learn from it for potential future cases of food contamination with mineral oil.

1.1 Risk assessment by EFSA

The risk evaluation by the EFSA was based on the analysis of 22 samples of contaminated sunflower oil with concentrations of mineral hydrocarbons reaching 7300 mg/kg in crude oils and 2000 mg/kg in refined oils. The chromatograms provided to the EFSA “revealed the presence of mainly medium- and long-chain hydrocarbons, with peaks around C₂₈–C₃₁. The shorter-chain hydrocarbons (< C₂₅) represented about 20% of the total hydrocarbons in crude sunflower oil. However, these compounds were removed by the refining process applied to produce refined vegetable oil for human consumption. The chromatograms of the refined oil indicated a complex mixture of linear and branched alkanes which were all high-boiling point compounds in the range of C₂₀–C₄₀ (high viscosity)” [1]. “No other contaminants were present in the samples”, in particular no additives for lubricating oils or pesticides, *i.e.* risk assessment was exclusively based on the hydrocarbons.

The EFSA used the highest level of contamination recorded in refined sunflower oil (2000 mg/kg of oil) and a daily consumption of 60 g vegetable oil for a 60-kg person, which resulted in a daily exposure to 2 mg/kg body weight (bw). This was compared to the acceptable daily intakes (ADI) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [4]. For mineral oil of high viscosity, an ADI of

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20 mg/kg bw was cited; for medium- and low-viscosity oil, the temporary ADI are 0.01 and 10 mg/kg bw, respectively, depending on the type of oil. Exposure corresponded to 10 or 20% of the ADI of the high-viscosity and the Class I medium- and low-viscosity oil, respectively, but to 200 times the ADI of the Class II and Class III oils. However, the “absence of medium-low viscosity compounds in the chromatograms of the refined sunflower oil . . . indicated the presence of only high viscosity compounds”.

In its opinion from 1995, the EU-Scientific Committee on Food (SCF), the predecessor of the EFSA, defined high-viscosity oil by a minimum viscosity, a maximum of 5% components with a boiling point below that of the *n*-alkane C₂₅, and an average molecular mass of no less than 480 Da (C₃₄-paraffins, 478 Da). Since viscosity cannot be determined for a contaminant in a food, the molecular mass distribution of the paraffins was used for the assessment.

1.2 Mineral aromatic hydrocarbons

The toxicological evaluations of the JECFA and the SCF were based on experimental data obtained from highly refined products (“highly refined paraffinic and naphthenic liquid hydrocarbons” and “white paraffinic mineral oils derived from petroleum-based hydrocarbon feedstocks”, respectively). “White” paraffin oils (considered “food grade”) are mineral oil fractions from which the aromatic hydrocarbons are extracted and which are subsequently hydrogenated to convert the residual aromatics to saturated hydrocarbons.

The question whether the mineral oil contaminating the Ukrainian sunflower oil was really of this quality was not addressed by the EFSA, presumably because of the lack of analytical evidence. If the mineral oil was added as a fraud, food-grade quality was not necessarily of high priority. In fact, to be clearly cheaper than sunflower oil, a rather cheap mineral oil product had to be selected.

Aromatic hydrocarbons in mineral oils have a composition strongly differing from those generated at high temperature, *e.g.* from incomplete combustion: A high percentage (usually more than 97%) is alkylated [5]. Instead of forming sharp signals in GC, they produce broad humps of poorly resolved components differing in the number of attached carbon atoms and isomers of these. Since neither mass spectrometry (MS) nor ultraviolet or fluorescence spectroscopic methods enable calibration of a response in the absence of standards of equal composition, the mineral aromatics must be measured by flame ionization detection (FID).

There is a wealth of data on food contamination by (polycyclic) aromatic hydrocarbons formed at high temperature (*e.g.* [6]), but little on that by aromatic hydrocarbons of mineral origin, which is probably related to the analytical difficulties. Moret *et al.* [7, 8] developed a complex on-line method enabling the measurement of the mineral aromatic hydrocarbons in foods and the characterization of their composition by ring number. From an edible oil or food extract,

the hydrocarbons were isolated by a rather large high-performance liquid chromatography (HPLC) silica gel column. The eluent of this fraction was evaporated in a miniaturized chamber, the residue separated by number of aromatic rings on an amino HPLC column, and these fractions were analyzed by GC-FID. This revealed the presence of mineral polycyclic aromatics (at least two rings) in the order of 10 mg/kg in various foods. For rice and linseeds (as well as linseed oil), the origin was the batching oil on jute bags they were packed in, but for other foods, such as fish and various edible oils, the sources were not identified. Alkylated mineral aromatics might be substantially less toxic than the non-alkylated parent compounds, but exposure seems to be higher by about three orders of magnitude.

This paper reports on results characterizing the mineral oils found in contaminated Ukrainian sunflower oils. An on-line HPLC-GC-FID method was used for determining the total concentration of the saturated and the aromatic mineral oil hydrocarbons in foods, from which the percentage of the aromatics in the mineral oil could be calculated.

2 Experimental

The mineral paraffin oil was “Paraffin viscous PH Eur, BP, USP 107160” from Merck (Darmstadt, Germany). The motor lubricating oil Total was from a local shop. Samples of contaminated sunflower oils were from Oleificio SABO (Manno, Switzerland), Stazione Sperimentale per le Industrie degli Oli e dei Grassi (Milano, Italy), and two companies that preferred remaining anonymous. None of the contaminated samples was from the retail market; they were all withheld as not suitable for human consumption.

The on-line LC-GC-FID method will be described in more detail elsewhere, but is outlined here. It involved an instrument from Thermo Scientific (Milano, Italy), consisting of a TriPlus autosampler, a Phoenix 40 dual syringe pump with three switching valves and a Trace gas chromatograph equipped with an on-column injector, a flame ionization detector and a switching valve for the regulation of the transfer.

n-Hexyl benzene (6B), *n*-nonyl benzene (9B), biphenyl (BP), perylene (Per), 5- α -cholestane (Cho), 1,3,5-tri-*tert*-butyl benzene (TBB), the *n*-alkanes C₁₂, C₁₄ and C₁₆, and PS-255 (a dimethyl polysiloxane) were from Sigma Aldrich (Buchs, Switzerland). The solution of internal standards contained 100 mg/L 6B, 9B, TBB and BP, 300 mg/L C₁₂, C₁₄ and C₁₆, as well as 500 mg/L Per and Cho in hexane. Hexane from Brenntag, Schweizerhall AG (Basel, Switzerland), was redistilled after passage through an aluminum oxide column (10 L hexane passed through 1.5 kg aluminum oxide 60, active basic, activity I, 63–200 μ m in a glass bottle; Merck, Darmstadt, Germany). Dichloromethane was from Baker (ultra resi-analyzed; Stehelin, Basel, Switzerland).

To 2 g sunflower oil in 10 mL hexane (200 mg for samples contaminated at >3000 mg/kg), 20 μ L standard solution was added. Depending on the level of contamination, 5–50 μ L was injected onto a 250 \times 2 mm i.d. Lichrospher Si 60 column (Grom, Rottenburg-Hailfingen, Germany) and chromatographed at 300 μ L/min with a gradient of eluent A (hexane) and B (dichloromethane). The gradient started with 100% A, increased B to 30% in 2 min and remained isocratic up to 6 min. Then, the column was backflushed with 100% B at 500 μ L/min for 9 min. It was reconditioned in forward flow for 10 min at 500 μ L/min A and another 10 min at 300 μ L/min.

The fractions of the saturated (2–3.5 min) and aromatic hydrocarbons (4–5.5 min; 450 μ L each) were transferred to GC through the on-column interface by the retention gap technique [9]. The GC system consisted of a 10 m \times 0.53 mm i.d. uncoated precolumn, silylated in the laboratory, connected to the vapor exit and a 15 m \times 0.25 mm i.d. separation column coated in the laboratory with a 0.12- μ m film of PS-255 by a T-piece union (part of the GC instrument). The fraction of the paraffins was transferred with 60 kPa helium as carrier gas at 65 $^{\circ}$ C, that of the aromatic hydrocarbons with 120 kPa helium at 50 $^{\circ}$ C. The vapor exit was closed at 3.55 and 5.6 min, respectively, after injection into HPLC. GC separation involved programming at 20 $^{\circ}$ C/min to 350 $^{\circ}$ C with 150 kPa helium, starting 6 min or 8 min after injection (paraffins and aromatics, respectively). The detector base was at 380 $^{\circ}$ C. The intermediate fraction (3.5–4 min) was also transferred, but merely served for cleaning the system (otherwise there was a memory effect of 1–2% for the paraffins) and verification of complete separation between the aliphatic and aromatic hydrocarbons.

The concentrations of the saturated and aromatic hydrocarbons were calculated using C_{16} and BP, respectively, as internal standards, applying a response factor of unity. The performance of the method was monitored by

verification standards. The area ratio of C_{12}/C_{14} checked for losses of volatile components during LC-GC transfer, and that of C_{14}/C_{16} for coelution of C_{16} with sample components. Separation of the aliphatic and the aromatic hydrocarbons had to be complete in the sense that neither Cho nor TBB were present in the intermediate fraction. TBB (little retained in HPLC) and Per (strongly retained, ensuring that benzopyrene and its alkylated derivatives would be included) had to be completely present in the fraction of the aromatic hydrocarbons, which meant that the area ratios TBB/BP and Per/BP had to correspond to that of a calibration by on-column injection. The measurement uncertainty was primarily determined by the accuracy of placing the baseline underneath the humps of unresolved material, *i.e.* it depended on the sample, but was below 20% for all samples reported here.

3 Results

3.1 Mineral oils of different qualities

As reference point, Fig. 1 shows on-line HPLC-GC-FID chromatograms of the saturated (upper chromatograms) and the aromatic hydrocarbons (lower chromatograms) of three mineral oil products considered typical of their type. The mineral hydrocarbons primarily or exclusively form a hump of unresolved components, either consisting of branched paraffins and cyclic naphthenes (here termed paraffins) or of alkylated aromatics. Since there is no chance of separating the components, a short (15 m) separation column was used with a fast temperature program (20 $^{\circ}$ C/min) in order to obtain a flat baseline and a high signal. The shape of the hump reflects the molecular mass distribution, which in turn reflects the distillation performed in the refinery. Since a non-polar GC stationary phase was used and the volatilities of the paraffins

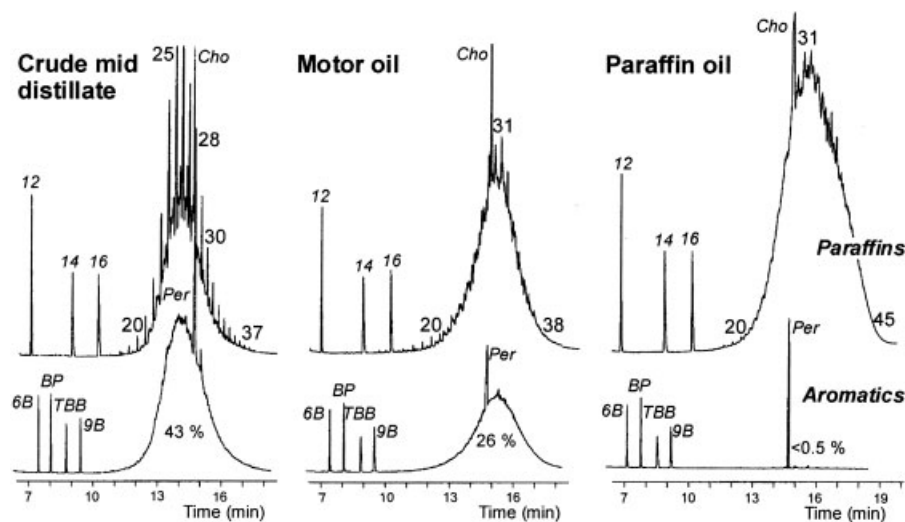


Figure 1. HPLC-GC-FID chromatograms of mineral oil products with different contents of aromatic hydrocarbons (%). Upper chromatograms, paraffins; lower chromatograms, aromatics of the same oil. Peaks labeled in italics, internal standards.

and aromatics correspond, the humps of the paraffins and the alkylated aromatics have virtually the same elution temperature and shape.

The sample on the left is a Russian crude mid distillate ranging from n -C₂₀ to n -C₃₇, centered on n -C₂₆. The n -alkanes are still present. Of this oil, 43% consisted of alkylated aromatics. The aromatics form a hump with hardly any component standing up as an isolated peak. The chromatograms in the center are typical of lubricating oils (Total, motor oil 2001). The oil was deparaffinated: The n -alkanes observed in the crude oil were largely removed; the large peaks on top of the humps are from the verification standards. The aromatic fraction is reduced to 26% of the oil by extraction. The oil on the right is a white paraffin oil certified as respecting pharmaceutical purity criteria. It must have gone through deparaffination, extraction and hydrogenation and contained less than 0.5% aromatics.

3.2 Sunflower oil

Figure 2 shows chromatograms of the natural components in a not substantially contaminated sunflower oil, with the paraffins on top and the aromatic hydrocarbons at the bottom. The oil on the left was crude whereas that on the right was refined. To avoid interferences, no internal standards were added.

Raw sunflower oils contain mono-, sesqui- and diterpene hydrocarbons, with alpha-pinene (not visible), calarene and kaurene as the predominant components of these classes. Since calarene and kaurene contain a double bond, they were sometimes partly eluted in the intermediate fraction between

the paraffins and the aromatic hydrocarbons, but not in the fraction of the aromatics. The natural paraffins show the pattern typical of all vegetable oils: a strong predominance of the odd-numbered n -alkanes, ranging from C₂₃ to C₃₅, and with a maximum usually at C₂₉ or C₃₁. The fraction of the aromatic hydrocarbons primarily shows a large peak for squalene (six double bonds).

Raffination, in particular deodorization, removes the terpenic components and most of the n -alkanes up to n -C₂₇. Often, part of the squalene is isomerized (primarily during bleaching), forming a hump of unresolved isomers. Immediately after squalene, peaks of the dehydroxylation of the sterols (sterenes, from bleaching and deodorization) are visible, with 3,5-stigmastadiene being the main degradation product from the main sterol, sitosterol [10].

A closer look at the paraffins reveals the presence of a low hump of unresolved material in the range of the plant n -alkanes. In fact, additional pre-separation with activated aluminum oxide to selectively remove the n -alkanes [11] enables to determine these mineral paraffins quantitatively. In the two samples shown, they amounted to 8 and 5 mg/kg.

Figure 3 shows analogous pairs of chromatograms for two contaminated Ukrainian sunflower oils. The raw oil (left chromatograms) contained 340 mg/kg mineral paraffins forming a broad hump of unresolved components from n -C₁₈ to n -C₃₈, centered on the n -alkane C₂₆. The mineral oil was deparaffinated; the n -alkanes on top of the hump correspond to those naturally present in sunflower oil (Fig. 2). The paraffins were accompanied by 100 mg/kg aromatics of the same molecular mass range, corresponding to 23% of the mineral oil. This mixture resembles that of the lubrication (motor) oil

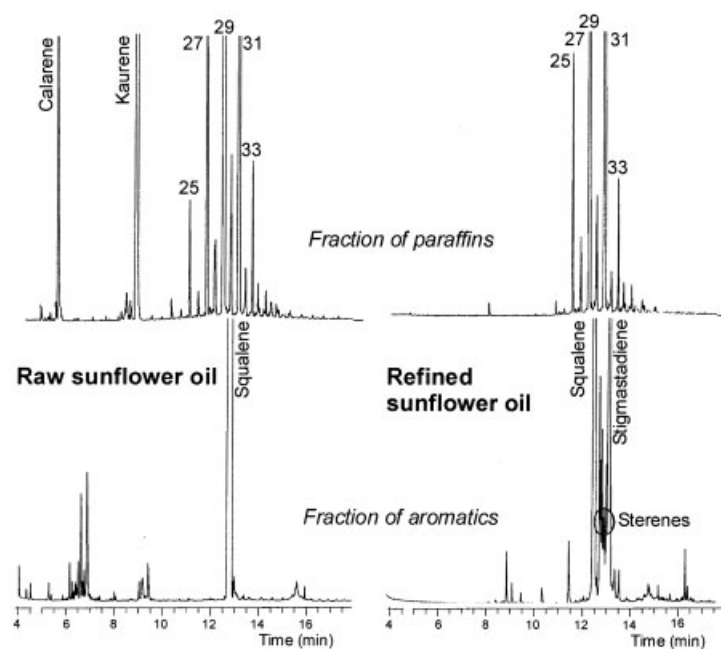


Figure 2. Natural components in the fraction of the paraffins (upper chromatograms) and the aromatic hydrocarbons (lower chromatograms) of raw (left) and refined (right) sunflower oil. No internal standards.

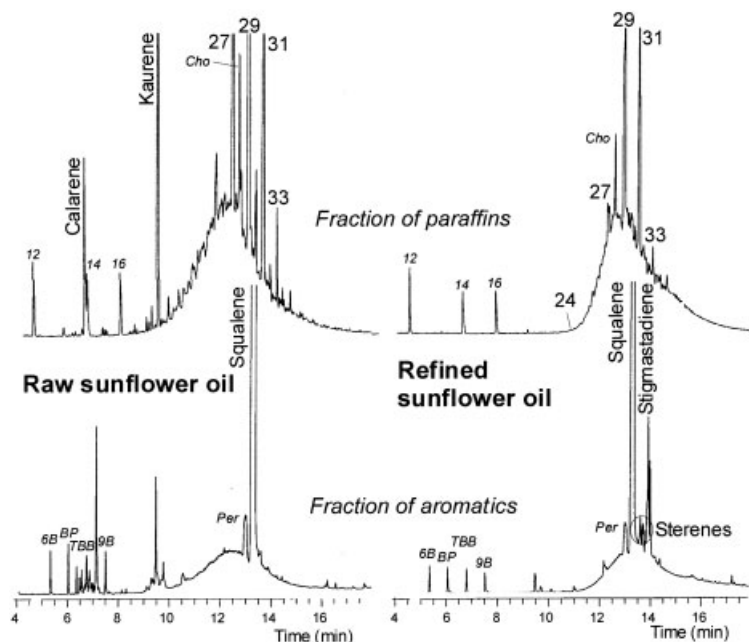


Figure 3. Contaminated Ukrainian raw and refined sunflower oils: 440 and 500 mg/kg mineral oil containing 23 and 28%, respectively, mineral aromatics.

shown in Fig. 1. The contamination of the sunflower oil amounted to a total of 440 mg/kg.

The refined sunflower oil on the right contained 360 mg/kg mineral paraffins. It was not produced from the crude oil on the left, but since the shape of the hump representing the high-molecular-mass components (right half) is rather exactly the same as that in the raw oil shown on the left, the contaminant was similar or may even have been the same. The low-molecular-mass components up to n -C₂₄ were totally eliminated by deodorization, and even the top of the hump was shifted by about two carbon atoms; approximately half of the contamination must have been removed during raffination. The 140 mg/kg mineral aromatics correspond to 28% of the contaminant. If the contaminant had been the same, this would indicate that the aromatic hydrocarbons were removed less efficiently than the paraffins, which seems plausible when considering the somewhat polar nature of the triacylglycerols. In fact, the hump of the aromatic hydrocarbons reaches further to the left (to the smaller molecular masses) than the paraffins.

3.3 Mineral oils contaminating Ukrainian sunflower oil

Table 1 lists the concentrations of the saturated and aromatic hydrocarbons in the 18 samples of contaminated Ukrainian sunflower oils analyzed. The sum of the two is considered as the concentration of the mineral oil. The concentration of the mineral oil in sample 4 must be considered as unknown since it originated from an (undisclosed) production process that enriched the mineral oil. The mineral oil composition was, however, not affected.

All samples contained mineral aromatic hydrocarbons. The maximum concentration of aromatics in a sunflower oil was 1800 mg/kg. It was found in a refined oil that was withdrawn from the market. Concentrations related to the total of the mineral oil ranged from 17 to 37% (average, 24%). The differences in the percentages of aromatics may be indicative of different mineral oils, but to some extent they are also related to deodorization under different conditions causing paraffins and aromatics to be removed differently.

All mineral oils were deparaffinated. Since all of them contained aromatic hydrocarbons in the range typical of lubricants or hydraulic oils, the common assumption is confirmed that the contaminant consisted of base oil for the manufacture of such technical products.

The composition of the mineral oil in terms of molecular mass distribution was similar, but not identical, for all samples. In the four crude sunflower oils analyzed, *i.e.* those containing the mineral oil in the original composition, clear differences were noted for three of them (Fig. 4), whereas oil 3 (not shown) seemed to contain the same mineral oil as oil 2. In the mineral oil 1 (top chromatograms), the hump ranged from n -C₂₀ to n -C₅₀ and the maximum was at the n -alkane C₃₀. In oil 2, the maximum was at n -C₂₆ (ranging from n -C₁₇ to n -C₄₂), and in oil 4 it was at n -C₂₈ (n -C₁₈ to n -C₄₂).

For the 14 refined sunflower oils, a comparison is more difficult because of the removal of the more volatile components and the resulting shift in the molecular mass distribution – both depending on the conditions of the deodorization. However, no contaminant was clearly different from the three shown in Fig. 4, in particular none reached higher molecular masses than oil 1.

Table 1. Concentrations of paraffins and aromatics in the 4 crude and 14 refined contaminated Ukrainian sunflower oils analyzed, the sum of the paraffins and aromatics, considered as mineral oil, as well as the percentages of the paraffins and aromatic hydrocarbons referring to the mineral oil.

Sunflower oil	Paraffins [mg/kg]	Aromatics [mg/kg]	Sum [mg/kg]	Paraffins [%]	Aromatics [%]
<i>Crude</i>					
1	350	85	435	80	20
2	340	100	440	77	23
3	2390	610	3000	80	20
4	?	?	?	67	33
<i>Refined</i>					
5	115	40	155	74	26
6	130	50	180	72	28
7	190	70	260	73	27
8	270	75	345	78	22
9	340	90	430	79	21
10	360	140	500	72	28
11	400	110	510	78	22
12	390	140	530	74	26
13	420	145	565	74	26
14	540	170	710	76	24
15	620	125	745	83	17
16	970	210	1180	82	18
17	1650	370	2020	82	18
18	3100	1800	4900	63	37

4 Conclusions

So far, mineral oil contamination of foods was analyzed through the paraffins, and this was also the data base for the risk evaluation available to the EFSA. However, neglecting the aromatics is a shortcoming, since the concentration of the contaminant may be substantially underestimated and, probably more importantly, the aromatics are toxicologically more relevant.

All 18 samples of the contaminated Ukrainian sunflower oils analyzed contained mineral aromatic hydrocarbons; the proportion of the aromatic hydrocarbons in the mineral oil ranged from 17 to 37%. This means that the contaminant was by far not a food-grade “white” oil, as evaluated for use as a food additive and assumed in the risk assessment of the EFSA. The most seriously contaminated refined sunflower oil contained 1800 mg/kg aromatic hydrocarbons (next to 3100 mg/kg paraffins).

The composition of the mineral paraffins in all contaminated sunflower oils was similar and corresponded to that described by the EFSA in April 2008. If the assumption holds true that the mineral oil was added as a fraud, always the same type of product was added, but the variations show that the mineral oil was not from a single source. If 100,000 t of sun-

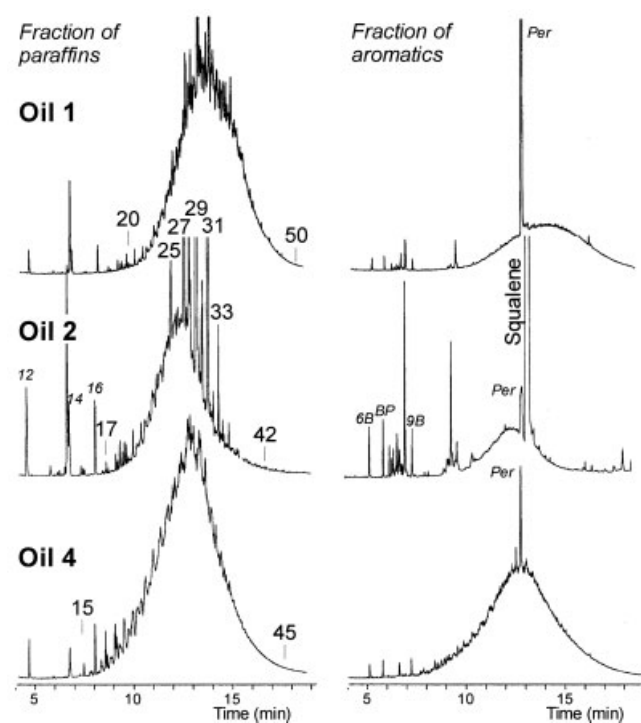


Figure 4. Fractions of the paraffins (left) and the aromatic hydrocarbons (right) in the crude sunflower oils numbered as in Table 1, showing that several mineral oil products of the same type were involved.

flower oil had been contaminated at an average concentration of 1000 mg/kg (no confirmed figures are available), the total amount of mineral oil added was 100 t. This oil was from at least three sources or batches of base oil.

Since the toxicological evaluation of the mineral aromatic hydrocarbons depends on the composition, the characterization of the aromatics is the next challenge. A corresponding paper involving two-dimensional GC (GC × GC) is in preparation.

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Conflict of interest statement

The authors have declared no conflict of interest.

References

- [1] EFSA: EFSA statement on the contamination of sunflower oil with mineral oil exported from Ukraine, May 29, 2008. www.efsa.europa.eu/cs/BlobServer/Statement/contam_statement_sunflower%20oil_en.pdf?ssbinary=true.
- [2] Summary minutes of the meeting of the Standing Committee on the Food Chain and Animal Health, Brussels, June 20, 2008. http://ec.europa.eu/food/committees/regulatory/scfcah/toxic/summary20062008_en.pdf.
- [3] K. Grob: Does the Ukrainian sunflower oil contaminated with mineral oil wake up sleeping dogs? An Editorial. *Eur J Lipid Sci Technol.* 2008, **110**, 979–981.
- [4] JECFA: Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food Additives, April 25, 2003. www.inchem.org/documents/jecfa/jecval/jec_1655.htm.
- [5] K. Grob, M. Biedermann, A. Caramaschi, B. Pacciarelli: LC-GC analysis of the aromatics in a mineral oil: Batching oil for jute bags. *J High Resol Chromatogr.* 1991, **14**, 33–39.
- [6] S. Moret, L. Conte: Polycyclic aromatic hydrocarbons in edible fats and oils: Occurrence and analytical methods. *J Chromatogr A.* 2000, **882**, 245–253.
- [7] S. Moret, K. Grob, L. S. Conte: On-line HPLC (LC)-solvent evaporation (SE)-LC-capillary GC-FID for the analysis of mineral oil polyaromatic hydrocarbons in fatty foods. *J Chromatogr.* 1996, **750**, 361–368.
- [8] S. Moret, K. Grob, L. S. Conte: Mineral oil polyaromatic hydrocarbons in foods, e.g. from jute bags, by on-line LC-solvent evaporation (SE)-LC-GC-FID. *Z Lebensm Unters Forsch.* 1997, **204**, 241–246.
- [9] K. Grob: *On-Line Coupled LC-GC*. Hüthig, Heidelberg (Germany) 1991.
- [10] K. Grob, M. Biedermann, A. Artho, J. P. Schmid: LC, GC, and GC-MS of sterol dehydration products. *Riv Ital Sost Grasse.* 1994, **71**, 533–538.
- [11] K. Fiselier, D. Fiorini, K. Grob: Activated aluminum oxide selectively retaining long chain *n*-alkanes. Part II. Integration into an on-line HPLC-LC-GC-FID method to remove plant paraffins for the determination of mineral paraffins in foods and environmental samples. *Anal Chim Acta* 2009, **634**, 102–109.