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Mineral oil paraffins in human body fat and milk

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Abstract

Paraffins of mineral oil origin (mineral paraffins) were analyzed in tissue fat collected from 144 volunteers with Caesarean sections as well as in milk fat from days 4 and 20 after birth of the same women living in Austria. In the tissue samples, the composition of the mineral paraffins was largely identical and consisted of an unresolved mixture of iso- and cycloalkanes, in gas chromatographic retention times ranging from *n*-C_{17} to *n*-C_{32} and centered at *n*-C_{23}/C_{24}. Since the mineral oil products we are exposed to range from much smaller to much higher molecular mass and may contain prominent *n*-alkanes, the contaminants in the tissue fat must be a residue from selective uptake, elimination by evaporation and metabolic degradation. Concentrations varied between 15 and 360 mg/kg fat, with an average of 60.7 mg/kg and a median of 52.5 mg/kg. Mineral paraffins might be the largest contaminant of our body, widely amounting to 1 g per person and reaching 10 g in extreme cases. If food were the main source, exposure data would suggest the mineral paraffins being accumulated over many years or even lifetime.

The milk samples of day 4 contained virtually the same mixture of mineral paraffins as the tissue fat at concentrations between 10 and 355 mg/kg (average, 44.6 mg/kg; median, 30 mg/kg). The fats from the day 20 milks contained <5–285 mg/kg mineral paraffins (average, 21.7; median, 10 mg/kg), whereby almost all elevated concentrations were linked with a modified composition, suggesting a new source, such as the use of breast salves. The contamination of the milk fat with mineral paraffins seems to decrease more rapidly than for other organic contaminants, and the transfer of mineral paraffins to the baby amounts to only around 1% of that in the body of the mother.
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1. Introduction

In the nineteen eighties, during routine control at the Official Food Control Authority of Zurich, a biscuit (Madeleine) was found which contained 1.5% paraffins of mineral oil origin (mineral paraffins) used as release agent, i.e. to facilitate the detachment of the finished piece from the baking dish. Rice from a second refining (after long-term storage) was turned shiny again by spraying mineral paraffins onto it, resulting in a concentration of 0.1–0.3% (Grob, unpublished work). This finding of occasionally massive food contamination with mineral paraffins initiated a broader investigation, with a first summary by Grob et al. (1992a).

Jute and sisal fibers were treated with batching oil to improve their processing properties. Brownish mineral oil fractions containing 20–25% aromatic hydrocarbons were used (Grob et al., 1991a; Moret et al., 1997).

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Concentrations of the mineral paraffins in foods transported and stored in jute, such as hazelnuts, cocoa beans (chocolate), linseeds and rice were typically in the range of 10–100 mg/kg (Grob et al., 1991b, 1992b, 1993). Food packaging materials released mineral waxes and oils at concentrations reaching several 100 mg/kg (Castle, 1991; Grob et al., 1991c, 1997; Jickells et al., 1994). Lubricating oils and release agents used in food industry often consisted of mineral paraffins and concentrations, e.g., in bread and bakery ware frequently exceeded 1000 mg/kg (Grob et al., 1991d; 1994). Printing inks often contained mineral paraffins as a solvent which reached 10–100 mg/kg in the foods packed in cardboard boxes (Droz and Grob, 1997). Some animal feeds contained used edible oils and fats (e.g. frying oils) from public waste collection sites not properly separating them from used motor oil and other technical products. They resulted in contaminated meat and eggs (Grob et al., 2001). Animal feeds may also contain mineral paraffins as a binder for minor components like minerals and vitamins in powder form. A large proportion of the edible oils were found to be contaminated with more than 10 mg/kg mineral paraffins (Wagner et al., 2001), with maximum concentrations of around 400 mg/kg (Moret et al., 2003). The broad contamination is thought to be from the atmosphere, i.e. lubricating oil emitted from diesel engines and other soot (Neukom et al., 2002), more specific and higher contamination from tractors moving around raw materials (olive pomace, grape seeds) before oil extraction. Fish often contains mineral paraffins and aromatics in the order of 100–1000 mg/kg referring to the fat (Moret et al., 1997). The source has not been identified.

Based on food consumption data and estimated concentrations in these items, Heimbach et al. (2002) estimated the mean dietary exposure to mineral paraffins in the US as 0.875 mg/kg body weight (bw). Half of this exposure was from white paraffin oils used as release agents in baking, for de-dusting of stored grain, in confectionaries and for coatings of fruits and vegetables. In Europe, most of these applications are illegal (de-dusting of grain, coating of fruits and vegetables) or were voluntarily stopped (use as release agents for bakery ware). Tennant (2004) estimated the mean and 97.5 percentile exposure in Europe as 0.39 and 0.91 mg/kg bw, respectively, for adults and as 0.75 and 1.77 mg/kg bw, respectively, for children. This would correspond to an average concentration in food of 25–60 mg/kg (1 kg food/d). Surveillance data by the Official Food Control of Zurich might have agreed with this in the early nineteen nineties, but since the major uses were stopped, exposure decreased substantially. More recent targeted analyses detected some foods containing around 50 mg/kg mineral paraffins, but the average was probably below 5 mg/kg and environmental contamination could well have become the predominant source (i.e. paraffins accompanied by aromatics and other materials).

Mineral paraffins are also used in many cosmetics and pharmaceutical products. Corresponding chromatograms were shown in Noti et al. (2003). In the extreme cases, these products virtually exclusively consist of mineral paraffins (paraffin oils, vaselines). No review on the exposure from this source was found in the scientific literature.

A summary of the toxicological findings regarding mineral paraffins was given by Noti et al. (2003). In 1989, the EU-Scientific Committee on Food concluded that "there was no toxicological justification for the continued use of mineral hydrocarbons as food additives". A temporary tolerable daily intake (TDI) of 0–0.005 mg/kg bw was set for oleum-treated mineral paraffins and of 0–0.05 mg/kg bw for hydrogenated products (SCF, 1989). Resulting limits in foods would have been in the range of 0.3–3 mg/kg, but were not imposed by legislation.

Perhaps in view of the broad use of mineral paraffins resulting in higher concentrations, the SCF revised its opinion and considered higher exposure safe provided the paraffins have a sufficiently high molecular mass not to be absorbed to a relevant extent (SCF, 1995). For mineral paraffin waxes, a group acceptable daily intake (ADI) of 0–20 mg/kg bw was allocated to highly refined products characterized by a minimum viscosity, a maximum of 5% components with a boiling point below that of the *n*-alkane C_{25} and an average molecular mass of no less than 500 Da. Regarding mineral paraffin oils, a temporary group ADI of 0–4 mg/kg bw was set for products specified by a minimum viscosity, a maximum of 5% components below the *n*-C_{25} and an average molecular mass of no less than 480 Da (C_{34}-paraffins, 478 Da).

In 2001, the SCF evaluated hydrogenated poly-1-decene as food additive, a synthetic substitute also called poly-alpha-olefines (PAO) containing 1.5% components with less than 30 carbon atoms (SCF, 2001). The material is poorly absorbed from the gastrointestinal tract (<1%) and an ADI of 0–6 mg/kg bw was established. In 2006, the European Food Safety Authority (EFSA) evaluated "waxes, paraffinic, refined, derived from petroleum based or synthetic hydrocarbon feedstocks", with an average molecular weight no less than 350 Da (about C_{32}), a minimum viscosity and a "content of hydrocarbons with carbon number less than 25, not more than 40% (w/w)". Owing to lack of toxicity data, a restriction of 0.05 mg/kg food was specified (EFSA, 2006).

The toxicological evaluation strongly differentiated by the molecular mass distribution. This corresponds to observations concerning uptake by animals. On the one hand, hens transferred 1.5–3% of the mineral paraffins centered at C_{21}–C_{24} alkanes added to the commercial feed into the eggs (Grob et al., 2001). On the other, cow milk or beef body fat usually contains no mineral oil material (detection limit, 3 mg/kg) of types used for lubrication, centered at C_{27}–C_{29} alkanes, although their feed is contaminated with it by the air (Neukom et al., 2002).

The sometimes apparently rather careless use of mineral paraffins raised the question about the relevance for humans, particularly regarding uptake from food, cosmetics, pharmaceutical products and possibly other sources. To this end, 33 samples of human milk were analyzed (Noti

et al., 2003). Related to the fat, the mean concentration was 95 mg/kg, strongly influenced by some extremely high values (180, 190, 700 and 1300 mg/kg). The median was at 26 mg/kg. Typical contents in the milk of the first days of breast feeding were around 50 mg/kg. In most cases, concentrations rapidly decreased with continuing breast feeding. In some others, however, they strongly increased, breast ointments being the source for at least some cases. Since the majority of the components are of less than C_{25}, these concentrations far exceed those considered safe by the SCF and EFSA. Particularly high exposure of the babies must be expected from mineral paraffins licked from the breast when used in (or as) breast salves and ointments.

This paper reports data from a project extending to human body fat samples. Samples of fat tissue were taken during Caesarean sections. From the same volunteers, human milk of days 4 and 20 after birth was collected. The composition and concentrations of the mineral paraffins were analyzed. An investigation of possible sources of the contamination through a questionnaire will be reported separately.

2. Experimental

2.1. Experimental design

Between October 2005 and June 2006, 144 women with an elective Caesarean section were included into the study at the Departments of Obstetrics and Gynecology of the Hospital of Bregenz and the Innsbruck Medical University, Austria. Inclusion and sample collection was performed after informed consent, in compliance with and approved by the Institutional Review Board. During the Caesarean section, 1 g of subcutaneous fat was removed after the delivery of the neonate when closing the abdominal wall. On days 4 and 20 post partum, samples of about 15 ml milk were collected at the end of breast feeding. The day 4 milk was collected using a pump (Symphony, Medela, Baar, Switzerland). The day 20 milk was collected either using a pump or by direct expression from the breast. To prevent contamination with mineral paraffins, women were instructed to avoid contact with hands and to refrain from using breast creams shortly before collecting the sample. Storage vials for fat and milk samples, the pump and the surgeons gloves were tested for the absence of mineral paraffins. All women filled in a questionnaire regarding their personal data, nutrition habits and customs of using cosmetics.

2.2. Determination of mineral paraffins

To about 1 g of tissue in a 25 ml Erlenmeyer flask with a glass stopper, 10 ml concentrated hydrochloric acid was added and heated to 80 °C during 30 min. After cooling, 300 mg fat (supernatant) was weighed into a 1.5 ml autosampler vial and filled up to 1.5 ml with hexane (Synopharm, Basel, Switzerland, redistilled).

To 10 g milk in an Erlenmeyer flask, 10 ml concentrated hydrochloric acid was added (for some samples 5 g milk and 5 ml acid) and heated to 80 °C during 30 min. The mixture was extracted twice with 10 ml and once with 5 ml redistilled pentane, sometimes using a centrifuge to improve phase separation. The pentane phases were combined in a tared flask and evaporated to dryness. The residue was weighed into a 1.5 ml autosampler vial and hexane was added to obtain a 20% w/v solution. For some exceptionally small samples and/or samples with a low fat content, only a 10% w/v solution could be prepared.

Extracts were analyzed by on-line normal phase high performance liquid chromatography (NPLC)-NPLC–gas chromatography (GC)–flame ionization detection (FID) essentially as described by Grob et al. (2001), using a fully automated instrument from Thermo Fisher (Milano, Italy). In short, 100 µl of the solution containing 20 mg fat was injected onto a 25 cm × 2 mm i.d. silica gel HPLC column. The breakthrough fraction (hexane, 300 µl/min) containing the hydrocarbons was transferred to a second 25 cm × 2 mm i.d. silica gel HPLC column to isolate the paraffins. The corresponding fraction (200 µl) was transferred to GC through the on-column interface by the retention gap technique (Grob, 1991), using a 7 m × 0.53 mm i.d. uncoated precolumn ahead of a solvent vapor outlet and a 5 m × 0.25 mm i.d. separation column coated in the laboratory with methylpolysiloxane PS-255 (Fluka, Buchs, Switzerland). Transfer and solvent evaporation occurred at an oven temperature of 72 °C (7 min, starting from injection into HPLC). Then the oven temperature was increased at 25 °C/min to 350 °C (5 min isothermal). The first NPLC column was backflushed with 1 ml methyl tert butyl ether (MTBE). For quantitation, a pure mineral paraffin oil used as release agent served as external standard. The detection limit was 3–5 mg/kg fat. The uncertainty was up to 30% at concentrations below 25 mg/kg and less than 20% above this level.

Numerous blanks were tested, working up water or cow milk samples virtually free of mineral paraffins. Great care was taken to avoid grease on the laboratory ware or the use of hand creams and similar products. The pumps and glass ware used to obtain the milk samples as well as gloves were tested by extraction with hexane.

3. Results and discussion

3.1. Gas chromatography of the paraffins

GC of mineral paraffins has been discussed in several papers cited in the Introduction. Fig. 1 shows a typical NPLC–NPLC–GC–FID chromatogram of a tissue fat containing 75 mg/kg mineral paraffins. The two-step preseparation ensured that virtually exclusively saturated hydrocarbons were transferred to GC. The large hump of unresolved material represents branched and cyclic hydrocarbons, as confirmed by mass spectrometry. As discussed previously, the mineral origin was derived from the absence in plant material and animals as well as by the presence of hopanes (Populin et al., 2004). A baseline was drawn underneath this hump (transferred from a blank chromatogram),

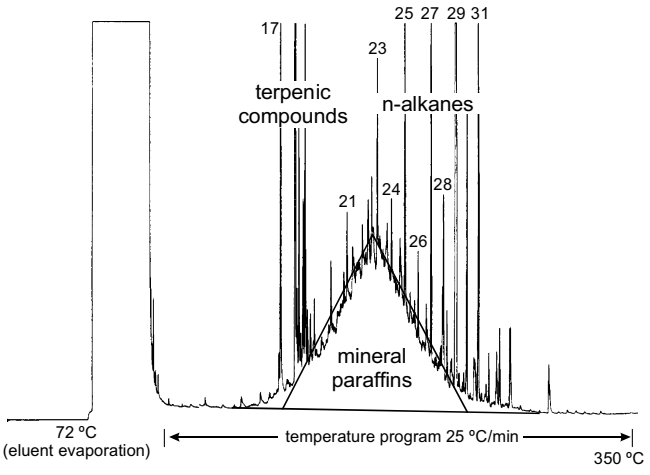

Fig. 1. Typical NPLC–NPLC–GC–FID chromatogram of a tissue fat. The mineral oil paraffins form the hump of unresolved components, the area of which is approximated by a triangle.

on top of which a triangle approximated its surface area. The latter was compared with that of a known amount of mineral paraffins.

The regular peaks were usually of secondary interest. Those between the C_{17}–C_{19} *n*-alkanes, in number and size varying from one sample to another, are commonly observed in extracts of plants, fish and animal products; they are assumed to represent terpenic components. The other main peaks are eluted with regular distances and represent odd-numbered *n*-alkanes, presumably from the surface waxes of plant materials, usually dominated by *n*-C_{29}. Even-numbered *n*-alkane in minor amounts are also of plant origin, but higher concentrations are indicative of mineral origin, where even and odd-numbered species are homogeneously distributed (e.g. Neukom et al., 2002; Wagner et al., 2001).

3.2. Tissue fat

Fig. 2 shows chromatograms of tissue fat samples spanning the range observed. The molecular mass distribution of the mineral paraffins (shape of the hump) in chromatogram A is as narrow as most commonly observed (see also below). That in B is the broadest of all 144 samples analyzed, broadened by at up to two carbon atoms towards larger molecular mass. Mostly the hump is centered on *n*-C_{23}–C_{24}, that in B slightly after *n*-C_{24}. This difference is surprisingly small when taking into account the broad variability of the mineral paraffins we are exposed to. Diesel oil is of far lower molecular mass, lubricating and hydraulic oil of larger mass (Neukom et al., 2002). The fractions typically used in food packaging materials and release agents consist of smaller alkanes, those in cosmetics span the whole range from far smaller to far larger species. Some contain substantial proportions of *n*-alkanes.

It is astonishing that the extract from all these mineral oil products into the human body fat is almost identical for all humans. This must be the result of highly selective processes: the more volatile components are lost by evaporation, the higher molecular mass components not taken up (with the same profile for the oral and the dermal route?) and the normal and certain branched alkanes are probably degraded together with those naturally present in food. This also means, however, that the composition of the mineral paraffins found in tissue fat does not provide information about the source.

Chromatograms C and D in Fig. 2 are from the tissue fats with the lowest and the highest content of mineral paraffins, shown at adjusted attenuations. Again there is no significant difference in the molecular mass distribution.

Fig. 3 shows the concentrations of the mineral paraffins in the 144 samples of tissue fat. They varied between 15 and 360 mg/kg, with an average of 60.7 mg/kg and a median of 52.5 mg/kg. 80% of the concentrations (10th–90th percentile) were in the narrow range between 30 and 100 mg/kg, which might also call for an explanation.

3.3. Milk fat: composition of paraffins

Fig. 4 compares the tissue fat (bottom) and the milk fat samples of days 4 and 20 of the same woman, selected as an example considered typical. The content of mineral paraffins in the milk fat at day 4 was somewhat below that of the tissue fat (55 compared to 85 mg/kg), which corresponds to expectations, since the sample was taken at the end of the breast feeding (see Section 3.4). The composition

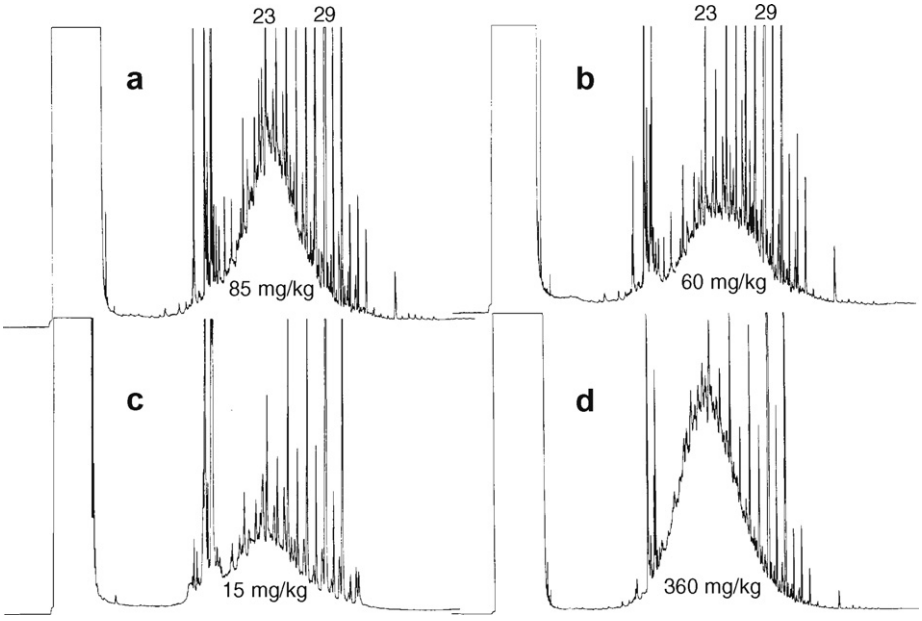

Fig. 2. Chromatograms of the extremes of the 144 tissue fat samples analyzed: a and b: narrow and broad fraction, c and d, minimum and maximum concentrations.

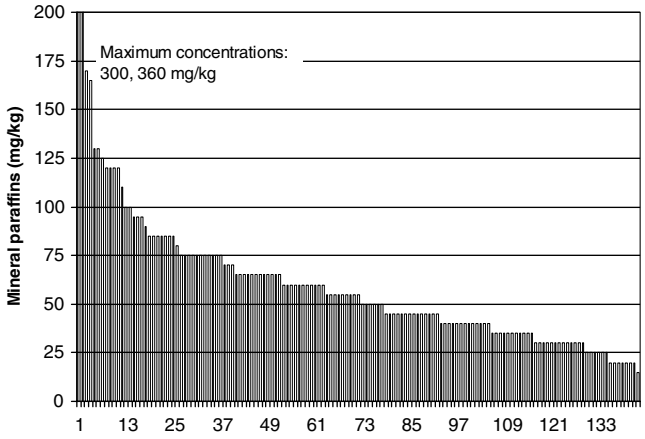

Fig. 3. Concentrations of the mineral paraffins in 144 samples of human tissue fat.

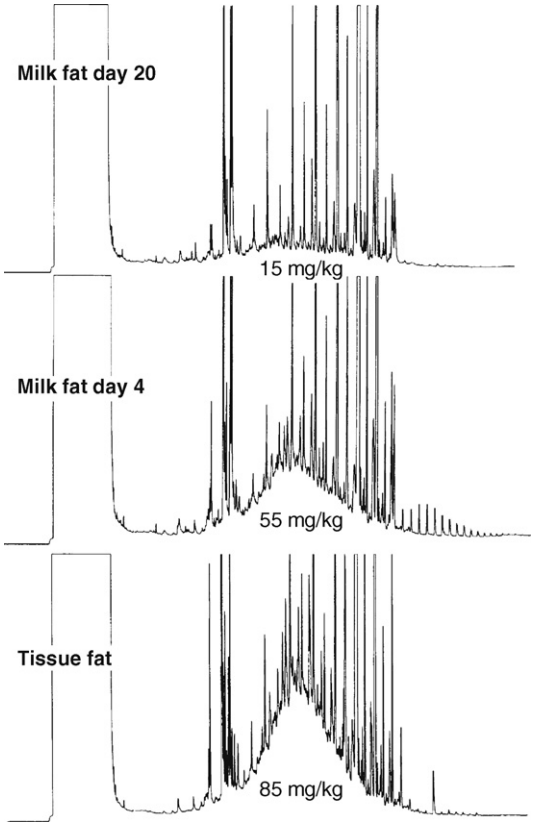

Fig. 4. Tissue fat as well as milk fats from days 4 and 20 of the same woman; example considered typical.

was virtually identical. Even the pattern of the normally shaped peaks corresponded rather well.

The milk fat from day 20 contained some 15 mg/kg mineral paraffins with an uncertainty of about 5 mg/kg primarily from interpreting the chromatogram. The hydrocarbons forming well shaped peaks once more matched those of the other two chromatograms. Their concentrations hardly decreased, indicating that the mineral paraffins were on the way to be exhausted, but the other, presumably natural hydrocarbons were re-supplied.

In the milk fat of day 4 shown in Fig. 5 the content of mineral paraffins slightly exceeded that in the tissue fat, but on day 20, it was clearly lower again. The example was chosen to point out the difference in the pattern of the signals on top of the hump of the mineral paraffins compared to Fig. 4: in addition to the *n*-alkanes with predominant odd-numbered chains (labeled in the top chromatogram) there are peaks eluted slightly earlier (marked by filled circles) between *n*-C_{22} and *n*-C_{28}. They are present in all three chromatograms and again their size remains fairly constant between the milk fats of days 4 and 20. Their identity and origin were not investigated; they are not observed in mineral oil fractions.

The tissue fat and the milk fat of day 4 shown in Fig. 6 correspond to those commonly observed. In the day 20 milk fat, however, the concentration of mineral paraffins is increased as compared to day 4 and the hump of unresolved iso- and cycloparaffins extends beyond *n*-C_{40}. Furthermore, there are large peaks for the *n*-alkanes C_{20}–C_{32} with the size of the even-numbered species closing up to the odd-numbered, indicating that most of these are also of mineral origin. The deviation from the normal composition and the relatively rapid appearance between day 4 and day 20 suggests a local contamination, such as from a vaseline-type mineral oil product used as breast salve (Noti et al., 2003). According to the questionnaire, this woman used lanolin on her breast, but it is open whether this particular lanolin contained mineral paraffins.

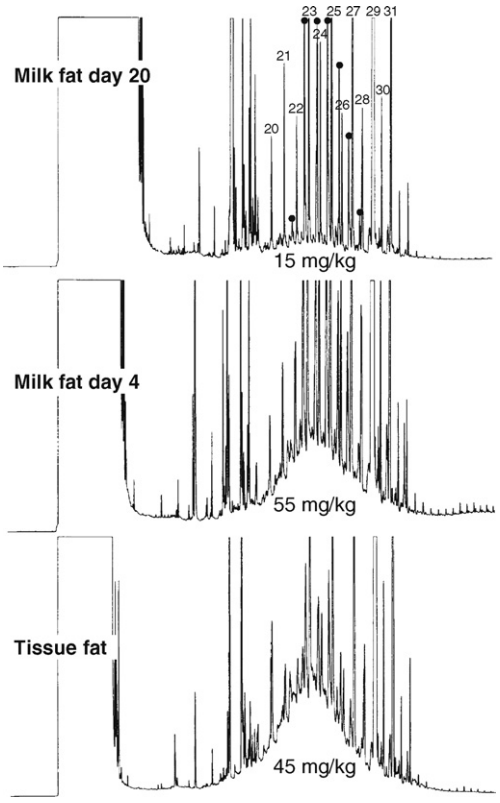

Fig. 5. Second set of frequently observed chromatograms, with additional peaks highlighted by filled circles.

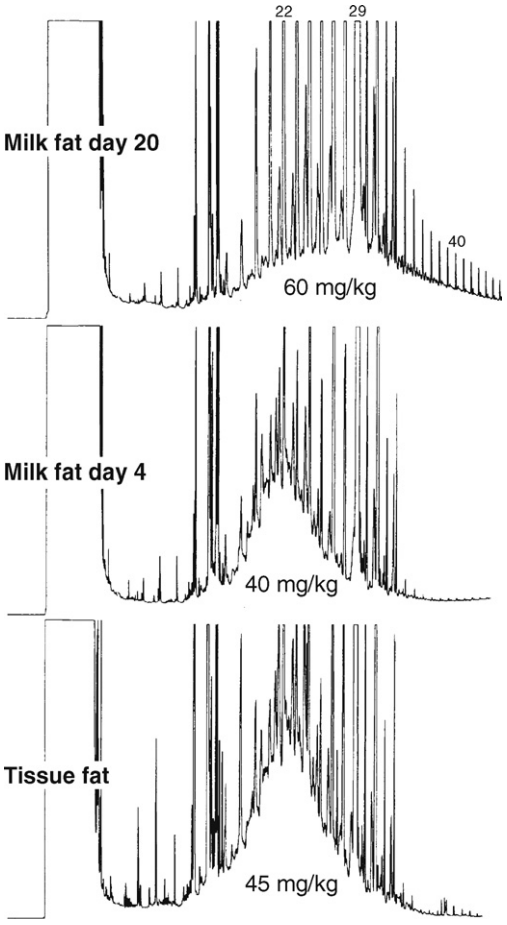

Fig. 6. Tissue and milk fat of day 4 showing the usual pattern of mineral paraffins. The milk fat of day 20, however, contained mineral paraffins at increased concentration and of a different composition. In this sample also most of the *n*-alkanes are of mineral origin as derived from the even-numbered species almost reaching the size of the odd-numbered.

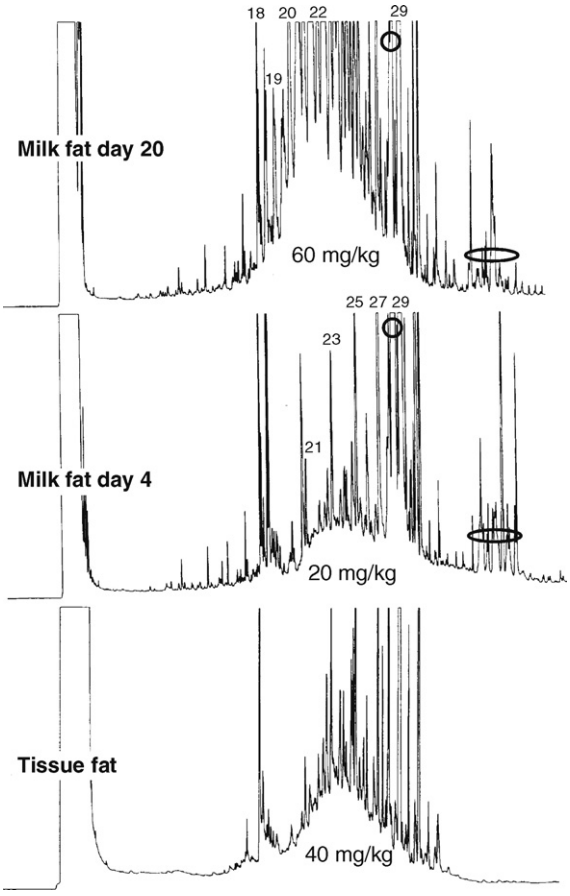

Fig. 7. Milk fats with non-identified hydrocarbons eluted in the region of the C_{40} alkanes (oval circles), a component eluted shortly before *n*-C_{29} (round circles) and (day 20 milk) a complex mixture in the range of the C_{19}–C_{25} *n*-alkanes.

Fig. 7 shows another example of milk fats containing unusual hydrocarbons. In the sample of day 4 there is a group of peaks eluted in the region of C_{40} (pointed out by oval circle). An additional large peak was eluted shortly before *n*-C_{29} (highlighted by a round circle). These components were not detected in the tissue fat, but were still present in the milk fat of day 20. It was the only case of this kind, and it is again assumed to be a local contamination.

In addition, the milk fat of day 20 contained large amounts of C_{17}–C_{26} hydrocarbons, i.e. of a molecular mass lower than the mineral paraffins usually observed in human milk. All *n*-alkanes C_{18}–C_{24} are found, including the even-numbered. As visible for C_{18} and C_{19}, they are closely co-eluted with other, unknown hydrocarbons. More distant, there were peaks showing up somewhat earlier and later than the *n*-alkanes. Those eluted earlier had the same positions as those pointed out in Fig. 5, but were present also at lower molecular masses. The simultaneous introduction of mineral *n*-alkanes and material not observed in mineral oil as well as the coincidence in the molecular mass range suggest an interrelation.

3.4. Contents of mineral paraffins in day 4 milk fat

The mean mineral paraffin content of the day 4 milk fats was 44.6 mg/kg, the median 30 mg/kg. This is similar to the results reported previously (Noti et al., 2003; not directly comparable, as the previous results were not focusing on day 4).

The slight decrease compared to the tissue fat (45 versus 60 mg/kg) corresponds to expectation. However, a more detailed examination of the results shows a more complex picture. As it seemed to facilitate the presentation, the discussion will start out from a first interpreting model. Since mineral paraffins are accumulated over a long period of time (see Section 4.2), their distribution in the body is assumed to be fairly even (remains to be confirmed). The milk fat is formed locally and there is an equilibration of the mineral paraffins between this milk fat and the surrounding tissue fat. The content in the milk fat would then tend to be lower than in the body fat. In fact, concentrations in milk fat rapidly decreased with each breast feeding (Noti et al., 2003), presumably because the local tissue fat is extracted and the replenishment is slow. On day 4, this effect should still be weak, since it is one of the first days

if not the first day of breast feeding after Cesarean section. However, concentrations also decreased during breast feeding (a factor of 2.5 was found previously) and, for ethical reasons, the milk samples were obtained at the end of breast feeding.

Fig. 8 compares individual paraffin concentrations in the fats of the tissue (bold line) and the day 4 milk for the 104 women for which both were available. The data was sorted by the tissue fat in decreasing order for the individuals in the *x*-axis. As expected by the above model, the majority of the concentrations determined in the milk fat were 2–4 times below those in the tissue fat, and the polynomial trend of the milk fat followed the tissue fat. However, there were also 10 milk fats substantially exceeding the content in the tissue fat. The maximum was 355 mg/kg (participant 8) and far exceeded the content of the tissue fat (120 mg/kg). In relative terms, the excess was even more pronounced for participants 57 and 94.

Since the model would not explain enrichment above the concentration in the tissue fat, a local supply was suspected, such as the use of breast salves and ointments containing mineral paraffins. For local applications with a short time difference (hours to days), it was noted that the uptake into the milk fat was less selective: the composition of the mineral paraffins was altered towards that applied to the breast and the *n*-alkanes were not degraded. Indeed, for participants 2, 20 and 81 (highlighted by an asterisk) the composition of the mineral paraffins clearly deviated from that normally observed, but no such effect was visible for the milk fats with the highest concentrations. This could be explained by a composition of the applied mineral paraffins incidentally being similar to that in the tissue fat or an older contamination having undergone metabolic changes (equilibration with the body fat, in particular with the abdominal fat analyzed, seems to be slow).

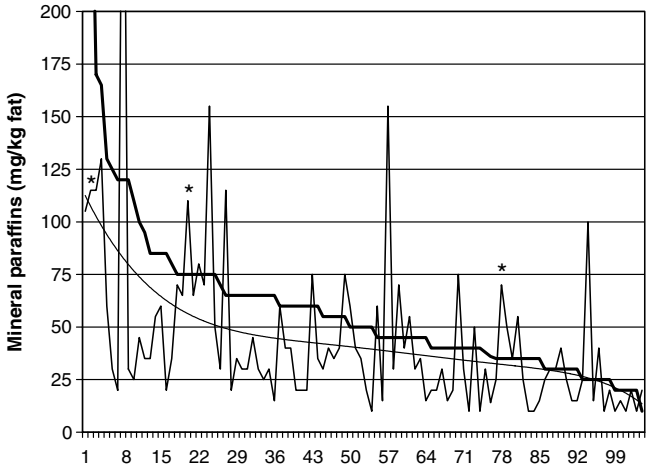

Fig. 8. Concentration of mineral paraffins in tissue fat (bold line) and the day 4 milk fat from the same woman, with the data for the 104 women (*x*-axis) sorted by the tissue fat. Dotted line: polynomic trend for the day 4 milk fats; asterisks, composition of the mineral paraffins deviating from that considered normal.

The fat content of the milks varied between 0.5 and 5.9 g/100 ml; the mean content was 2.4 g/100 ml. On day 20 it ranged from 0.5 to 8.5 g/100 ml, with an average of 3.7 g/100 ml. There was no clear correlation between the fat content and the concentration of mineral paraffins in this fat.

3.5. Contents of mineral paraffins in day 20 milk fat

The content of mineral paraffins in the milk fat from day 20 varied between less than 5 mg/kg (detection limit) and 285 mg/kg. Assuming a concentration of 2.5 mg/kg for the samples containing less than 5 mg/kg mineral paraffins, the average concentration was 21.7 mg/kg and the median 10 mg/kg.

As known from the previous study and shown in Figs. 4 and 5, the mineral paraffin content usually drops with every day of breast feeding and is little above the detection limit on day 20, resulting from depletion of the tissue fat near the sites the milk fat is formed and transported. This decrease is faster than that described in literature for PCBs, dioxins and other persistent bioaccumulative components (LaKind et al., 2001). In fact, 55 from 82 day 20 milk fats contained 15 mg/kg mineral paraffins or less. There were, however, also 14 samples containing between 30 and 285 mg/kg.

Since the samples from day 20 were taken at home without supervision, direct contamination of the milk, either from contact with the breast skin or fingers contaminated with mineral paraffins, cannot be ruled out. Using the pump, the milk spurts out and should have no contact with the skin. Also milk obtained by manual pressing should not be in contact with the breast skin except of the pores of the nipples where it spurted out.

The day 20 milk fat of the woman with the highest content on day 4 (355 mg/kg) was still at 95 mg/kg, but this time the molecular mass distribution was distorted, suggesting addition from a local source. The highest concentration in a day 20 milk fat (285 mg/kg) was from a woman with only 40 mg/kg mineral paraffins in the tissue fat and 15 mg/kg in the day 4 milk fat. Fig. 9 presents the comparison of the two milk fats from a given woman in the same stile as Fig. 8: the day 20 results are laid over those from day 4 and sorted by decreasing contents on day 4. There is little correlation: the high contents on day 20 often fall on low contents on day 4 and vice versa. Asterisks show that for all samples with high concentrations the shape of the hump of mineral paraffins or the presence of even-numbered *n*-alkanes indicated additional contamination.

It is concluded that the elevated concentrations in the milk samples from day 20 are from new contamination. In the most drastic case reported previously (Noti et al., 2003), the milk fat from a woman with serious problems regarding inflammation and desperately experimenting with ointments had a content of mineral paraffins increasing from 40 on day 7 to 1300 mg/kg on day 20.

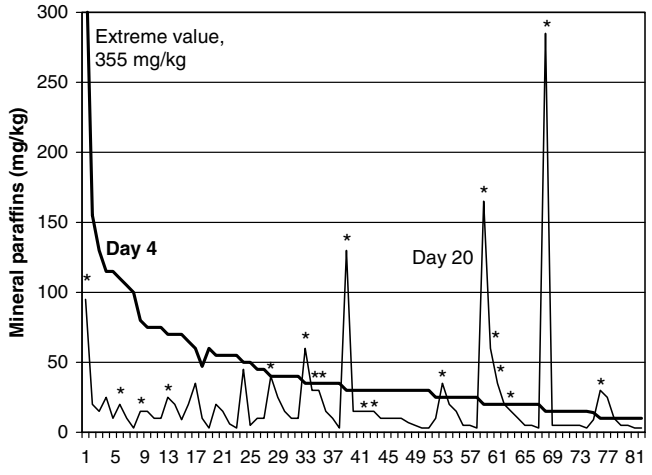

Fig. 9. Concentrations of mineral paraffins in milk fat of day 20 laid over decreasing contents in the fat of the day 4 milk (bold line). Asterisks indicate abnormal composition of the mineral paraffins.

4. Conclusions

4.1. Analytical data

The project provides the first data on mineral paraffins contaminating human body fat and links these with the contamination of the human milk. The results can be summarized by the following three points:

1. Abdominal tissue fat of 19–47 year old women living in Austria contained 15–360 mg/kg mineral paraffins, with an average of 60 mg/kg.
2. All tissue fats contained mineral paraffins of virtually the same composition, i.e. primarily iso-alkanes centered at C_{23}/C_{24} *n*-alkanes and ranging from about *n*-C_{17} to *n*-C_{32}. Since the mineral oil products we are exposed to range from much smaller to substantially higher molecular mass, this composition is indicative of efficient selection processes. This also means, however, that it does not help us identifying the source(s) of the contamination.
3. At the beginning of breast feeding, the concentrations of mineral paraffins in the milk fat are similar to those in the body fat, but then they rapidly decrease and are at <5–15 mg/kg on day 20. For milk fats with higher contents, fairly recent local contamination is assumed, such as through crèmes, salves and ointments. The maximum concentration encountered in this project was 355 mg/kg (it was 1300 mg/kg in the study of Noti et al., 2003).

4.2. Model calculations

The results leave many open questions calling for further investigations. The following three calculations based on hypotheses may provide interesting starting points.

Largest contaminant of our body? The total amount of mineral paraffins contaminating the human body fat can be estimated based on the assumptions that the concentration is the same throughout the body (not confirmed) and that it is largely independent of the body weight (see separate report). Then a slim person with 60 mg/kg mineral paraffins in 5 kg body fat contains 0.3 g. For a heavier person with 30 kg fat this is almost 2 g. If the 30 kg body fat were contaminated at the maximum level found (360 mg/kg), the total content of mineral paraffins would exceed 10 g. This suggests that the mineral paraffins are the largest known contaminant in the human body.

Long-term accumulation: It might be interesting to relate this content of the human body fat to the estimated exposure from foods. Tennant (2004) estimated the mean daily exposure to 0.4 mg/kg bw, but this seems high from the food control in Switzerland. Using tritium-labeled mineral paraffins, Ebert et al. (1966) determined an uptake of unchanged paraffins into rats from oral administration of 1.5% after 5 h, a decrease to 0.3% after 2 days and to 0.1% after 21 days. 1.5–3% of the mineral paraffins fed to hens were recovered in the eggs (Grob et al., 2001). Combining a high estimate of exposure of 20 mg/d from food with a high uptake of 1% and a neglect of elimination results in an incorporation of 0.2 mg/d. It would take 5000 days (some 14 years) to accumulate the 1 g of mineral paraffins estimated above. This result may be interpreted in two ways (or a combination of these). Firstly, the mineral paraffins found in the tissue fat accumulate over long times, perhaps life time. If this were true, most of the mineral paraffin material observed in adults of today would originate from times with much higher exposure from food. Secondly, it may indicate that sources other than food are more important, such as creams, salves, lipsticks and pharmaceuticals. Even inhalation of incompletely burned diesel fuel and heating oil or lubricating oil from the exhaust of diesel engines could be important. In this context it is interesting to note that the body fat of animals is clean (detection limit of about 5 mg/kg fat) unless the animal was raised with contaminated feed (known for hens, pigs and cattle, Grob et al., 2001).

Mineral paraffins transferred to babies: The amount of mineral paraffins fed to babies could be approximated by assuming a mean content in the milk fat during the first 10 days of 40 mg/kg and that the concentrations becomes negligible then (supposing no additional local contamination). If the baby drinks on average 800 ml milk per day with a mean fat content of 3%, it totally ingests 240 g fat containing 9.6 mg mineral paraffins. The rate of uptake by the baby might be high, since it is the selection of paraffins which already the mother accumulated and did not metabolize. The mother transfers roughly 1% of her mineral paraffins to her baby, confirming a local depletion, probably little more than that of her breasts.

4.3. Conclusions for regulatory measures

Taking into account that most mineral paraffins found in tissue and milk fat are of a mass below C_{25}, the concentrations exceed those considered safe for foods by the SCF and EFSA (TDI of 0.005–0.05 mg/kg bw; SCF, 1989) by 2–3 orders of magnitude. It must, furthermore, be taken into

consideration that the mineral paraffins accumulated by the mother might be those with the worst properties, i.e. those picked up and resisting degradation and elimination. Owing to the uncertainty in the toxicological evaluation, this is not necessarily alarming, but means that the risk should be better assessed.

The compositional information on the mineral paraffins accumulated by the human body may serve as an indication of which types of mineral oil products should be avoided. As a first observation, the mineral paraffins accumulated over long-term (those found in the tissue fat) are virtually free of *n*-alkanes. Since many of the mineral oil products we are exposed to contain large amounts of *n*-alkanes, this suggests that our metabolism removes them. In fact, our food contains substantial amounts of (odd-numbered) natural *n*-alkanes.

The accumulated alkanes are typical for mineral paraffin oils. The metabolism may remove some branched alkanes and just leave a residue of those most resistant to degradation – the lacking resolution by the analysis did not provide evidence on this aspect. However, it seems safe to assume that contamination of our body can be prevented if mineral paraffin oils including components in this range of molecular mass are avoided. In GC, the critical components are eluted between *n*-C_{18} and *n*-C_{28}. As the branched and cyclic alkanes are eluted at up to 2 carbons lower retention time than the corresponding *n*-alkanes, the molecular masses range from about *n*-C_{18} to *n*-C_{30}. This is in line with the opinions of the SCF and EFSA that mineral paraffins of low molecular mass should be avoided, but it might be necessary to review the limit at C_{25}.

One of the main questions remaining to be answered concerns the sources of the mineral paraffins accumulated in our body. It is known that animals take up paraffins from feeds. It also seems that paraffins from breast salves are transferred to human milk through the breast skin, which suggests that they could also be absorbed through other skin. Nothing is known about mineral paraffins inhaled from ambient air, except that animals breathing the same air do not seem to take them up.

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