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Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials

In the frame of Commission Recommendation (EU) 2017/84

S. Bratinova, E. Hoekstra (Editors)

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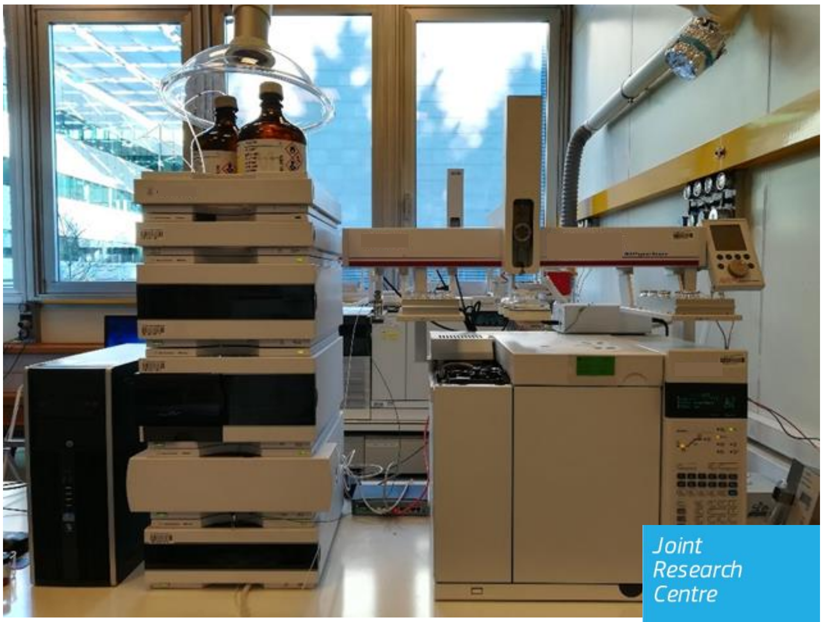

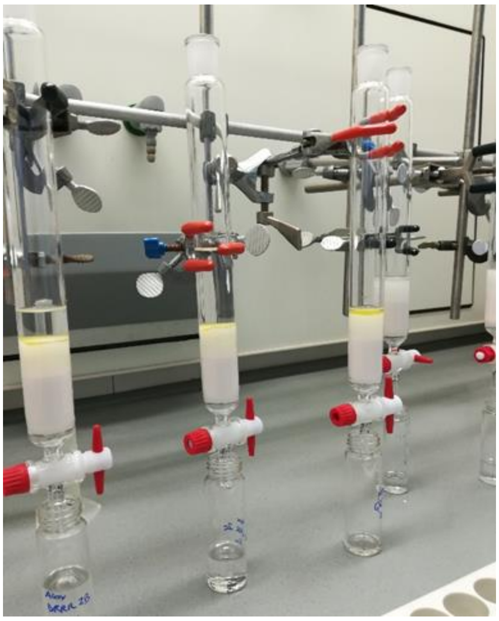

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Executive summary

This guidance document covers specific directions for sampling and analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in food and FCM in the frame of Recommendation (EU) 2017/84 for the monitoring of mineral oils.

It provides guidance on the minimum performance requirements of the analytical methods fit for MOSH/MOAH monitoring. The guidance should be used by all stakeholders involved in the determination of mineral oil hydrocarbons in food and FCM, i.e. food inspectors, official control laboratories, laboratories in industry and laboratories of non-governmental organisations.

This guidance aims to support the generation of reliable data for the occurrence of both fractions - MOSH and MOAH - and to enable reporting by laboratories that are already familiar with the analytical approaches and have proven their analytical performance in relevant proficiency testing (PT) schemes.

For laboratories that are not familiar with MOSH/MOAH analysis, this guidance gives the minimum performance requirements and references to current analytical approaches described in the scientific literature. It does not provide standard operating procedures.

1 Introduction

Consumers are exposed to a range of mineral oil hydrocarbons (MOH) via food[1]. Major sources of MOH in food are food packaging and additives, processing aids, and lubricants. Technical grade MOH contains up to about 50 % mineral oil aromatic hydrocarbons (MOAH); approved food grade mineral oil saturated hydrocarbons (MOSH) (white oils) are reported to contain less than 1 % of MOAH. Estimated MOSH exposure ranges from 0.03 to 0.3 mg/kg b.w. per day, with higher exposure in children. Except for white oils, exposure to MOAH is about 20 % of that of MOSH.

In 2012, the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA), on request by the Commission, issued an Opinion[1] concluding that the potential human health impact of groups of substances among the MOH vary widely.

Occurrence data were available only for a limited number of food groups and only from a few countries. At that time MOAH measurements did not exist for the majority of the samples. Until 2009, only the MOSH fraction was routinely analysed. Then it was possible to extend the method to the MOAH fraction[2]. MOAH, however, may act as genotoxic carcinogens, while some mineral oil saturated hydrocarbons (MOSH) can accumulate in human tissue and may cause adverse effects in the liver. As some MOAH are considered mutagenic and carcinogenic, it is important to organise monitoring of MOH to better understand the relative presence of MOSH and MOAH in food commodities that are major contributors to dietary exposure.

Commission Recommendation (EU) 2017/84 requests the Member States to monitor animal fat, bread and rolls (including fine bakery ware), breakfast cereals, confectionery (including chocolate and cocoa), fish meat, fish products (canned fish), grains for human consumption, ices and desserts, oilseeds, pasta, sausages, tree nuts, vegetable oil. It is required to sample food and pre-packaged food and to analyse the food and, if relevant, food contact materials (FCM) as well for the presence of mineral oil and to report the results to EFSA.

The analysis of MOH in food and FCM, especially in food with high fat content, is very demanding in terms of methodology and interpretation. It requires harmonisation amongst laboratories in terms of definitions, performance characteristics and data reporting to EFSA.

According to the Recommendation (EU) 2017/84 *"to ensure the reliability of the obtained analytical data, Member States should ensure the availability of suitable analytical equipment and gain sufficient experience in the analysis of MOH both in food and in FCM before generating analytical results. To ensure the uniform application of this recommendation, the European Union Reference Laboratory for FCM (EU-RL) should provide further guidance to the competent authorities of the Member States and other interested parties, including guidance on information that could be collected during investigations, as well as methods of sampling and analysis … the Member States should collaborate with the EU-RL to jointly develop that guidance in accordance with their needs for developing analytical capabilities."*

Following the Recommendation, this guidance document aims at giving directions to the Member States with respect to sampling, describing the performance characteristics of the analytical approaches, as well as defining the way the results should be reported to EFSA.

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[1] EFSA (2012) Scientific Opinion on Mineral Oil Hydrocarbons in Food. EFSA Journal 10(6), 2704.

[2] Biedermann M, Fiselier K, Grob K. (2009) J Agric Food Chem 57, 8711.

2 Scope

This guidance document has been developed for supporting the implementation of Commission Recommendation (EU) 2017/84. It provides guidance on sampling, analysis and reporting of the results for the content of total MOSH and MOAH and various carbon fractions of MOSH and MOAH. This guidance shall be used by food inspectors, official control laboratories, commercial laboratories (independent and industry) and laboratories of non-governmental organisations that monitor mineral oil in food and FCM.

This guidance aims to

1. facilitate harmonised sampling of food and FCM for MOSH and MOAH analysis;
2. facilitate harmonised reporting to EFSA by laboratories that are already familiar with the analytical approaches and have proven their analytical performance in relevant proficiency testing (PT) schemes;
3. give the essential performance requirements for the methods to be applied in MOSH/MOAH analysis;
4. give references to current analytical approaches described in the scientific literature for laboratories that are not familiar with the analytical methodology

This guidance does ***not*** aim to provide standard operating procedures.

If other guidance or standard operating procedures are necessary, these would be developed in the frame of future planned hands-on training courses.

This guidance should enable stakeholders to sample, analyse and report mineral oils in food and, if relevant, in FCM in a harmonised manner.

3 Sampling

Recommendation (EU) 2017/84 refers explicitly to Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for sampling food, and relating to the control of lead, cadmium, mercury, inorganic tin, inorganic arsenic, 3-MCPD and polycyclic aromatic hydrocarbons in food.

Only the following sections of this Regulation are relevant for sampling procedures for mineral oil in food: Annex Part A; Annex Part B: sections B.1.1 (just for official control); B.1.2 to B.1.6, B.1.7 (first paragraph); B.1.8; B.2 (not the last paragraph related to tin B.2.2); and B.3.

Since Recommendation (EU) 2017/84 does not concern official controls, strict adherence to Regulation (EC) No 333/2007 is not obligatory in the context of mineral oil monitoring.

In the following paragraph the guidance applies to sampling, where the main focus is on collecting from different suppliers as many products as possible in a given food category as listed in Recommendation (EU) 2017/84.

Guidance for sampling

As the focus of the Guideline is on monitoring and not official control, it is not obligatory to follow sections B.1.2, B.1.4 and B.1.5 for the sampling of mineral oil in lots and sub-lots.

Moreover, it is not obligatory to adopt section B.1.6, particularly in taking samples for enforcement, defence and referee purposes.

If unused packaging material from the same batch that was used to package the food is still available at the food business operator, then it should also be sampled as it may provide useful information to identify the source of any contamination that is found in the packaged food.

The person performing sampling should take all necessary precautions to avoid contamination of the sample. For example, the use of cosmetics such as hand creams should be avoided.

The sample collection tools should be free from mineral oil contamination.

Unpackaged food should be sampled in containers which are inert for mineral oil. Only containers that are impermeable to MOH, do not release interfering substances and do not adsorb MOH, should be used. Glass or polyethylene terephthalate (PET) containers have the identified properties and are most preferred. Each new batch of sample containers should be checked for mineral oil contamination. If a mineral oil contamination is detected, the containers should be washed before use with purified n-hexane and dried at the highest temperature possible. Glass sample containers could also be annealed, preferably at 400 °C. Mineral oil contamination of sample containers needs to be checked for each new batch after such treatment.

> NOTES:
>
> Polyolefin sample containers, made of, e.g. polyethylene or polypropylene, may release polyolefin oligomeric hydrocarbons (POH). These containers are not suitable unless appropriate precautions such as lining with aluminium foil are taken to prevent contamination of the samples.
>
> Metal sample containers and aluminium foil may have a mineral oil film on their surface due to their production. These containers would only be suitable upon ensuring that they are free of mineral oil residues. The mineral oil residues could be removed by rinsing with purified n-hexane.
>
> Paperboard boxes are generally not suitable even for the secondary packaging of the samples.

After collecting the sample, the sample container should be closed with a Polytetrafluoroethylene (PTFE)-layered lid or a glass stopper. Otherwise, the sample container must be covered first with aluminium foil before being sealed with its cap or stopper. The aluminium foil also needs to be checked for residual mineral oil contamination on its surface. No rubber rings should be used to close the container.

Pre-packaged food or FCM should be wrapped in aluminium foil at the point of sampling and kept wrapped until being analysed to prevent cross-contamination. If the pre-packaged food sample is brought into the laboratory without being wrapped in aluminium foil, this should be documented.

All contamination of the sample, e.g. by the use of tape or adhesives (paper/plastic labels) or contact with paper or paperboard, should be prevented. However, the sample must remain properly identifiable, e.g. by using a permanent marker.

If glass sample containers cleaned in a washing machine are reused, then the efficiency of the washing procedure should be checked. Reused sample containers do not need to be checked for residual mineral oil contamination if the washing procedure was effective in removing such residues.

No tape or adhesives (paper/plastic labels) should be used to fix the aluminium foil that covers the pre-packaged food.

The sample identification number should be written on the aluminium foil using a permanent marker.

Recording of information during sampling

The following information on the food sample should be recorded. The relevant EFSA reporting data elements are mentioned in brackets.

EFSA requirements [3] laid down in the Standard Sample Description on Food and Feed (SSD1) are:

- Laboratory sample code (S.01) - expressed by a unique sample identification number, not longer than 20 characters.
- Country of sampling (S.04) – This is the country where the food was selected for laboratory testing.
- Country of origin of the product (S.06) - This is the country where the food originated.
- Area of origin for fisheries or aquaculture activities code (S.08) - FAO Fisheries areas.
- EFSA Product code (S.12) - Food products should be described according to the FoodEx catalogue of the Standard Sample Description (SSD). It is mandatory to report, at least, level 2 of the FoodEx code. It is strongly encouraged to classify the food samples at the most detailed hierarchical level available (FoodEx level 3 and 4). This is particularly needed for food groups like "Food for infants and small children" and "Products for special nutritional use", where any available additional descriptions shall be provided.

  Specific attention needs to be given to the reporting of data on cereal grains. It is essential to make a clear distinction between grains as harvested (unprocessed grains of undefined use, not for human exposure assessment), grains for human consumption, and grains as feed.
- Product full-text description (S.14) – This is essential to check if the EFSA product code (FoodEx code) given by the data provider is consistent with the text description. This will avoid any possible mistakes in coding and additional clarification requests. Also, as this is a free text element, the information could be provided even in the national language. The original description of the sample from the national database can be copied here. It should be avoided to repeat just the FoodEx description. Moreover, any additional information that does not belong to any of the other SSD fields should be reported in S.14.

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[3] EFSA (2010) Standard sample description for food and feed. EFSA Journal 8(1), 1457

- Packaging (S.16) - Describe the container or wrapper that holds the product, e.g. multi-layer material or inner bag incl., further information on the material of layers; the presence of a barrier and assembled packaging material.
- Product treatment (S.17) - It is mandatory to indicate explicitly if the original sample is treated or not, especially if it is a dehydrated product.
- Product comment (S.21) - Additional information on the product, particularly preparation details if available.
- Year, month and day of expiry (S.25, S.26, S.27) – Best-before date or use by year or other indications of the expiry date.
- Year, month and day of sampling (S.28, S.29, S.30) - If the sample is the result of sampling over a given period, this field should contain the year, month or day when the first sample was collected.
- Sampling strategy (S.33) - It is mandatory to describe how the sample was selected from the population being monitored or surveyed.
- Programme type (S.34) - The sampling programme type must be reported to indicate the type of control programme or other types of the source to which the sample belongs.
- Sampling method (S.35) - It is mandatory to define the way the samples were collected for analysis. In the case of aggregated samples, the number of the incremental samples should be reported.
- Sampling point (S.39) - Point in the food chain where the sample was taken.
- History of the food or pre-packaged sample - e.g. about possible contamination sources during food processing or contact with secondary packaging, transport boxes, jute bags, batching oils[4] (R.32).

Additional information:

- Article number.
- European Article Numbering (EAN) code[5].
- Batch or lot number.
- Total mass of aggregated food sample.
- Labels (physically or photocopy) - In the context of pre-packaged food in paper and board, the mass of the food and the packaging needs to be determined.
- Mass of packaged food sample (if possible).
- Mass of packaging material (if possible).

Table I summarises the minimum number of incremental samples to be taken and the mass of the incremental samples depending on the mass of the (sub) lot of the non-packaged products.

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[4] Toolbox for Preventing the Transfer of Undesired Mineral Oil Hydrocarbons into Food (2017) German Federation of Food Law and Food Science (Bll e.V). https://www.bll.de/download/toolbox-for-preventing-the-transfer-of-undesired-mineral-oil-hydrocarbons-into-food

[5] https://en.wikipedia.org/wiki/International_Article_Number

Table I Minimum number of incremental samples to be taken and the mass or volume of the incremental samples depending on the mass of the lot of the non-packaged products

product type	lot mass (ton)	no. sublot	(sub)lot mass (kg)	min. no. of incremental samples	min. amount of incremental sample (g or ml)
bulk products	≥ 100	≥ 1	> 500	10	100
	< 100	1	> 500	10	100
	< 100	1	≥ 50 and ≤ 500	5	200
	< 100	1	< 50	3	330
other products	≥ 15	≥ 1	> 500	10	100
	< 15	1	> 500	10	100
	< 15	1	≥ 50 and ≤ 500	5	200
	< 15	1	< 50	3	330
bulk homogeneous liquid products		≥ 1		3	330

4 Analysis

4.1 Description of the mineral oil hydrocarbons

Mineral oil hydrocarbons (MOH) are a complex mixture of hydrocarbons, which originate from crude mineral oils or which are produced from coal, natural gas or biomass through Fischer-Tropsch synthesis.

MOH does not include hydrocarbons:

- naturally occurring in food: such as n-alkanes of odd numbered carbons (from C_{21} to C_{35}) or natural olefins of terpenic origin (such as squalene, sterene or carotenoids).

- such as POH (polyolefin oligomeric hydrocarbons) potentially migrating from plastic packaging (e.g. polyethylene or polypropylene packaging) or synthetic isoparaffins with short and long side chains used e.g. in synthetic lubricants and adhesives.

MOH are divided into two main types - MOSH and MOAH.

Mineral oil saturated hydrocarbons (MOSH)[6]

MOSH comprise paraffins (open chain hydrocarbons) and naphthenes (cyclic hydrocarbons), which are mostly highly alkylated and originate either directly from mineral oil or are formed during refining by hydrogenation of aromatic compounds or other conversion processes.

Paraffins (open chain hydrocarbons) are distinguished from naphthenes (hydrocarbons with at least one saturated ring). Paraffins can be grouped into the linear n-alkanes (those with at least about 20 carbons are forming waxes) and the branched hydrocarbons, usually being liquids. Naphthenes tend to be highly alkylated and originate either from mineral oil or from hydrogenation of aromatics.

Mineral oil aromatic hydrocarbons (MOAH)[6]

MOAH contain at least one aromatic ring. They include polyaromatic compounds, but should be distinguished from the compounds commonly termed polyaromatic hydrocarbons (PAH), such as benzopyrenes, which are formed at high temperatures. PAH are only slightly alkylated and can be analysed as individual substances, whereas MOAH are usually alkylated to more than 98 %[7], consist of large numbers of compounds and form broad chromatographic signals (humps) with hardly any sharp peak signal on top.

Polyolefin oligomeric hydrocarbons (POH)[6]

POH are oligomers of polyolefins, such as polyethylene, polypropylene and polybutylenes. FCM uses comprise plastic bags, containers or films, sealable heat layers (e.g. in aluminium bags) and other lamination as well as adhesives and plasticisers. The POH that are potentially relevant for human health and determined by the described method are at the low end of the molecular mass range of the oligomers. They largely consist of branched hydrocarbons, sometimes mono-unsaturated hydrocarbons and are eluted from the HPLC column in the MOSH fraction. POH may sometimes be distinguishable from MOSH by their chromatographic pattern, but it is difficult (and for some POH impossible) to differentiate and chromatographically separate them from the MOSH if both are present[8].

Poly Alpha Olefins (PAO)[8]

Similar to POH, PAO are isoparaffins with short main hydrocarbon chains and long side chains of synthetic origin. Usually they can be identified by their characteristic chromatographic pattern.

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[6] Biedermann M., Grob K. (2012). Journal of Chrom. A 1255, 56

[7] Grob K., Biedermann M., Caramaschi A., Pacciarelli B. (1991) J. High Resolut. Chromatogr. 14, 33

[8] Biedermann M., Grob K. (2012) Journal of Chrom. A 1255, 76

4.2 Definition of the measurands

For the analytical determination of the MOSH/MOAH content in food and FCM measurands have to be defined, which reflect the MOSH/MOAH descriptions above.

MOSH

The total MOSH measurand is defined as the total mass fraction of MOSH – expressed in mg MOSH / kg sample – after separation from MOAH and removal of all possible interferences in the extract, as quantified by integration of the whole signal interval in the GC/FID chromatogram between the retention times of the peak start of n-C_{10} and the peak end of n-C_{50} after subtracting the identified sharp peaks not belonging to MOSH and using cyclohexylcyclohexane (CyCy) as internal standard (IS).

Another hydrocarbon could be used as IS, provided its response factor is identical. Other detection techniques are acceptable, provided that equivalent results are demonstrated.

The MOSH fraction may include polyolefin oligomeric hydrocarbons[9] and hydrocarbons from poly alpha olefins (PAOs)[9] in case where their separation/substraction is impossible. The presence of POH and/or POA should be clearly reported.

MOAH

The total MOAH measurand is defined as the total mass fraction of MOAH – expressed in mg MOAH / kg sample – after separation from MOSH and removal of all possible interferences in the extract, as quantified by integration of the whole signal interval in the GC/FID chromatogram between the retention times of the peak start of n-C_{10} and the peak end of n-C_{50} after subtracting the identified sharp peaks not belonging to MOAH and using 1- or 2-methylnaphthalene as IS.

Another hydrocarbon could be used as IS, provided its response factor is identical. Other detection techniques are acceptable, provided that equivalent results are demonstrated.

4.3 Background of MOH analysis

Over the past decade several approaches have been suggested for the determination of MOSH and MOAH in FCM and food, all of them having certain advantages and drawbacks. MOSH/MOAH separation and its subsequent determination could be achieved by applying on-line LC-GC-FID, off-line HPLC followed by GC-FID or manual off-line separation of MOSH/MOAH followed by GC-FID.

It is not possible to separate the mineral oils into single components because they typically contain a complex mixture of alkanes and other compounds. The combination of LC, which separates MOSH from MOAH, and GC-FID for quantification allows for an appropriate determination of the MOSH and MOAH content. In the GC-FID chromatograms of the MOSH and MOAH fractions, further fractions can be defined based on the retention time of the corresponding n-alkanes under the same chromatographic conditions. It has been decided in agreement with EFSA to collect data for mineral oils up to n-C_{50} atoms in their molecules in order to reflect the composition of some lubricant oil with heavier oil fractions. Until recently, all the reported data included only hydrocarbons up to n-C_{40}.

On-line LC-GC has the advantages of high separation efficiency, high sample throughput, reduced solvent consumption and sample manipulation, thus enhancing the reproducibility of the method. On-line LC–GC–FID analysis enables the re-use of the same LC column. Solvent consumption is lower than with most conventional liquid chromatographic sample preparation methods, including solid

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[9] Biedermann-Brem, Kasprick N., Simat T., Grob K. (2012) Food Addit. Contam. A 29, 449.

phase extraction (SPE). On-line coupling to GC is integrating sample preparation into the final analysis and is fully automating rather complex procedures. As it is a closed system, it also avoids contamination during sample preparation, which is of particular importance for analytes that are widely present in laboratories, such as mineral oil hydrocarbons. On the other hand, the sensitivity is limited by the capacity of the LC column. Dedicated instrumentation and skilled operators are required.

An LC column could also be applied for a pre-separation in the off-line mode by collecting the MOSH and MOAH fractions using automated fraction collectors. LC separating columns with a larger internal diameter (4.6 mm instead of 2 mm) could be used for this approach. A larger sample could be injected into the LC column compared to on-line coupling. In order to achieve similar detection limits as in on-line coupling, a fifth of the fraction should be injected into the GC. Nevertheless it requires larger volume injection in the GC system even if the fractions are significantly enriched beforehand. This is the greatest challenge of this technique, in addition to contamination-related problems that could occur during the collection of the MOSH and MOAH fractions.

The third possibility is to follow a pure "off-line" method, using a glass column filled with silica/$AgNO_3$ to separate the MOSH and MOAH fractions[10]. The method is very time consuming and requires strict measures to prevent contamination of the sample from the consumables and the environment.

Flame ionisation detection (FID) is neither sensitive nor selective. It provides almost identical responses to all hydrocarbons, making it a preferred detector for MOSH/MOAH quantifications. However, due to the lack of selectivity, additional sample preparation techniques to eliminate interferences and to enrich both MOSH and MOAH[7] fractions may need to be applied.

Until now, most of the data on the MOSH and MOAH content in foods and FCM were produced after applying chromatographic separation using automated on-line coupled LC-GC and quantification by FID.

Today, the LC-GC-FID method is referred to as the method of choice for the quantification of mineral oils in routine analysis[1,11]. Many private laboratories apply this on-line method since it has clear advantages over the “manual” off-line methods[12], despite the need for a sophisticated instrument.

With difficult samples and matrices, further characterisation of the MOSH/MOAH fractions can be performed by using additional analytical techniques, e.g. GC-MS, LC-GC-FID/MS or GCxGC-FID/MS[13,14,15]. However the need for further characterisation must be decided on a case by case basis by an experienced analyst.

4.4 Outline of the analytical approach

As a general recommendation, the methods published by Kantonales Labor Zürich and BfR[6,9,11] can be followed for determination of the MOSH/MOAH content in food and FCM. Also other approaches, complying with the performance requirements as defined in section 4.6 could be applied.

In short MOSH and MOAH are extracted from the sample matrix using an organic solvent after the addition of internal and verification standards. The extract is submitted to isolation and separation of the MOSH and MOAH fractions. MOSH and MOAH fractions are separated on a HPLC silica gel column or a glass column filled with silica/$AgNO_3$ using e.g. a n-hexane/dichloromethane gradient.

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[10] The compendium of the Federal Institute for Risk Assessment (BfR) and the Cantonal Laboratory of Zurich (KLZH) (2012) “Determination of mineral oil hydrocarbons in food and packaging material”. https://www.bfr.bund.de/cm/343/messung-von-mineraloel-kohlenwasserstoffen-in-lebensmitteln-und-verpackungsmaterialien.pdf

[11] Biedermann M, Munoz C, Grob K. (2017) J Chrom. A 1521, 140

[12] Fiselier K., Grundböck F., Schön K., Kappenstein O., Pfaff K., Hutzler C., Luch A., Grob K. (2013) J. Chrom. A 1271, 192

[13] Biedermann M., Grob K. (2015) Journal of Chrom. A 1375, 146

[14] Spack L., Leszczyk G., Varela J., Simian H., Gude T., Stadler R. (2017) Food Additives & Contaminants: Part A 34(6), 1052

[15] Populin T. , Biedermann M., Grob K., Moret S., Conte L. (2004) Food Additives and Contaminants 21(9), 893

Each fraction is transferred in large volume either on-line or off-line to a GC pre-column. Solvent vapours are discharged via a solvent vapour exit located between the uncoated pre-column and the GC separation column or by using a solvent vent in a PTV injector. Volatile components are retained by solvent trapping applying a partially concurrent eluent evaporation. High boiling components, spread over the entire length of the flooded zone, are refocused by the retention gap technique.

The signal area in the FID chromatogram attributed to MOSH/MOAH is calculated by integration of the chromatogram covering the range of ≥n-C_{10} to ≤n-C_{50}, taking the baseline of the blank into account. Sharp peaks ***above*** the hump, attributed to the naturally occurring n-alkanes in food (primarily with odd-number carbon atoms in their molecules from n-C_{21} to n-C_{35} and hydrocarbons of terpenic origin) need to be cut out from the hump signal. POH and PAO signals have to be subtracted if possible.

The calculations of the MOSH and MOAH mass fractions ($w_{MOSH/MOAH}$) are performed by using the following equation:

$$w_{MOSH/MOAH} = \frac{A_i \times m_{IS} \times 1000}{A_{IS} \times m}, \text{ where}$$

A_i is the signal area attributed to MOSH or MOAH (total or C-fraction) after the elimination of the identified sharp peaks above the hump and if possible, elimination of POH and/or POA signals;

A_{IS} is the peak area of the internal standard CyCy (MOSH) or 2-MN (MOAH) or an equivalent IS;
m_{IS} is the mass of the internal standard added to the sample in mg;
m is the mass of the test portion, in g.

If POH and PAO are present and cannot be excluded in the mass fraction calculation, then the test result shall be accompanied by a note that POH and/or PAO are present.

Some samples may contain odd-numbered n-alkanes in the range of n-C_{21} to n-C_{35} in such quantities that the chromatograms of the MOSH fraction are severely overloaded and that those signals might overlap with the mineral oil hump. In this case, it is recommended to use an additional clean-up technique. For instance, aluminium oxide strongly retains long-chain n-alkanes. Often MOH contain exclusively branched and cyclic components, which are retained much less by aluminium oxide. Therefore, the use of aluminium oxide enables the removal of paraffins. Caution: **This auxiliary method is removing waxes which contain large amounts of >C_{25} n-alkanes.**

Epoxidation is a purification step that may be necessary for the quantification of MOAH. This purification step allows the elimination of olefins like squalene, which elute within the MOAH fraction and interfere with its quantification (e.g. olive oil, palm oil). Epoxidation also removes certain olefins co-eluting with the MOSH fraction. Therefore, epoxidation may also be used as a purification step for the MOSH fraction as well. So far, the epoxidation step is the best compromise to remove olefins even though it is in some cases not fully quantitative and the efficiency may be sample dependent. Depending on the sample, this reaction may induce the epoxidation of a part of the MOAH or the incomplete removal of interfering olefins.

Saponification followed by extraction could also be applied to difficult samples with a high fat content. Saponification has the advantage that it can be applied to all food types (dry and wet, with either a low or a high fat content), avoiding the need to remove water before the extraction step. Furthermore, it efficiently removes high amounts of fat, but it can be very solvent- and time-consuming.

Further lowering of the quantification limits by enrichment of the MOAH and/or MOSH fractions could be necessary for some samples.

For facilitating the choice of the required auxiliary methods, a decision tree is proposed for removing interferences in the MOSH/MOAH fraction and obtaining the requested sensitivity via concentration (Figure 1). Each step is accompanied by an example chromatogram shown in Annex I. The proposed decision tree is mainly related to the on-line LC-GC-FID method.

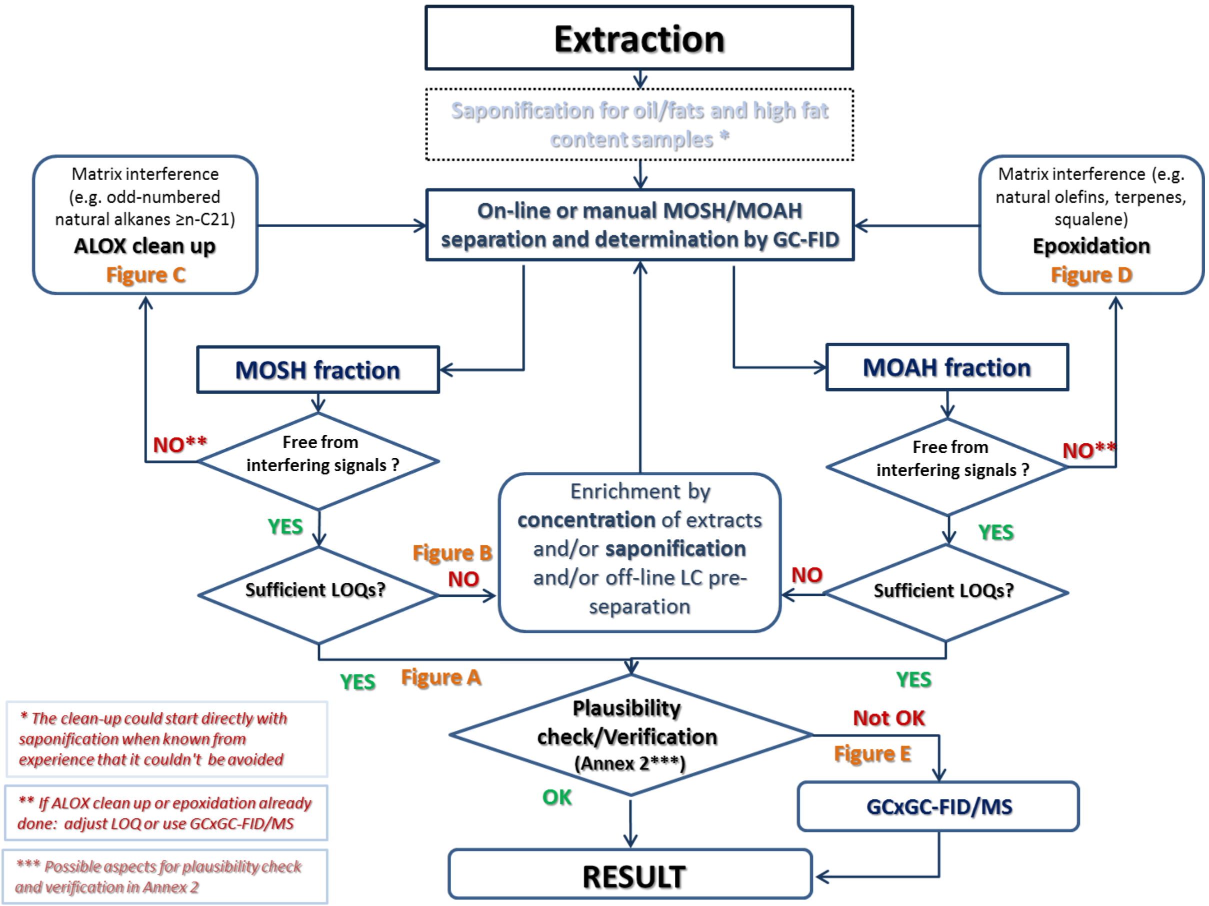

Figure 1 **Decision tree on the use of auxiliary methods. Figures A-E can be found in Annex I. ALOX is aluminium oxide.**

Experienced operators are required for a correct interpretation of the GC chromatograms. Annex II could be used as a starting point in this complicated process. Knowledge about the sample and the potential peak patterns of interferences is essential, e.g. for avoiding an overestimation of MOAH by the presence of non-aromatic compounds in the respective retention time intervals.

If an interference is suspected even after purification, the characterisation of the MOSH or MOAH fraction has to be verified by using additional analytical methods, such as (LC-)GC-MS or GCxGC-FID/MS.

4.5 Verification of the method performance

GC performance

Since the MOH analysis includes hydrocarbons of up to n-C_{50}, laboratories should use a temperature programme and a GC column which allow to determine mineral oil of up to n-C_{50} without significant column bleeding, e.g. DB-1 0.1 µm, 15 m x 0.25 mm i.d. or MXT-1 0.25 µm, 15 m x 0.25 mm i.d. The response ratio of n-C_{50} to n-C_{20} should be between 0.8 and 1.2.

It should also be noted that graphite/vespel ferrules (which are commonly used in GC) will not withstand the temperatures required for analysis up to n-C_{50}.

Selection of internal and verification standards[6, 9]

In principle, many hydrocarbons are suitable as internal standards for the quantification of the MOSH and MOAH fraction by FID since the response factors are very similar. The same holds for verification standards, which control whether the analytical procedure or the LC or GC column are performing well.

MOSH

Cyclohexylcyclohexane (Cycy) is a suitable internal standard. It is not found in relevant quantities in mineral oils and packaging. Cycy is eluted just before n-C_{13} from apolar GC columns (coated with dimethylpolysiloxanes). This separation is usually incomplete on dimethylpolysiloxanes with 5% phenyl substitution.

To keep the loss of internal standard under control, a verification standard of n-C_{11} could be added in the same amount as Cycy. In case of losses of volatiles, n-C_{11} is likely to be lost to a greater extent than Cycy. Caution need to be applied when the n-C_{11} area is smaller than Cycy: it may indicate loss of Cycy by evaporation or that Cycy was co-eluted with a sample component.

A second verification standard, n-C_{13}, could be added at half of the amount of Cycy. It is eluted closely after Cycy, and it creates a typical pair of peaks that is easily recognised. Furthermore, it enables the verification of the chromatographic separation of CyCy and n-C_{13} for each analysis.

MOAH

The standards for MOAH analysis might be selected analogously. The closely eluting pair of 1- and 2-methylnaphthalene (MN) is easily recognised as internal standards. The two peaks should be of the same area: a difference could indicate co-elution of the larger peak with a sample component.

n-Pentyl benzene (5B) can be used to monitor the losses of volatiles.

1,3,5-Tri-tert-butyl benzene (TBB) was historically introduced as a marker for the start of the MOAH fraction, however it turned out in the context of MOH analysis in cosmetic, that di(2-ethylhexyl) benzene (DEHB) is more suitable as it elutes together with the first MOAH. It is proposed as a marker for the start of the MOAH fraction in a recent publication[11].

(TBB)/DEHB and perylene (Per) could verify the start and the end of the MOAH fraction.

4.6 Quantification

MOSH and MOAH are quantified according to the equation mentioned in Section 4.4.

Sub-fractions of MOSH and MOAH (so-called C-fractions) in the chromatograms are defined by the position of the elution signals of n-alkanes from the GC column. The following MOSH and MOAH C-fractions are defined:

MOSH:	**MOAH:**
total MOSH	Total MOAH
MOSH ≥n-C_{10} to ≤n-C_{16}	MOAH ≥n-C_{10} to ≤n-C_{16}
MOSH >n-C_{16} to ≤n-C_{20}	MOAH >n-C_{16} to ≤n-C_{25}
MOSH >n-C_{20} to ≤n-C_{25}	MOAH >n-C_{25} to ≤n-C_{35}
MOSH >n-C_{25} to ≤n-C_{35}	MOAH >n-C_{35} to ≤n-C_{50}
MOSH >n-C_{35} to ≤n-C_{40}	
MOSH >n-C_{40} to ≤n-C_{50}	

Each C-fraction starts at the retention time of the peak end of the first n-alkane of the range and stops at the retention time of the peak end of the second n-alkane of the range. Only the C-fraction

≥n-C_{10} to ≤n-C_{16} starts at the retention time of the peak start of n-C_{10} and stops at the retention time of the peak end of n-C_{16}.

The parameters "total MOSH/MOAH" should be determined by integration of the whole signal interval in the chromatogram, starting at the retention time of the peak start of n-C_{10} and ending at the retention time of the peak end of n-C_{50} after the elimination of the identified sharp peaks above the hump and if possible, elimination of POH and/or POA signals.

Voluntary is the reporting of the additional mass fractions of MOSH Humps and MOAH Humps. For this voluntary parameter, several MOSH/MOAH humps can be reported and can be characterised by the nearest n-alkane where the hump starts, the nearest n-alkane for the top of the hump and the nearest n-alkane where the hump ends. The area of the hump (e.g. $A_{n\text{-}C20,n\text{-}C23,n\text{-}C27}$) is calculated from the integration of the hump and the mass fraction is calculated using the formula in Section 4.4. If humps are overlapping, the minimum (Y2) between the tops of the humps (Y1 and Y3) should be used as the end of the first hump or starting for the following hump (see **Figure 2**).

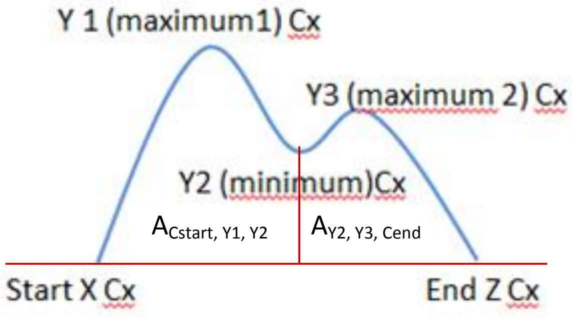

Figure 2 **Integration of overlapping humps with areas $A_{Cstart, Y1, Y2}$ and $A_{Y2, Y3, Cend}$**

4.7 Performance requirements of the analytical methods

The final analytical procedure should undergo thorough validation to prove its fitness for purpose. Guidance for laboratory method validation can be found, for instance, in the Eurachem guide[16].

Table II defines the performance requirements for the analysis of MOSH and MOAH fractions, concerning the maximum allowable limit of quantification (LOQ) for each C-fraction (LOQ-max), the target LOQ for each C-fraction (LOQ-t), acceptable ranges for recovery (R_{rec}) of mineral oil from samples, and the intermediate precision for different types of samples. The measurement uncertainties and limits of quantification depend on the type of samples and the kind of sample preparation applied.

Limit of quantification

The LOQ that can be reached with a method is significantly influenced by the fat content of the sample, since the capacity of the LC column to retain lipids is limited and this determines the amount of sample which can be injected. Therefore additional sample preparation steps are necessary in some cases to reach the LOQ-t or LOQ-max.

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[16] Eurachem Guide (2014) The Fitness for Purpose of Analytical Methods A Laboratory Guide to Method Validation and Related Topics. https://www.eurachem.org/index.php/publications/guides/mv

The LOQ derived from the chromatographic signal of MOSH or MOAH can be influenced by the signal shape. As a rule, *ca.* 50-100 ng MOSH or MOAH should be injected on the GC separation column. The LOQ can be estimated taking into account the sample preparation steps, including the enrichment step and the mass of the sample.

For different types of food matrices, there are two LOQs defined: a ‘target-LOQ’ (LOQ-t) and a ‘maximum-LOQ’ (LOQ-max). The LOQ-t is the analytically achievable quantification limit for the majority of matrices of each food category, whereas the LOQ-max should not be exceeded for any analytical methods used in MOSH or MOAH analysis. The LOQ is differentiated for the fat content of the food and for paper and board. The laboratory should aim to achieve the LOQ-t mentioned in Table II.

Recovery

The method applied for the determination of the MOSH and MOAH in food and FCM shall ensure that the recovery (R_{rec}) is within the acceptable range as indicated in Table II.

In the chromatographic methods used for the determination of MOSH and MOAH, mineral oil concentrations are automatically corrected for recovery through the use of internal standards (IS) for quantification. The ratio (signal (analyte) / signal (IS)) is assumed to be constant throughout the analytical process, which compensates for eventual losses of the analyte.

For certain foods (solid foods and packaging materials), inclusions could severely hamper the extraction process of mineral oil or render it virtually impossible. In this case, the internal standard does not represent the mineral oil sufficiently as it will not have been penetrated into the inclusions.

The following matrices are critical:

- Foods with crystalline components, such as products with high sugar content (e.g. powdered drinks, infant formula) and pasta.
- Paperboard: Ethanol improves the extraction of small molecules by swelling.
- Plastics: readily permeable plastics (e.g. polyolefins) are extracted much faster than effective barrier materials.

A check can be carried out by rinsing the sample extracted under normal conditions (removal of residual extract) and carrying out subsequent extractions under harsher conditions (several times, longer extraction times, higher temperature).

Intermediate precision

The requirements on intermediate precision, also called within-laboratory or in-house precision, of analytical results of MOSH and MOAH for foods with different fat content are indicated in Table II.

The intermediate precision should be calculated following the Eurachem guide[16].

Table II Performance requirements for MOSH and MOAH analysis: maximum LOQ for each C-fraction (LOQ-max), target LOQ for each C-fraction (LOQ-t), acceptable ranges for recovery (R_{rec}) of mineral oil from samples, and intermediate precision

Categories	**Associated foods** #	**LOQ - max [mg/kg]**	**LOQ -t [mg/kg]**	**R_{rec} [%]**	**interme-diate precision [%]**
Dry, low-fat content (< 4% fat/oil)	bread and rolls; breakfast cereals; grains for human consumption; pasta, products derived from cereals	0.5	0.1	80 - 110	15
Higher fat/oil content (> 4% fat/oil)	fine bakery ware; confectionery (incl. chocolate) and cocoa; fish meat, fish products (canned fish); oilseeds; pulses; sausages; tree nuts	1	0.2	70 - 120	20
Fat/oils	animal fat (e.g. butter); vegetable oils	2	0.5	70 - 120	20
Paper and Board	Reporting only up to C_{35} (extraction optimised up to C_{35})	10	5	80 - 110	10

In some cases, a shift to another category may be necessary due to different fat content. This has to be stated and justified for each case.

5 Reporting of results

Results shall be reported:

- in mg/kg;
- with two significant figures (e.g. 150, 15, 1.5 or 0.15 mg/kg); and
- rounded using the rules in section B.2 of ISO 80000-1:2009.

For each report's result, a short description of the analytical steps in the applied procedure shall be reported.

The analyst should specify how the reported expanded measurement uncertainty was estimated.

Data has to be reported in the EFSA database. The following data elements are relevant, either mandatory or recommended, and required to report to EFSA[17]. For the datasets generated in the past and fulfilling the requirements laid down in the chapters before, a symbol "0" could be entered when there are no data for some of the mandatory elements, in order to be able to proceed.

The data elements refer to the overall guidance on Standard Sample Description on Food and Feed [3] (SSD1). Those data providers using the guidance on Standard Sample Description ver. 2.0[18] (SSD2) must request the converted reporting requirements to the EFSA support (data.collection@efsa.europa.eu). The version SSD1, explained in this document, will be supported by EFSA in 2019. From the year 2020 onward, only SSD2 specifications will be supported.

Code	Data elements	Description/requirement	Comment
S.01	Laboratory sample code	Laboratory sample code must be identified by a unique sample identification number, not longer than 20 characters.	Mandatory for EFSA
S.03	Language	The language used to complete the free text fields of the table must be specified.	Mandatory for EFSA
S.04	Country of sampling	The country of sampling is the country where the commodity was selected for laboratory testing.	Mandatory for EFSA
S.06	Country of origin of the product	The country of origin is the country where the commodity originates. It is particularly important to avoid the code “unknown (XX)”, especially when reporting data from raw agricultural commodities. If it is still unknown, provide info in S.14	Mandatory for EFSA
S.08	Area of origin for fisheries or aquaculture activities code	Fisheries or aquaculture area specifying the origin of the sample (FAO Fisheries areas).	Mandatory for this guidance, for relevant food

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[17] EFSA (2017) Specific reporting requirements for contaminants and food additives occurrence data submission. EFSA supporting publication EN-1262. and its annex "Specific_Requirements_2016_data_Annexes.xlsx"

[18] EFSA (2013) Standard Sample Description ver. 2.0. EFSA Journal 11(10), 3424

S.12	EFSA Product code	Food products should be described according to the FoodEx catalogue of the SSD. It is envisaged that detailed information on the different food groups will be needed to perform the exposure assessment. It is mandatory to report at least the level 2 of the FoodEx code. It is strongly encouraged to classify the food samples at the most detailed hierarchical level available (FoodEx level 3 and 4). This is particularly needed for food groups like “Food for infants and small children” and “Products for special nutritional use”, where any available additional descriptions shall be provided. Specific attention needs to be given to the reporting of data on cereal grains. It is essential to make a clear distinction between grains as harvested (unprocessed grains of undefined use, not for human exposure assessment), grains for human consumption and grains as feed. For this reason, three distinct groups are available within the FoodEx catalogue. Be aware that the Feed codes starting with “F” are not in use any longer’; “G” codes should be used instead.	Mandatory for EFSA
S.14	Product full-text description	Product full-text description is essential to check if the EFSA product code (FoodEx code) given by the data provider is consistent with the text description. This will avoid any possible mistakes in coding and additional clarification requests. In addition, as this is a free text element, the information could be provided even in the national language. The original description of the sample from the national database can be copied here. It should be avoided to repeat only the FoodEx description. Moreover, any additional information that does not belong to any of the other SSD fields should be reported in S.14.	Mandatory for this guidance
S.16	Packaging	Describe container or wrapper that holds the food product. A common type of packaging: paper or plastic bags, boxes, tinplate or aluminium cans, plastic trays, plastic bottles, glass bottles or jars. Relevant packaging that cannot be described here should be reported in code R.32	Mandatory for this guidance. See SSD list annexed. Refer to PRODPAC list in the annexe
S.17	Product treatment	It is mandatory to clearly indicate if the original sample is treated or not, especially if it is a dehydrated product (select: "Dehydration", T131A); in the absence of this information, the status “as consumed” will be assumed.	Mandatory for EFSA. See SSD list. Refer to PRODTR list.
S.21	Product comment	Additional information on the product, particularly home preparation details if available. When the analysis is carried out after the preparation of the product (e.g. reconstitution of powders or cooking processes) report “analysed=as consumed”. If the analysis is performed on the product before the preparation of the report, “analysed=as purchased”. It is recommended to report the conditions used for the preparation “comment=deep fried in oil”.	Mandatory for this guidance, if relevant

S.25	Year of expiry	Best before-year or use by-year or other indications of the expiry year.	Mandatory for packaged food
S.26	Month of expiry	Best before-month or use by-month or other indications of expiry month.	Mandatory for packaged food
S.27	Day of expiry	Best before-day or use by-day or other indications of the expiry day.	Mandatory for packaged food
S.28	Year of sampling	Year of sampling. If the measure is the result of sampling over a given period, the field should contain the year when the first sample was collected	Mandatory for EFSA
S.29	Month of sampling	Month of sampling. If the measure is the result of sampling over a given period, this field should contain the month when the first sample was collected.	Mandatory for this guidance
S.30	Day of sampling	Day of sampling. If the measure is the result of sampling over a given period, this field should contain the day when the first sample was collected.	Mandatory for this guidance
S.32	Programme legal reference	Reference to the legislation for the program defined by programme number.	Mandatory for this guidance; use “N304A” and insert: COMMISSION RECOMMENDATION (EU) 2017/84
S.33	Sampling strategy	It is mandatory to describe how the sample was selected from the population being monitored or surveyed.	Mandatory for EFSA “ST20A“ Selective, “ST30A” Suspect, “ST10A” Objective, “ST90A” other
S.34	Programme type	The programme type must be reported to indicate the type of control programme or other types of the source to which the sample belongs.	Mandatory for EFSA; use: K009A (Official (EU) programme)
S.35	Sampling method	It is mandatory to define the way the samples were collected for analysis. In the case of pooled samples, the number of the sample should also be provided in ‘Number of samples (S.36). (The default value of the number of the sample is "1").	Mandatory for EFSA; use: N011A (According to Reg. 333/2007)
S.39	Sampling point	Identify in the food chain where the sample was taken. (Doc. ESTAT/F5/ES/155 “Data dictionary of activities of the establishments”).	Mandatory for EFSA. See SAMPNT catalogue.
L.02	Laboratory accreditation	In accordance with Article 12 of Regulation 882/2004 and with Article 37 of Regulation (EU) 2017/625 from 14th of December 2019, laboratories designated for official controls must be accredited to ISO/IEC 17025	Mandatory for EFSA
R.01	Result code	The result code should be unique for each record. If the laboratory does not provide it, then, it must be created, e.g. by merging the sample code with the parameter code. For example, a sample (Samp001) has been analysed for Mercury (RF-00000170-CHE) and Cadmium (RF-00000150-CHE). The result code created could be: Samp001_170 and Samp001_150.	Mandatory for EFSA
R.02	Year of analysis	It is mandatory to report the year of analysis. If the analysis has been performed over a period of time, the completion date of analysis should be stated.	Mandatory for EFSA
R.03	Month of analysis	Month when the analysis was completed.	Mandatory for this guidance

R.04	Day of analysis	Day when the analysis was completed.	Mandatory for this guidance
R.06	Parameter code	It is mandatory to code the contaminants using the code from the SSD PARAM catalogue. For the voluntary parameter MOSH Hump and MOAH Hump, several humps can be reported. The reporting format allows you to include for each hump the nearest starting C number of the respective n-alkane, the nearest top C number of the respective n-alkane and the nearest ending C number of the respective n-alkane, e.g.: The 3rd hump for MOSH with C_{start}=20, C_{top}=24 and C_{end}=30 must be reported with the following code RF-00007456-PAR `#humpId=3$Cstart=20$Ctop=24$Cend=30`. If humps are overlapping, the minimum (Y2) between the tops of the humps (Y1 and Y3) should be used as the end of the first hump or starting of the following hump (see **Figure 2**).	Mandatory for EFSA; MOSH: • RF-00000397-ORG (total MOSH) • RF-00007450-PAR (MOSH >C9 to ≤C16) # • RF-00007451-PAR (MOSH >C16 to ≤C20) • RF-00007452-PAR (MOSH >C20 to ≤C25) • RF-00007453-PAR (MOSH >C25 to ≤C35) • RF-00007454-PAR (MOSH >C35 to ≤ C40) • RF-00007455-PAR (MOSH >C40 to ≤C50) MOAH: • RF-00000398-ORG (Total MOAH) • RF-00007457-PAR (MOAH >C9 to ≤C16) # • RF-00007458-PAR (MOAH >C16 to ≤C25) • RF-00007459-PAR (MOAH >C25 to ≤C35) • RF-00007481-PAR (MOAH >C35 to ≤C50) Voluntary: RF-00007456-PAR (MOSH Hump n) RF-00007462-PAR MOAH Hump n) # read (MOSH ≥C10 to ≤C16) and (MOAH ≥C10 to ≤C16) since integration starts with n-C_{10}.

R.08	Parameter type	It is mandatory to indicate whether the parameter reported is an individual parameter or parameter sum.	Mandatory for EFSA; The reported parameters are all individual parameters so use: P001A (Individual)
R.10	Analytical method code	It is mandatory to specify the analytical instrument used	Mandatory for EFSA; manual method GC-FID, use: F040A (but mention in R.11 the LC or SPE part) or on-line method LC-GC-FID , use F675A.
R.11	Analytical method text	A detailed description of the whole analytical method	Mandatory for this guidance; please describe here: - Description of sample preparation methods - Description of auxiliary methods used, e.g. epoxidation, aluminium oxide clean-up, enrichment, saponification.
R.13	Result unit	It is obligatory to report the unit of measurement for the values reported in, “Result LOQ”, “Result Value” and “Result uncertainty” . Beware that the unit of measurement should be consistent for all the elements.	Mandatory for EFSA; results need to be reported in mg/kg (G061A)
R.15	Result LOQ	For the management of left-censored data, it is highly recommended to report at least the LOQ of the analytical method regardless of what is selected in data elements Result value (R.18) and Type of result (R.27).	Mandatory for this guidance
R.18	Result value	If the ‘Type of the result (R.27)’ is "Numerical value"	Mandatory for EFSA, if the Type of result (R.27) is equal to VAL. It has to, otherwise be left empty.
R.22	Result value uncertainty	It is recommended to provide the expanded uncertainty (95% confidence interval) associated with the concentration measurement.	Mandatory for this guidance
R.24	% of fat in the original sample	Percentage of fat in the original sample if the results are expressed on a 'fat weight' basis	Highly recommended for this guidance. Mandatory if the percentage of fat is mentioned on packaged food
R.25	Expression of result	For this result, it is mandatory to report the results in the whole weight.	Mandatory for EFSA; whole weight (B001A)
R.27	Type of result	The data elements, “Result value”, “Result qualitative value” and “type of result” are used to describe different types of results of the analysis; therefore, reporting the “Type of result” is mandatory for all contaminants. If a certain C-fraction, e.g. $>C_{40}$ to $\leq C_{50}$, has not been analysed in the past, a zero can be introduced for that fraction.	Mandatory for EFSA; For total MOSH and MOAH and their C-fractions “VAL” (‘numeric value’) or “LOQ” (‘less than LOQ’) are mandatory.

	Attachment ID	Identifier of the image providing additional information on the analysis performed.	Recommended for samples with unclear sources. Report here the web address (Unified Resource Location -URL) of the image of integrated LC-GC-FID chromatograms of MOSH and MOAH in the reported sample/result. Only one document can be reported. In case multiple documents must be linked to the same record, use a compressed archive (e.g. zip files). The image can be loaded to a repository made available by EFSA upon request to data.collection@efsa.europa.eu

R.32	Comment on the result	When the analysis is carried out after the preparation of the product (e.g. dehydrated product reconstituted “as consumed” (infant and follow-up formulae, coffee grounded) or food cooked as consumed (pre-cooked French fries/potato product for home cooking), it is recommended to report the conditions used for the preparation.	Mandatory for this guidance; please report here: • explanation on how LOQ was derived for the total MOSH and/or MOAH and their C-fractions • specify how the reported expanded measurement uncertainty was estimated • explain the deviation of reporting different C-fractions ;e.g. C_{40}-C_{50} not analysed or C_{24} instead of C_{25} • further description of the material and layers that could not fit in the code S.16: the presence of a barrier, assembled packaging material • Identify sources and description of strategy indicating them, e.g.: 1. analysis of FCM, analysis of the food at different stages of the supply chain; 2. the presence of mineral oil identifiers, e.g. pristane, phytane, hopanes in the MOSH, or identifiers of the use of recycled paper (DIPN) by GC-MS or GCxGC-FID/MS; 3. If PAO, POH or waxes of mineral origin have been identified, this shall be reported

The following example is provided to indicate how to report the different analytical results for the same sample for MOSH analyses (MOAH analysis can be reported in the same way changing the parameter codes). Only the most relevant variables are indicated to explain how to code the analysis results. In the parameter code, the English description of the code, reported between brackets, is indicated exclusively for simplifying the reading, and should not be included in the file.

Laboratory sample code (S.01)	IT_MYLB_2018_01
EFSA Product code (S.12)	A.01.001028
Product full text description(S.14)	Yoghurt, cow milk, plain

Result code (R.01)	Parameter code (R.06)	Result unit (R.13)	Result LOQ (R.15)	Result value (R.18)	Type of result (R.27)
IT_MYLB_2018_01/01	RF-00007450-PAR (MOSH >C9 to ≤C16)	G061A		10	VAL
IT_MYLB_2018_01/02	RF-00007451-PAR (MOSH >C16 to ≤C20)	G061A		5.0	VAL
IT_MYLB_2018_01/03	RF-00007452-PAR (MOSH >C20 to ≤C25)	G061A	0.2		LOQ
IT_MYLB_2018_01/04	RF-00007453-PAR (MOSH >C25 to ≤C35)	G061A	0.2		LOQ
IT_MYLB_2018_01/05	RF-00007454-PAR (MOSH >C35 to ≤ C40)	G061A		25	VAL
IT_MYLB_2018_01/06	RF-00007455-PAR (MOSH >C40 to ≤C50)	G061A		5.0	VAL
IT_MYLB_2018_01/07	RF-00007456-PAR (#humpId=1$Cstart=10$CTop=16$Cend=21)	G061A		15	VAL
IT_MYLB_2018_01/08	RF-00007456-PAR (#humpId=2$Cstart=34$CTop=37$Cend=40)	G061A		25	VAL
IT_MYLB_2018_01/09	RF-00007456-PAR (#humpId=3$Cstart=40$CTop=42$Cend=44)	G061A		5.0	VAL
IT_MYLB_2018_01/10	RF-00000397-ORG (Total MOSH)	G061A		45	VAL

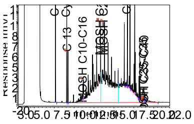
List of abbreviations

DIPN	diisopropylnaphthalene
EAN	European Article Numbering
EURL	European Union Reference Laboratory
FID	flame ionisation detector
GC	gas chromatography
GCxGC	comprehensive two-dimensional gas chromatography
LC	liquid chromatography
LOQ	limit of quantification
MOAH	mineral oil saturated hydrocarbons
MOSH	mineral oil aromatic hydrocarbons
MS	mass spectrometry
NMR	nuclear magnetic resonance
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PAO	poly alpha olefins
POH	polyolefin oligomeric hydrocarbons
PT	proficiency testing
SPE	solid phase extraction
SSD	Standard Sample Description

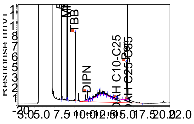
Annex I

Figure A: **on-line LC-GC: sufficient LOQ (rice)**

MOSH



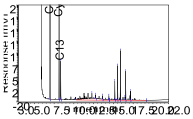
MOAH



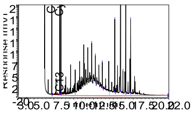
MOSH [mg/kg]						**MOAH [mg/kg]**			
>=C10-≤C16	>C16-≤C20	>C20-≤C25	>C25-≤C35	>C35-≤C40	>C40-≤C50	>=C10-≤C16	>C16-≤C25	>C25-≤C35	>C35-≤C50
0.21	**2.2**	**3.1**	**2.3**	**<0.1**	**<0.1**	**<0.1**	**1.2**	**0.39**	**<0.1**

Figure B: **insufficient LOQ (rice)**

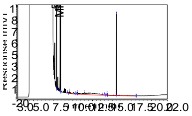
MOSH before enrichment



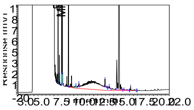
MOSH after enrichment



MOAH before enrichment



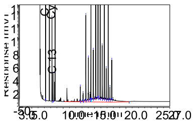
MOAH after enrichment



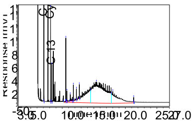
MOSH [mg/kg]						**MOAH [mg/kg]**			
>=C10-≤C16	>C16-≤C20	>C20-≤C25	>C25-≤C35	>C35-≤C40	>C40-≤C50	>=C10-≤C16	>C16-≤C25	>C25-≤C35	>C35-≤C50
0.20	**0.67**	**0.58**	**0.31**	**<0.1**	**<0.1**	**<0.1**	**0.41**	**<0.1**	**<0.1**

Figure C: **Aluminum oxide treatment (olive oil) + Enrichment**

MOSH before alox pre-separation



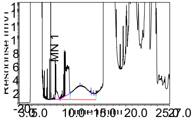
MOSH after alox pre-separation

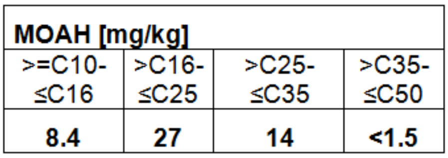


MOSH [mg/kg]					
>=C10-≤C16	>C16-≤C20	>C20-≤C25	>C25-≤C35	>C35-≤C40	>C40-≤C50
<0.1	**0.52**	**2.3**	**6.5**	**1.2**	**0.69**

Figure D: **Epoxidation (olive oil, Panettone)**

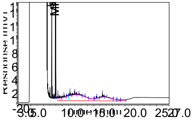
MOAH before epoxidation (olive oil)

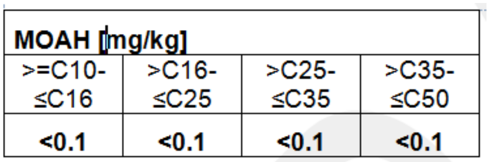




MOAH [mg/kg]			
>=C10-≤C16	>C16-≤C25	>C25-≤C35	>C35-≤C50
8.4	**27**	**14**	**<1.5**

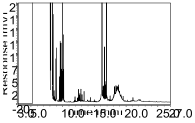
MOAH after epoxidation (olive oil)





MOAH [mg/kg]			
>=C10-≤C16	>C16-≤C25	>C25-≤C35	>C35-≤C50
<0.1	**<0.1**	**<0.1**	**<0.1**

MOAH before epoxidation (Panettone)



MOAH after epoxidation (Panettone)

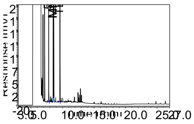
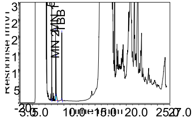
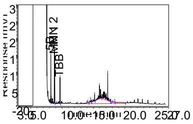


Figure E: **interferences after epoxidation (olive oil): higher LOQ or characterization by GCxGC-FID/MS**

MOAH before epoxidation





MOAH [mg/kg]			
>=C10-≤C16	>C16-≤C25	>C25-≤C35	>C35-≤C50
<1	**≤2**	**≤11**	**≤4**

Reported as “≤” results, as verification (e.g. by GCxGC-FID/MS) needed, as MOAH hump does not correspond with MOSH hump. Results of this example cannot be reported to EFSA as LOQ higher than LOQ-max.

Annex II

Verification in the MOSH/MOAH analysis

<table>
<tr><th></th><th>MOSH</th><th>MOAH</th></tr>
<tr><td>First check</td><td colspan="2">Are the humps typical for mineral oil fractions (e.g. width)?
Correspondence of the molecular mass distribution of MOSH and MOAH
Is the ratio of MOSH and MOAH concentration reasonable?</td></tr>
<tr><td>Treatment of riding peaks</td><td>Homogeneous distribution of n-alkanes → MOSH (e.g. waxes of mineral oil origin); all others (terpenes, natural alkenes/alkenes) eliminated
Dominating odd-numbered n-alkanes: natural alkanes subtracted; removable by activated aluminium oxide in case of overloading</td><td>Riding peaks are usually not MOAH: all to be subtracted, e.g. resin acid derivatives from paperboard, polystyrene oligomers</td></tr>
<tr><td rowspan="3">Natural olefins</td><td>In on-line LC-GC, monounsaturated olefins are eluted in the MOSH fraction; diunsaturated ones in the MOSH or MOAH fraction</td><td>Squalene and sterenes form single signals around C_{28}-C_{29} (removable by epoxidation)</td></tr>
<tr><td colspan="2">Isomerization products of squalene and carotenes form humps more narrow than MOH (removable by epoxidation)</td></tr>
<tr><td>No non-aromatic unsaturated hydrocarbons expected in mineral oil</td><td></td></tr>
<tr><td>Polyolefin oligomeric hydrocarbons (POH) or other synthetic hydrocarbons</td><td>- typical patterns of riding peaks for PP and some PE, but in mixtures oligomers of LDPE are difficult to distinguish from MOSH
  - even-numbered n-alkanes or alkenes: PE POH
  - homologue row of iso-alkanes ΔC3: PP POH
- series of narrow humps (e.g. adhesives, PAO from synthetic lubricants)
- plausible sources?</td><td>MOAH-fraction contains polyunsaturated synthetic hydrocarbons, occurring as series of narrow humps, e.g., in adhesives. Manual method: monounsaterated POH may shift into the MOAH fraction</td></tr>
<tr><td>Identification of source</td><td colspan="2">Transfer through gas phase: limited volatility range (at RT up to about C_{24})
Transfer from FCM: check extract from FCM
Ratio MOSH/MOAH: depending on degree of refining
Environmental contamination is typically free of MOAH</td></tr>
<tr><td>Compounds indicative of sources</td><td>- pristane, phytane (GC-FID)
- multibranched alkanes (GCxGC-FID/MS)
- hopanes, steranes (GC-MS, GCxGC-FID/MS)</td><td>- DIPN identifier for recycled paper and board (typical peak pattern in GC-FID or GC-MS)
- Methyl dibenzothiophene possible identifier for non or little refined oils (GC-MS, GCxGC-FID/MS)</td></tr>
<tr><td>Cases of doubt</td><td colspan="2">Pattern by GCxGC-FID/MS</td></tr>
</table>

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