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Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials – 2nd Edition

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

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Table of Contents

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Executive summary

Four years after the first edition, drafting this second edition of the guidance document was triggered both by DG SANTE's request to update the instructions for reporting and by the need to reflect the experience acquired in the field of the mineral oil analyses .

As in the first edition, this guidance document provides specific recommendations for sampling and analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in food and food contact materials (FCM) in the frame of Recommendation (EU) 2017/84 for the monitoring of mineral oils. In addition, it addresses the requirements resulting from the Joint Statement of the Member States (dated April 21, 2022) and the clarifications to the statement (dated October 19, 2022) regarding the presence of MOAH in food, including food for infants and young children.

Minimum performance requirements are specified for the analytical methods fit for MOSH and MOAH monitoring and control. The guidance should be used by all stakeholders concerned, i.e. food inspectors, official control laboratories, laboratories in industry and laboratories of non-governmental organisations.

This guide aims to support the reporting of reliable data when quantifying both MOSH and MOAH fractions by laboratories that are familiar with the analytical approaches and that have demonstrated satisfactory analytical performance in relevant proficiency testing (PT) schemes. In addition, this guide provides references to current analytical approaches described in the scientific literature to laboratories that are not familiar with MOSH/MOAH analysis, even though it does not provide any standard operating procedures.

1 Introduction

Consumers are exposed to a range of mineral oil hydrocarbons (MOH) present in the food chain [1]. Major sources of MOH in food are migration from FCM, unintentional contamination by non-food grade lubricants, environment, or the use of refined (food grade) mineral oils as additives and processing aids agents. 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 body weight per day, with higher exposure in children [1]. 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. This Opinion is currently being revised to include recent literature data and updated risk assessments, and will be available for public consultation beginning of 2023.

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.

According to Commission Recommendation (EU) 2017/84 [2]*"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 (EURL-FCM) 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."*

Four years after the first edition [3], the need for a second edition was identified due to the experience acquired in the field of mineral oil analyses and triggered by the DG SANTE request to update the instructions for reporting.

2 Scope

This guidance document was developed to support the implementation of Commission Recommendation (EU) 2017/84 [2]. It provides guidance on sampling, analysis and reporting of the results for the content of total MOSH and MOAH.

This guide specifies minimum performance requirements for the analytical methods fit for MOSH and MOAH monitoring and control. This guide aims to

- harmonise sampling of food and FCM for MOSH and MOAH analysis;
- harmonise reporting for MOSH/MOAH content;
- harmonise integration of MOAH chromatograms and proper limit of quantification (LOQ) evaluation;
- recommend method performance characteristics for MOSH/MOAH analysis; and
- provide relevant literature references.

This guidance does ***not*** provide any standard operating procedures.

Relevant standard operating procedures for groups of food commodities were recently developed/improved for infant formula and for edible oils and fats. These methods were ring-trial validated by the JRC and CEN, respectively and the final reports are due in 2023.

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

3 Sampling

Recommendation (EU) 2017/84 [2] refers explicitly to Regulation (EC) No 333/2007 [4] 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. However, only few sections of this Regulation are relevant for sampling procedures for mineral oil in food, e.g. Annex Part A; Annex Part B: sections B.1.1 (for official control); B.1.2 to B.1.6, B.1.7; B.1.8; B.2; and B.3.

The following section will give guidance on sampling that may be relevant both to monitoring MOH in food and FCM and to official controls.

Guidance for sampling

When unused packaging material from the same batch that was used to package food is still available at the food business operator, it should 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 (e.g. 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 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 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 samples, 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 sealing with a 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 analysis in order to prevent cross-contamination. Pre-packed food should be sampled as close as possible to the best-before-date. Any pre-packaged food sample brought into the laboratory without aluminium foil wrapping should be properly 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.
The efficiency of the washing procedure should be checked whenever glass sample containers cleaned in a washing machine are reused. These containers do not need to be checked for residual mineral oil contamination if the washing procedure is proven to be 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, according to EFSA's requirements [2] laid down in the Standard Sample Description on Food and Feed (SSD1):

- ☐ Laboratory sample code - expressed by a unique sample identification number.
- ☐ Country of sampling – where the food was selected for laboratory testing.
- ☐ Country of origin of the product - where the food originated from.
- ☐ Area of origin for fisheries or aquaculture activities code - cf. FAO Fisheries areas.
- ☐ EFSA Product code - Food products should be described according to the FoodEx catalogue of the Standard Sample Description (SSD).

  Note: 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 – This is essential to check if the EFSA product code (FoodEx code) given by the data provider is consistent with the text description.
- ☐ Packaging - short description of 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 - indicate explicitly if the original sample is treated or not, especially if it is a dehydrated product.
- ☐ Product comment - Additional information on the product, particularly preparation details if available.
- ☐ Year, month and day of expiry – Best-before date or use by year or other indications of the expiry date.
- ☐ Year, month and day of sampling - 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 - describe how the sample was selected from the population being monitored or surveyed.

- ☐ Programme type - 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 - specify 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 - 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 [5].

Additional information:

- ☐ Article number.
- ☐ European Article Numbering (EAN) code [6].
- ☐ 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 (when available).
- ☐ Mass of packaging material (when available).

Table 1 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. sub-lot	(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
			50 to 500	5	200
			< 50	3	330
other products	≥ 15	≥ 1	> 500	10	100
	< 15	1	> 500	10	100
			50 to 500	5	200
			< 50	3	330
bulk homogeneous liquid products		≥ 1		3	330

4 Analysis

4.1 Definition of the measurands

For the analytical determination of the MOSH/MOAH content in food and FCM, measurands should be defined, which reflect the MOSH/MOAH descriptions from the most recent EFSA's opinion [1].

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 (when needed), as quantified by integration of the whole signal interval in the GC/FID chromatogram between the retention times (RT) of the peak start of n-C_{10} and the peak end of n-C_{50} separated on an apolar GC column (dimethylpolysiloxanes with ≤5 % phenyl substitution) (i) after trimming of the identified sharp peaks not belonging to MOSH, (ii) after subtraction of the reagent blank, and (iii) using cyclohexylcyclohexane (Cycy) as internal standard (IS)".

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

The MOSH fraction may include polyolefin oligomeric hydrocarbons (POH) and hydrocarbons from poly alpha olefins (PAOs) [7] when their separation/subtraction 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 (if needed), 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} separated on an apolar GC column (dimethylpolysiloxanes with ≤5 % phenyl substitution) (i) after trimming the identified sharp peaks not belonging to MOAH, (ii) after subtraction of the reagent blank, and (iii) using 1- or 2-methylnaphthaline (1-MN, 2-MN) as IS".

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

4.2 Background of MOH analysis

MOSH/MOAH separation and its subsequent determination can 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 [8, 9, 10].

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 (to separate MOSH from MOAH) and GC-FID (for quantification) allows for an appropriate determination of the MOSH and MOAH content. It has been decided to collect data for mineral oils falling into the volatility range of n-C_{10} up to n-C_{50} atoms in their molecules, though contamination with heavier oil fraction may occur in some type of foods. The decision was driven by the impossibility to demonstrate lack of discrimination above n-C_{50} with the current instrumental setup.

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 phase extraction (SPE). On-line coupling to GC integrates part of the sample preparation into the final analysis, which however is a rather complex procedure. As the on-line system is a closed system, it

prevents 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.

In the off-line mode, an LC column with larger internal diameter (4.6 mm instead of 2 mm) can be used for MOSH/MOAH fractionation. A larger sample could then 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 "off-line" method uses a glass column/cartridge filled with silica/$AgNO_3$ to separate the MOSH and MOAH fractions [8]. The method is 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. However, it provides almost identical responses to all hydrocarbons, making it a preferred detector for MOSH/MOAH quantifications. Due to the lack of selectivity, additional sample preparation techniques to eliminate interferences and to enrich both MOSH and MOAH [9-10] fractions may need to be applied.

Today, the LC-GC-FID method is referred to as the method of choice for the quantification of mineral oils in routine analyses [1, 11]. Many 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 should be performed by using additional analytical techniques, e.g. LC-GC-FID/MS [13], GCxGC-FID/MS [14-15] or LC-GCxGC-TOFMS/FID [16], which, furthermore may give the possibility to quantify MOAH grouped by number of aromatic rings in line with the EFSA recommendation to be published in 2023. Research on quantification by using comprehensive two-dimensional gas chromatography is ongoing in that field [15, 16].

4.3 Outline of the analytical approach

As a general recommendation, the methods published by Kantonales Labor Zürich and BfR [8-11] can be applied to determine the MOSH/MOAH content in food and FCM. In addition, other approaches, complying with the performance requirements (see Section 4.6) can be used.

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. an n-hexane/dichloromethane gradient. 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. Volatile components are retained by solvent trapping applying 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 total MOSH/MOAH measurands represents an unresolved signal (hump). It is measured by integration of the chromatogram covering the range of elution of ≥ n-C_{10} to ≤ n-C_{50}, and subtracting the reagent blank. Sharp peaks above the MOSH 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), as well as all sharp peaks above the MOAH hump need to be cut out from the signal (see annex 1 for details). When the MOSH

chromatogram indicates the presence of POH and/or PAO, their peaks should not be subtracted from the MOSH signals and the quantitative result should be reported as mixture of MOSH/POH, MOSH/PAO or MOSH/PAO/POH. When MOSH, POH and PAO are chromatographically distinguishable, the quantitative result for MOSH, POH and PAO can be reported separately.

The calculation of the MOSH or MOAH mass fraction ($w_{MOSH/MOAH}$) is performed as follows:

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

Where:

- A_i is the signal area attributed to the **corrected hump** (MOSH or MOAH) after correction for the background, trimming of identified sharp peaks above the hump (if necessary);
- A_{IS} is the peak area of the internal standard (Cycy for MOSH, 1-MN for MOAH) or 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.

Assigning the signal area belonging to MOSH or MOAH is not an easy task in the presence of interferences and when the baseline has an offset over the whole or part of the RT range. Additional guidance for the integration is provided in Section 4.5.

Some samples may contain **natural odd-numbered paraffins** 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 such cases, it is recommended to use an additional clean-up for the MOSH fraction. For instance, the use of **aluminium oxide (ALOX)** retains long-chain n-alkanes and to a much less extent branched and cyclic components, and enables a selective removal of natural paraffins (Figure 1).

Caution: This auxiliary method removes also mineral oil waxes containing large amounts of n-alkanes with C numbers above n-C_{25}. Recovery of MOSH after ALOX is often lower, when compared to MOSH determined without ALOX clean-up.

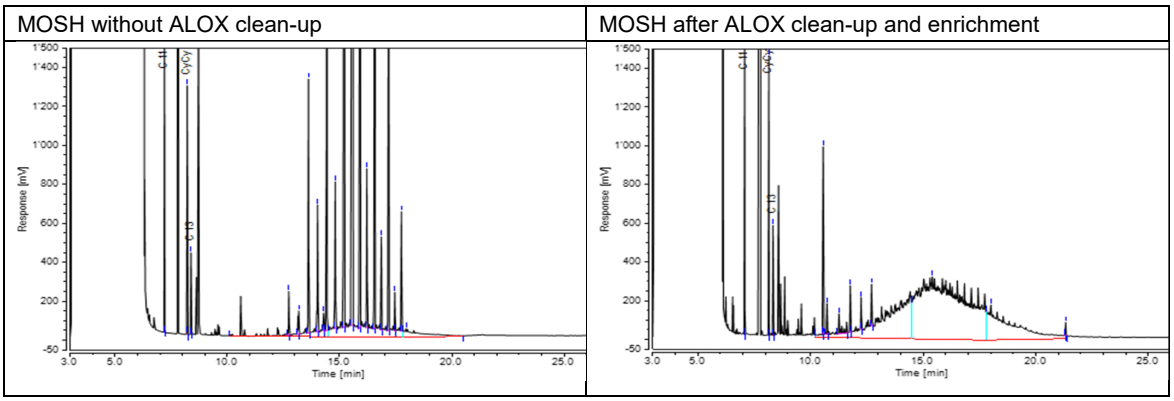


Figure 1 - Effect of the clean-up of the MOSH fraction extract on ALOX column in olive oil (MOSH C10-C50: 11.2 mg/kg).

Epoxidation (EPOX) is a purification step that may be required for the quantification of MOAH [17, 18]. 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 be used as a purification step for the MOSH fraction as well. Depending on the sample, the reaction may induce the epoxidation of a part of the MOAH or an insufficient removal of interfering components. Figure 2 illustrates the effect of epoxidation for the determination of MOAH in olive oil or in panettone.

According to Nestola [19], **epoxidation with meta-chloroperoxybenzoic acid (*m*CPBA) in ethanol** at room temperature is the method of choice to remove the interfering olefins. However, this method does not perform efficiently with products with high fat contents (e.g. palm oil). Up to 40 % MOAH may be lost depending on the matrix and the MOAH composition, particularly including 3-7 rings polycyclic aromatic compounds, due to their partial epoxidation. Despite these shortcomings, the epoxidation step is the best compromise to remove olefins. Research is on-going to further improve this method.

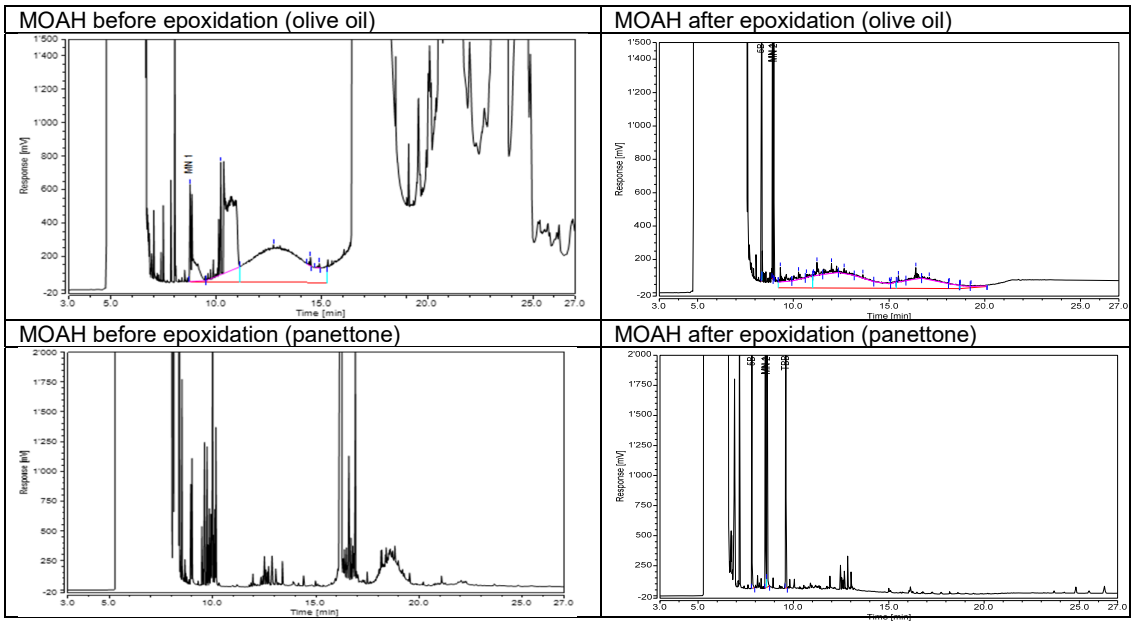


Figure 2 - Effect of epoxidation in olive oil (spiked) or panettone samples (MOAH C10-C50, olive oil: 49 mg/kg; MOAH C10-C50, panettone: <0.4 mg/kg.

Recently, Nestola proposed an alternative epoxidation approach [20] using hydrogen peroxide/formic acid instead of *m*CPBA to remove more efficiently the interferences from edible oil extracts. The reagent blank obtained by using performic acid was claimed to show lower interferences, when compared with the one obtained with *m*CPBA, ever after *m*CPBA prior cleaning. Similarly, underestimated total MOAH contents are obtained. Investigations are ongoing to evaluate the applicability of this method to different matrices.

Saponification (SAPO) preceding (or followed by) extraction is necessary to be applied to some high fat content foods. It removes the lipids and allows sample enrichment. Saponification may improve analytical sensitivity (lower LOQ) when determining MOSH/MOAH in edible oils and fats and some other high fat containing samples. However, it may induce slightly different distributions of the internal and verification standards for the MOAH fraction (1,3,5-tri-tert-butylbenzene (TBB) and MNs) in the course of the extraction with hexane.

Alternatively **enrichment/clean-up** from polar substances of the MOSH/MOAH fractions by offline chromatography on an **activated silica gel column** with larger capacities to retain lipids could be necessary for some samples to increase the analytical sensitivity and reduce LOQs, as shown in Figures 3 and 4.

To facilitate the choice of the required auxiliary methods, a **decision tree** (Figure 5) is developed for removing interferences in the MOSH/MOAH fraction and obtaining the required sensitivity via enrichment, mainly related to the on-line LC-GC-FID method.

Experienced operators are required to correctly interpret the GC chromatograms. Annex 1 could be used as a starting point in this demanding process. Knowledge about the sample and the potential peak patterns of the interferences is essential, e.g. to avoid 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 GCxGC-FID/MS.

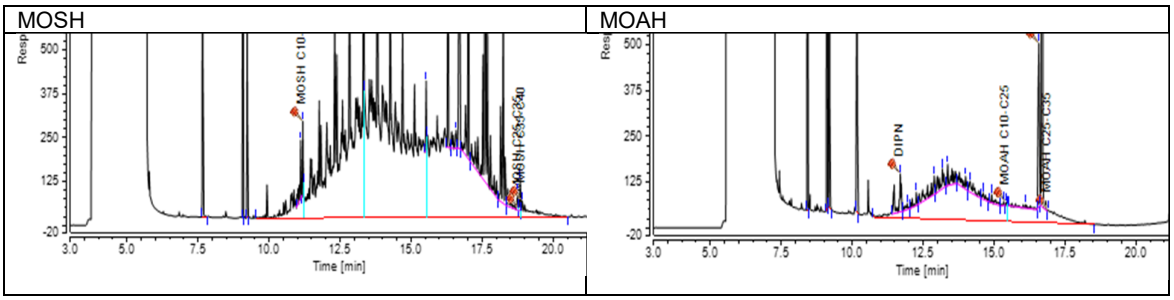


Figure 3 - MOSH/MOAH in rice chromatograms, where no further clean-up is required (MOSH C10-C50: 7.8 mg/kg, MOAH C10-C50: 1.6 mg/kg).

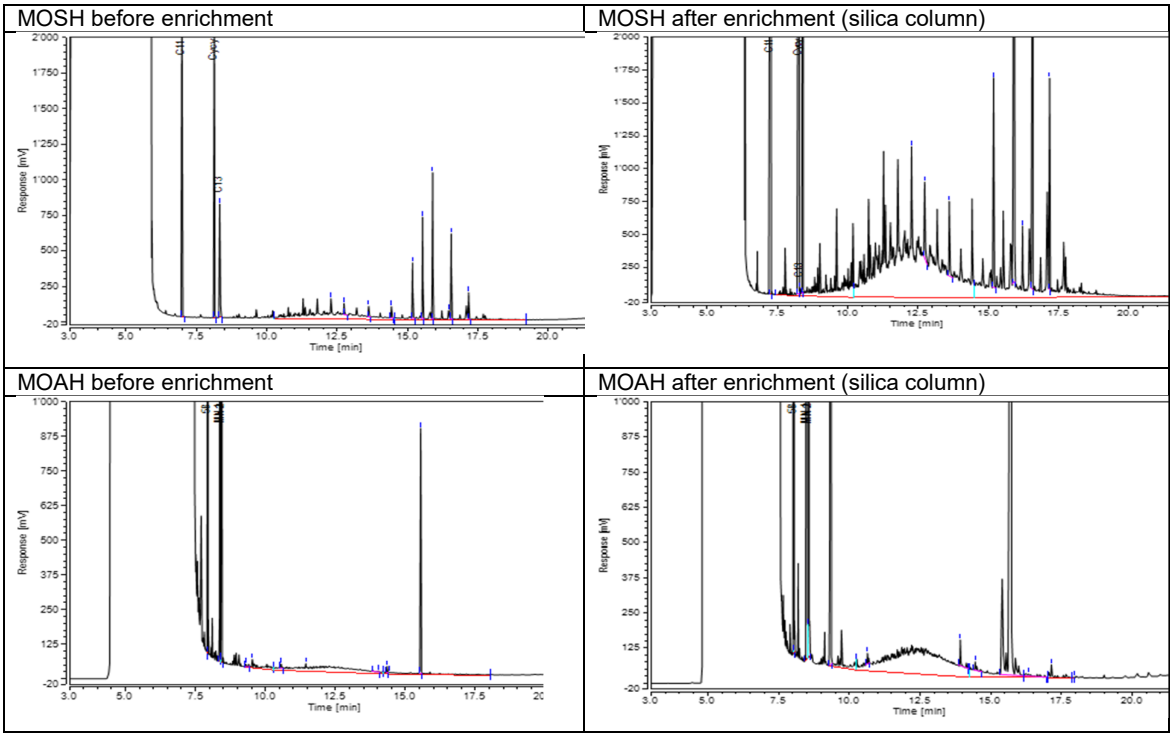


Figure 4 - MOSH/MOAH in rice chromatograms presenting the effect of enrichment on a silica column (MOSH C10-C50: 1.8 mg/kg, MOAH C10-C50: 0.4 mg/kg).

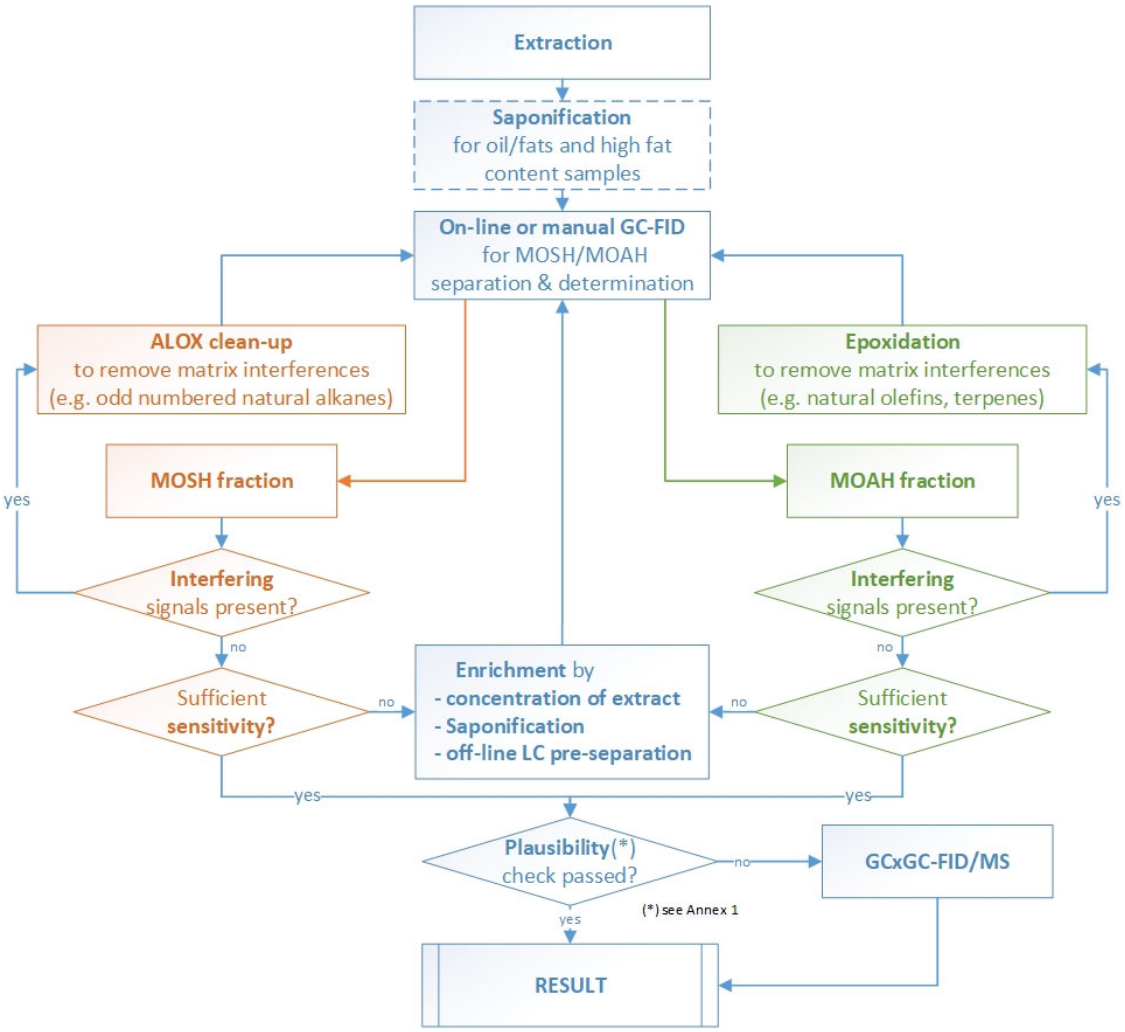


Figure 5 Decision tree on the use of auxiliary methods.

4.4 Verification of the method performance

GC performance

Since the MOH analysis includes hydrocarbons up to n-C_{50}, laboratories should

- use a temperature programme and a GC column which allow the elution of 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. ;
- guarantee an absence of discrimination between low and high boiling compounds by keeping the response ratio of n-C_{10} to n-C_{20} and n-C_{50} to n-C_{20} between 0.8 and 1.2.
- check the performance of the on-line LC-GC-FID system by verifying the recovery of a well characterised mineral oil with a known composition, such as Gravex 913, Shell SN500*. More information is available in the JRC report [21].

Selection of internal and verification standards **[9]**

Quantification of MOH is performed using a flame ionisation detector (FID), which provides an equal response per unit mass for hydrocarbons and sufficient dynamic range to cover the capacity of the capillary column. The addition of a single internal standard hydrocarbon at a known amount is therefore sufficient for the instrumental calibration.

A standard mixture of substances is used to control the analysis of MOH. The mixture includes internal standards for the quantification of MOSH and MOAH and a series of verification standards for the optimisation and monitoring of LC separation of MOSH and MOAH fractions. Additional verification standards are added to identify interferences on internal standards in GC analysis and/or loss of volatile components during sample preparation or during GC injection. All checks are based on the ratio of peak areas of the different standards as described below.

The internal standards listed below are chosen based on the optimisation of the analytical procedures by Kantonales Labor Zürich [9]. Other substances could be used as standards when new evidences will be available. As a general rule, substances contained in the same amount should have the same peak area. The following specification of ratios of the internal standards should therefore be seen as examples. If other concentrations are selected, the ratio must be adjusted accordingly.

When complete saponification is applied and the extraction of mineral oils is performed in hexane in the absence of fat layers, the ratio TBB vs 1- or 2-MN is above 1 with a mean value around 1.15, even after a second hexane extraction of the aqueous saponification solution. This was confirmed during the two ring trial validation studies for the determination of MOSH/MOAH in edible oil and fats by CEN and in infant formula by the JRC organised in 2021/22. It might be attributed to the difference in distribution between the aqueous and hexane phases of the two IS belonging to two different classes of substances (2-ring non-branched and single ring branched aromatic hydrocarbons). In such cases, the quantification is performed *vs* TBB as the efficiency of the extraction of 1-MN and 2-MN in hexane is lower. Consequently, when saponification is applied, the results reported *vs* TBB should be reported with uncertainty reflecting the contribution of the saponification step.

Table 2. Examples for internal and verification standards for MOSH and their ratio

Step	IS	Comment
Quantification	Cyclohexyl-cyclohexane (Cycy)	Used as internal standard for quantification of MOSH since it is not present in relevant quantities in mineral oils or in food and packaging extracts. Cycy is eluted just before n-C_{13} from apolar GC columns (coated with dimethylpolysiloxanes). This separation could be incomplete on more polar columns, such as dimethylpolysiloxanes with 5 % phenyl substitution.
Verification of correct LC elution		Since the start of MOSH elution from the LC column depends on the void volume of the column and is not affected by column aging, it does not require a verification standard. Silica gel LC columns may show a certain size exclusion effect, causing the n-C50 to be eluted slightly earlier than n-C20. The proper start of the MOSH fraction is checked by monitoring the ratio of n-C50 to n-C20. The end of the MOSH fraction is determined by Cycy. (Cycy exhibits slightly more retention on the LC column than cholestane [11]). Its peak area is monitored against n-C11 (proper peak area ratio 1:1) and n-C13 (2:1). Cycy must be absent in the MOAH fraction.
Verification of the IS loss	n-C11	The verification standard is n-C11, which is present at the same amount of Cycy. Since n-C11 is more volatile than Cycy, a correct peak area ratio of 1:1 between n-C11 and Cycy will exclude a loss of volatiles during sample preparation or during GC large volume solvent split injection.
Verification of the presence of interferences of Cycy	n-C13,	n-C_{13} is added at half the amount of n-C_{11} and Cycy. It is eluted closely after Cycy, creating a typical pair of peaks easily recognised at the beginning of the GC chromatogram. A response ratio for n-C13 to Cycy that is much different than 2:1 could indicate an interference with Cycy, which would affect the quantification of MOSH.

Table 3. Examples for Internal and verification standards for MOAH and their ratio

Step	IS	Comment
Quantification	1 or 2-methyl-naphthalene (1-MN/2-MN)	1-MN or 2-MN are used as internal standards for quantification of MOAH since they are not present in relevant quantities in mineral oils or in food and packaging extracts. The peak pair is eluted at the beginning of the GC chromatogram and easily recognised. On a non-polar GC column, 1-MN is eluted after 2-MN.
Verification of correct LC elution	1,3,5-tri-tert-butylbenzene (TBB)	TBB is the verification standard for the start of the elution of the MOAH fraction. It is added at the same amount as the MNs. A correct peak area ration of 1:1 between TBB and 1-MN or 2-MN will indicate a correct collection of the MOAH fraction. TBB was historically introduced as a marker for the start of the MOAH fraction [9], however it turned out in the context of MOH analysis in cosmetics, that di(2-ethylhexyl) benzene (DEHB) is more suitable as it elutes together with the first MOAH such as long-chain alkylated benzenes. It is proposed as a marker for the start of the MOAH fraction in publication [13].
	Perylene	Perylene is the verification standard for the end of the elution of the MOAH fraction. It is added in double the amount of the other standards. A correct peak area ratio of 2:1 between Per and 1-MN will indicate a correct collection of MOAH fraction. Alternatively, its elution may be also monitored by the LC UV-detector. Note: in case epoxidation is performed the ratio is not reliable anymore due to losses of Per.
Verification of the presence of interferences of 1-MN or 2-MN	1-MN or 2-MN	A correct peak area ratio of 1:1 between 2-MN and 1-MN is used to exclude interferences on 1-MN or 2-MN. In case of a deviating peak ratio, further evaluation of the proper peak areas must be done by comparing these with the peak areas of the other verification standards (5-PB, TBB, or DEHB). A peak without interfering signal must be chosen as internal standard.
Verification of volatiles loss	5-pentyl-benzene (5-PB)	5-PB is a verification standard present at the same amount as the MNs. Since 5-PB is more volatile than 1-MN, a correct peak area ratio of 1:1 between 5-PB and 1-MN will exclude a loss of volatiles during sample preparation or during GC large volume solvent split injection.

RT mix

In order to define the position of the beginning and the end of the MOSH/MOAH to be quantified (C10-C50) and to ensure the absence of discrimination, a Retention Time mix (RT mix), containing at least the n-alkanes listed below, should be injected in the two channels of a dual channel GC (if present) to account for the difference in the GC columns and other factors.

- (n-C10) n-decane (124-18-5)
- (n-C11) n-undecane (1120-21-4)
- (n-C13) n-tridecane (629-50-5)
- (n-C20) n-eicosane (112-95-8)
- (n-C50) n-pentacontane (6596-40-3)

4.5 Integration of the chromatograms and quantification

Total MOSH (n-C10-C50) and total MOAH (n-C10-C50) are quantified according to the equation mentioned in Section 4.3.

The integration of the MOSH/MOAH hump is a critical step contributing to the large variability of the results.

The "total MOSH/MOAH content" (n-C10-C50) is determined by integrating the chromatogram,

- from the retention time of the beginning of the n-C10 peak;
- to the retention time of the end of the n-C50 peak;
- after the trimming of the riding peaks (when necessary – see Annex 1) above the hump(s); and
- after the subtraction of/adjustment for the reagent blank (baseline).

The obtained “corrected hump” should be an unambiguously identified smooth hump.

More details about the integration of MOAH chromatograms can be found in the JRC Report [22]. A short overview is given hereafter.

Note: This Guidance focuses on the MOAH fraction (i) as the one of toxicological concern [23] and (ii) in line with the Joint statement of 21 April 2022 [24] of the Member States regarding the presence of Mineral Oil Aromatic Hydrocarbons in food, including food for infants and young children

Internal standards - When the peaks of 1-MN and 2-MN are not fully resolved, they should be separated by a vertical line down to the baseline before integration. The straightforward valley-to-valley integration does not represent the full area of the IS peak, as the area under the red triangle is not included (see Figure 6). Depending on the resolution, this contribution could be negligible. However it must be considered with caution, since the calculation of the final mass fraction is directly correlated with the estimation of the IS (cf. one point calibration).

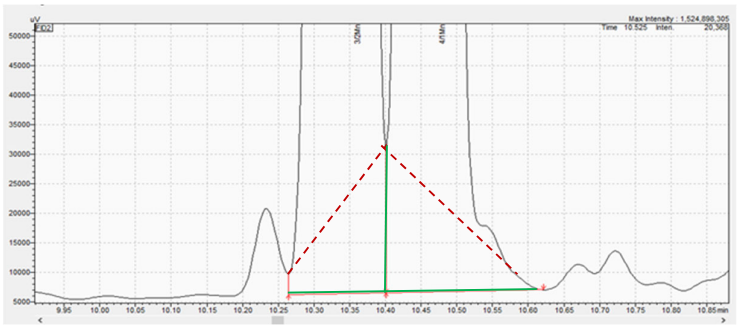


Figure 6
Correct (green line) and incorrect (red line) integration of the internal standards 2- and 1-MNs

When integrating the MOAH hump, the general aim is to obtain a hump with a smooth Gaussian-like distribution (consisting of one or several Gaussian distributions), with no riding peaks.

NOTE. In rare cases long-chain alkylated benzenes can be identified using GC×GC analyses as well-defined single peaks riding over the MOAH hump. In such cases they must be included in the MOAH quantification and the result must state their inclusion.

Subtraction of superimposed humps - The presence of superimposed interfering humps in MOAH chromatograms should be avoided as much as possible. This can be checked by comparing the MOSH and MOAH chromatograms. When such superimposed humps are identified they should be subtracted from the MOAH chromatogram (Annex 2).

Baseline correction – Adjust/shift the reagent blank baseline to the background of the sample in order to compensate for any signal offset.

Visual check/validation - To verify the proper integration for some difficult/challenging samples, it is recommended to visualise the test sample chromatogram with the reagent blank chromatogram subtracted.

4.6 Performance requirements of the analytical methods

According to ISO 17025, any measurement procedure to be accredited, should be properly validated to prove its fitness for purpose. Guidance for method validation can be found in the Eurachem guide [25]. Recommended method performance characteristics for the determination of total MOSH and MOAH content in different food categories are presented in Table 4, including relative intermediate precisions (RSD_{ip}), recovery (R_{rec}) ranges and maximum limits of quantification (LOQ).

4.6.1 Limit of quantification

The limit of quantification (LOQ) of a method is significantly influenced by the fat content of the sample, since the capacity of the LC column to retain lipids is limited, thus determining the amount of sample that can be extracted and injected. In some cases, additional sample preparation steps are therefore necessary to increase the analytical sensitivity, resulting in a lower LOQ.

Harmonised LOQ estimation from in-house method validation study

The determination of the "corrected unresolved signal (hump)" lying between the baseline (reagent blank) and a forest of overriding peaks and/or overlaying humps is challenging as described in 4.5. However, for a given MOSH/MOAH content, the width and shape of the corresponding hump is influenced by the volatility range of the hydrocarbons.

What is the lowest content one can reliably report? A harmonised approach for the estimation of the LOQ is summarised hereafter, based on the recommendations of the JRC Guide on LOD/LOQ [26].

The guide recommends three approaches to estimate the limit of detection (LOD), based on:

The standard deviation from 10 **replicate blanks** (or low concentration pseudo blanks).	Matrix specific for each subgroup of matrices, such as e.g. sunflower-, rapeseed-, palm-, olive oil.
The standard error of the intercept of a dedicated **calibration** at low concentration.	Matrix specific (similar to above).
The standard deviation of the differences in signal abundance of replicates (low-spiked blank samples and/or the native blank sample).	Representative of the group of matrices investigated.

Well-characterised MO having mono-modal distribution covering approximately half of the RT span, (e.g. Gravex, Shell SN500*) should be used for spiking the relevant blank matrices. Other MO's having similar distributions (RT span) regardless of the volatility of the MO components (position of the hump in the chromatogram) are also suitable.

The following relation between LOD and LOQ is recommended, in accordance with Regulation (EU) 333/2007 [4] and the JRC guide [26]:

$$x_{LOQ} = 3.3^* x_{LOD}$$

The spiking level selected for the determination of LOQ should comply with the following criteria:

- the hump of the spiked sample should be **unambiguously distinguished** from the blank and fulfil the requirements from the plausibility check in Annex 1:
- the signal to noise ratio (S/N) at the maximum of the hump (apex) should be greater than 10: **S/N > 10**;

resulting in a relative standard deviation of the intermediate precision (RSD_R) < 20%.

With some matrices where interferences still disturb the integration of the corrected MOSH or MOAH humps, results should be reported as lower than the LOQ* value for that specific sample even though it will be over the max legal LOQ.

4.6.2 Recovery

The measurement procedures used for the determination of MOSH and MOAH in food and FCM must show recoveries (R_{rec}) falling within the ranges specified in Table 4.

In the chromatographic methods applied for the determination of MOSH and MOAH, the use of an internal standard (IS) allows for the automatic correction for recovery. The signal ratio (analyte to IS) is assumed to be constant throughout the analytical process, thus compensating for any losses of the analyte. However, this is not valid when ALOX or EPOX auxiliary methods are used, for which significant decrease in content (up to 40 %) of MOSH (after ALOX) or MOAH (after EPOX) were observed in some samples. In such cases, results should be reported "as is" without any recovery corrections.

In certain food commodities (e.g. infant formula, packaging materials) the MO contamination could be present in heterogenic parts of the food that are difficult to extract. In such cases, the IS may not be able to mimic sufficiently the behaviour of the MO, as the IS would be better extracted than the MO. A check for an effective recovery after extraction must be carried out at the method development stage by subsequent extraction of the sample residue under standard conditions and performing subsequent extractions under harsher conditions (for a longer extraction time; at a higher temperature).

4.6.3 Intermediate precision

The acceptance criteria for intermediate precision (also called within-laboratory precision) when determining MOSH and MOAH in food commodities with different fat content are indicated in Table 4. The intermediate precision should be calculated as recommended in the Eurachem guide [25].

Table 4. Performance requirements for total MOSH and total MOAH analysis: maximum analytical LOQ (max LOQ) of the method, acceptable ranges for recovery (R_{rec}) of mineral oil from samples, and relative standard intermediate precision (RSD_{ip})

Categories	**Associated foods (#)**	**Max LOQ [mg/kg]**	**R_{rec} range [%]**	**RSD_{ip} [%]**
Dry, low-fat content (< 4% fat/oil)	bread and rolls; breakfast cereals; grains for human consumption; pasta, products derived from cereals	0.50	80 - 110	15
Higher fat/oil content (4% - 50% fat/oil)	fine bakery ware; confectionery (incl. chocolate) and cocoa; fish meat, fish products (canned fish); oilseeds; pulses; sausages; tree nuts	1.0	80* - 110	20
Fat/oils > 50% fat	animal fat (e.g. butter); vegetable oils	2.0	80* - 110	20
Paper and Board	Reporting only up to C_{35} (extraction optimised up to C_{35})	10	80 - 110	10

(#) In some cases, a shift to another category may be required. This has to be stated and justified for each case.

*Could be lower when applying ALOX for MOSH or EPOX for MOAH

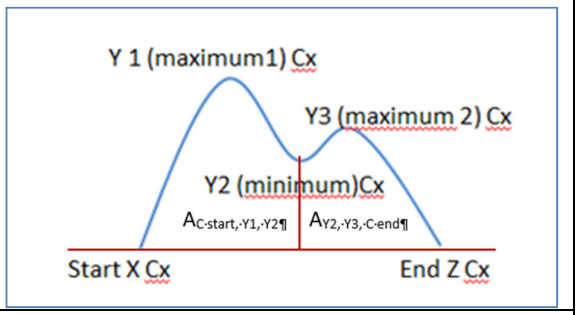
5 Reporting of results

Results shall be reported:

- in mg/kg total MOSH (nC10-nC50) or MOAH (nC10-nC50);
- with two significant figures (e.g. 150, 15, 1.5 or 0.15 mg/kg);
- rounded in accordance to section B.2 of ISO 80000-1:2009.

A short description of the analytical steps applied shall accompany each result. The information listed below should be provided by the reporting laboratory (if needed/requested) to ensure the comparability of results.

When reporting to EFSA, the reporting format should comply with the EFSA's requirements [27] and it has to contain at least the following information:

Sample preparation	Was saponification applied?
	Was an enrichment by off-line LC pre-separation applied?
	Was ALOX clean-up applied for the MOSH fraction?
	Was EPOX clean-up applied for the MOAH fraction?
	What EPOX agent was used? mCPBA or performic acid?
MOSH/MOAH separation	Manual or on-line
Instrumentation used	Specify the analytical instrument used
Analytical method	Short description of the whole analytical procedure used
Hump description	Several humps can be reported. 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. For example. The 3rd hump for MOSH with C_{start}=20, C_{top}=24 and C_{end}=30 should be reported Note: 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 and start of the next hump. 
LOQ	in mg/kg, evaluated during the in-house method validation
Result value	in mg/kg MOSH (POH or POA present?) or MOAH
Measurement uncertainty	It is recommended to provide the associated expanded uncertainty (with a coverage factor k = 2, corresponding to 95% confidence interval).
Internal standard	Specify the IS used for quantification (TBB, 1-MN or 2-MN for MOAH ; CyCy for MOSH, other?)
Raw data	Provide a snapshot image of the chromatogram properly zoomed in (see examples in the Annex 2)

List of abbreviations

ALOX	clean up by column with Aluminium oxide
EPOX	clean up by epoxidation
EURL	European Union Reference Laboratory
FID	flame ionisation detector
GC	gas chromatography
GCxGC	comprehensive two-dimensional gas chromatography
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
mCPBA	meta-chloroperoxybenzoic acid
MO	mineral oil
MOSH	mineral oil saturated hydrocarbons
MOAH	mineral oil aromatic hydrocarbons
MS	mass spectrometry
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

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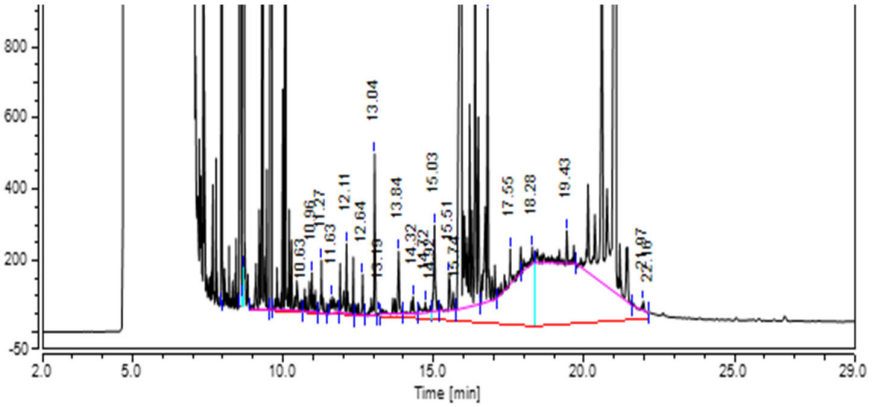
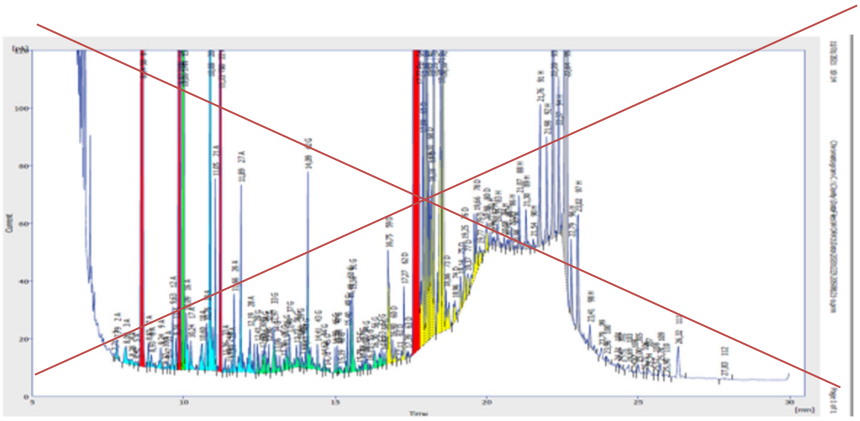
Annex 1. Verification in the MOSH/MOAH analysis

	MOSH	MOAH	
1st check	✓	✓	Is the hump shape/width typical for mineral oil fractions? Individual mineral oil distillates exhibit Gaussian-like humps covering certain homologues of hydrocarbon isomers, MOH can be mixtures of individual distillates. Mineral oil waxes exhibit a homologue row of mainly n-alkanes with certain isomers eluting in between.
	✓	✓	Correspondence of molecular mass distribution of MOSH and MOAH
	✓	✓	Is the ratio of MOSH and MOAH concentrations reasonable? Depending on the degree of refining of the distillate, the MOAH-content ranges from 0 to up to 50 %. Aromatic extracts, used as e.g., extender oils for rubbers, may contain > 90 % MOAH.
Riding peaks	✓		A homologues series of n-alkanes is considered MOSH. ➔ remove all other known discrete signals, e.g., terpenes, mono- and some di-unsaturated olefins, natural alkenes
	✓		Dominating odd-numbered n-alkanes ➔ remove natural alkanes by integration or using activated aluminium oxide in case of overloading
		✓	No riding peaks are expected for MOAH. ➔ remove all riding peaks (e.g., resin acid derivatives from paperboard, some di-unsaturated olefins, polystyrene oligomers, siloxanes) Exception: When the riding signals are proven to be MOAH, e.g., by GC×GC, riding peaks are not removed.
Natural olefins		✓	Polyunsaturated olefins; squalene and sterenes form single discrete signals around C28-C29 Isomerization products of squalene and carotenes form humps that are narrower than MOH ➔ remove by epoxidation in case of overloading and/or co-elution with MOAH hump
	✓		Non-aromatic unsaturated hydrocarbons are not expected in mineral oil and, if present, will be quantified together with MOSH.
POH & other synthetic hydrocarbons	✓		Typical patterns for PP and some PE oligomers - even-numbered n-alkanes or alkenes: PE POH - homologue row of iso-alkanes and alkenes ΔC3: PP POH ➔ If signals are clearly identified as POH only, POH can be

			integrated separately and reported as such. ➔ If non-distinguishable from or mixed with MOSH, POH are integrated together with MOSH. The result is reported as mixture of MOSH and POH.
	✓		Synthetic hydrocarbons: series of narrow humps (e.g. adhesives, PAO from synthetic lubricants) ➔ proceed as described for POH
		✓	MOAH-fraction contains polyunsaturated synthetic hydrocarbons, occurring as series of narrow humps. Manual method: monounsaturated POH may shift into the MOAH fraction ➔ proceed as described above for POH
Identify source	✓	✓	Transfer through gas phase: limited volatility range at RT up to C_{24}
	✓	✓	Transfer from FCM - check extract from FCM
	✓	✓	Ratio MOSH/MOAH - depending on degree of refining
	✓	✓	Environmental contamination is typically free of MOAH
Typical compounds	✓		pristane, phytane (GC-FID) multibranched alkanes (GC×GC-FID/MS) hopanes, steranes (GC-MS, GC×GC-FID/MS)
		✓	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, GC×GC-FID/MS)
In doubt	✓	✓	Check pattern by GC×GC-FID/MS to differentiate MOSH and MOAH from interferences. ➔ Report qualitative composition of MOSH and MOAH fraction

Annex 2. Example for correct and incorrect trimming





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