# Determination of Mineral Oil Hydrocarbons in Food and Packaging by online HPLC-GC-FID and GCxGC-TOF-MS

**Background**

Mineral oil hydrocarbons (MOH) are distillation products of crude oil or coal tar. They contain straight and branched aliphatic and cycloaliphatic (Mineral Oil Saturated Hydrocarbons, MOSH) as well as alkylated, partially hydrogenated aromatic hydrocarbons (Mineral Oil Aromatic Hydrocarbons, MOAH). Heterocycles may also be present. MOHs range from volatile and readily degradable hydrocarbons to poorly soluble, poorly volatile and poorly degradable high-molecular compounds from lubricating greases and oils. MOH from printing inks and recycled cartons are able to migrate into packaged food in high quantities. However, MOH in different compositions can also enter food through many other pathways. Some examples are: Contamination with "Batching Oil" from jute bags (hazelnuts, rice, cocoa or coffee beans), release agents in bakery products, paraffin oil for fining rice and as dust binder as well as hydraulic oils from dosing systems (food industry).

**Toxicological classification**

For a health assessment, an adequate characterisation of the composition of mineral oil mixtures is currently lacking. It is therefore not yet possible for BfR to make a complete risk assessment. Independent of the risk assessment, however, these contaminations are generally undesirable in food.

Paraffins are one of the quantitatively most significant contaminations in the human body. Current studies show an accumulation in various organs in the molecular weight range of about n-C20 to n-C46. Saturated hydrocarbons are not carcinogenic, but in high doses lead to increased cholesterol levels and increased levels of inflammation markers (Stroes et al. N *Engl J Med* 2019; 380:89-90).

Aromatic hydrocarbons are potentially genotoxic and carcinogenic (Opinion on human food exposure to 'petroleum hydrocarbons', European Food Safety Authority (EFSA), June 2012). The estrogenic efficacy of the MOAH fraction has been demonstrated by BfR in in vitro studies (Luch et al. 2016, *PLOS ONE* 11(1): e0147239). However, it is necessary to distinguish mono- and diaromatic compounds from tri-aromatic and higher condensed MOAH (Grob, *J. Agric. Food Chem.* 2018, 66, 27, 6968-6974). With regard to mineral oil mixtures with a high aromatic content (MOAH), according to the BfR, intake should be avoided altogether, since it cannot be ruled out that the MOAH fraction contains carcinogenic aromatic compounds.

It is estimated that between 0.03 and 0.3 mg of saturated hydrocarbons (MOSH) per kilogram of body weight are ingested daily through food, and the intake may be higher in children (EFSA, 2012). EFSA estimates that the intake of aromatic hydrocarbons (MOAH) ranges from 0.006 to 0.06 mg per kilogram of body weight. For a child weighing 10 kg, this means a daily intake of up to 3 mg MOSH and 0.6 mg MOAH.

**Legal classification**

The temporary ADI of 0.01 mg/kg body weight for mineral oils of classes II and III was withdrawn at the JECFA Seventy-sixth meeting in June 2012 (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 913(2002)).

Since April 2011, a temporary migration limit for aromatic-free mineral oils (i.e. MOSH, used as a formulating agent for paper production) in the molecular weight range of n-C10 - n-C16 of 12 mg/kg has been in place, set by BfR. For the fraction of n-C17 - n-C20, a migration limit of 4 mg/kg food for saturated hydrocarbons was established in November 2012, also by BfR (BfR recommendation XXXVI).

The 4th draft of the 22nd Ordinance amending the Commodities Ordinance (Mineral Oil Ordinance) of 07.03.2017 provides for maximum quantities for the transition of MOAH from food contact materials and articles made of paper, cardboard or paperboard produced using recycled paper. According to this, food contact materials and articles may only be placed on the market if the maximum level of 0.5 mg/kg of food or 0.15 mg/kg of food simulant is observed for the transition from MOAH (if necessary by using a functional barrier). Up to this level, a transition is deemed not to have taken place and a functional barrier is considered suitable. The draft was submitted to the EU Commission in August 2020 with minor changes for the notification process.

Furthermore, the GMP Regulation (EC) No. 2023/2006 stipulates that every manufacturer must ensure that "*materials and articles intended to come into contact with food [...]* (are) *appropriate to their intended use by not endangering human health*".

Last but not least, the Contaminants Regulation (EC) No. 1881/2006 demands that for "*contaminants which are considered to be genotoxic carcinogens [...], maximum levels should be set at a level which is as low as reasonably achievable (ALARA)* ". The ALARA principle therefore also applies to MOAH.

Furthermore, the GMP Regulation (EC) applies. On 24 March 2019, Mr. Helling (Saxon State Ministry of Social Affairs and Consumer Protection) presented the BLL guidance values for the first time. They apply to vegetable fats and oils (except tropical oils), bakery products/cereals, chocolate or confectionery and nuts (for details see Fig.1).

**LAV and Lebensmittelverband:**
**Common MOH-Benchmark levels**
**(June 2020)**

| No. | Product group<br>Food category<br>(consumer products) | MOSH<br>and<br>analogues<br>[mg/kg]<br>$C_{10}$-$C_{50}$ | MOAH<br>[mg/kg]<br>$C_{10-C50}$ | Notes on use<br>(Notes on the food groups recorded /<br>on non-recorded products and limitations /<br>if appropriate on reasons, basis of the data or other special features)<br>**MOH-Benchmark Levels must always be used in conjunction with the described definition.** |
|---|---|---|---|---|
| 1 | Vegetable oils,<br>(such as rapeseed oil,<br>sunflower oil, linseed oil,<br>olive oil)<br>(excluding oils/fats of tropical plants and soya oil) | **13** | **n.q.**[2] | these benchmark levels are not intended for application to oils/fats that have been extracted from tropical plants (e.g. coconut oil) due to an insufficient base of statistical data (in Dec. 2018) |
| 2 | Bread and biscuits, fine pastries, cereal products and cereal-based products, cereals, rice, pasta | **6** | **n.q.**[3] | not to raw commodities or raw doughs |
| 3 | Confectionery (sugar confectionery except chewing gum), chocolate and cocoa-based confectionery | **9** | **n.q.**[3] | |
| 4 | Nuts, tree nuts, oilseeds, coconut, peanuts and dried fruit, including mixtures thereof | 4 | **n.q.**[3] | |

*n.q. – not quantifiable, i.e. contents < limit of quantification (here: $LOQ_{max}$ in mg/kg in accordance with the JRC Guidance on sampling, analysis and data reporting for monitoring of mineral oil hydrocarbons in food and food contact materials, Valid as of 2019)*

[2] $LOQ_{max}$ for every fraction (cf. JRC Technical Report[1]) for fats/oils is equivalent to 2 mg/kg
[3] $LOQ_{max}$ for every fraction (cf. JRC Technical Report[1]) for low-fat foods < 4% fat is equivalent to 0.5 mg/kg; > 4% fat is equivalent to 1 mg/kg

Fig. 1: Overview of BLL guidance values; Source: German Food Association, https://www.lebensmittelverband.de/download/benchmark-levels-moh-in-foods

**Analytics**

The investigation of the MOH is based on the international DIN EN 16995:2017 "Determination of saturated petroleum hydrocarbons (MOSH) and aromatic petroleum hydrocarbons (MOAH) with online HPLC-GC-FID" as well as on the compendium of the Federal Institute for Risk Assessment (BfR) and the Cantonal Laboratory Zurich (KLZH) for the measurement of MHC in food and packaging materials and the JRC "Guidance on sampling, analysis and data reporting" (published 2019).

The JRC Handbook, in line with its title, contains specific instructions for sampling and analysis of saturated mineral oil hydrocarbons (MOSH) and aromatic mineral oil hydrocarbons (MOAH) in food and food contact materials, as recommended in the EU Regulation (EU) 2017/84 on the monitoring of mineral oils. Minimum requirements are set for the implementation of appropriate analytical methods in the context of MOSH / MOAH monitoring. These guidelines should be used by all those involved in the determination of mineral oil hydrocarbons in food and food contact materials, i.e. food inspectors, official control laboratories, laboratories in industry and private laboratories. This guide is intended to help generate reliable data on the presence of both MOSH and MOAH and to enable reporting by laboratories already familiar with analysis.

DIN SPEC 5010:2018-05 is the publicly available specification for the investigation of migration from paper, board and cardboard using Tenax® as adsorbent. With this method the migration potential of food contact materials can be estimated.

The MOH are extracted from the sample with an organic solvent. Complex samples, e.g. tea, fat-rich food such as chocolate or fats/oils, are additionally purified with various auxiliary techniques (e.g. with activated aluminium oxide or an epoxidation step) before the measurement. Afterwards the MOH are determined by means of online HPLC-GC/FID. The normal phase HPLC retains interfering lipids and separates the MOSH fraction from the MOAH fraction. The respective fraction (MOSH/MOAH) is then detected by FID. Quantification is performed using internal standards added prior to extraction.

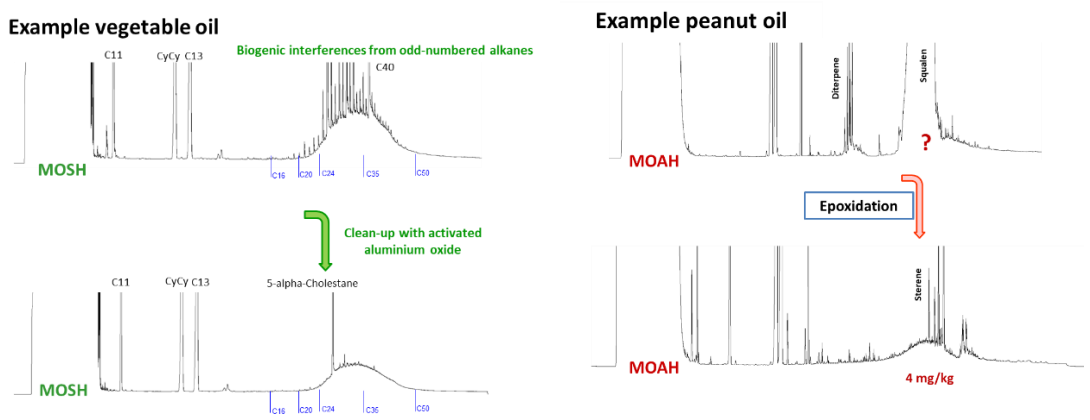



Fig.2: HPLC-GC-FID chromatograms of MOSH and MOAH in an olive oil and a peanut oil

It is important to adapt the sample preparation to the existing matrix. Water containing matrices and paper packages must be extracted with ethanol added. The ethanol serves as a solubilizer, which makes the apolar analytes in the sample accessible to the extraction agent n-hexane at all.

In order to achieve the determination limits for fats/oils and fat-rich matrices required by the JCR, in deviation from DIN EN 16995, saponification of the samples is absolutely necessary to separate fats from the MOH and not to overload the silica gel column. This is the only way to ensure sufficient enrichment of the sample extracts.

The odd-numbered biogenic n-alkanes from n-C23 upwards are removed from the sample extract with activated aluminium oxide (annealed at 500 °C for 16 h). The basic aluminium oxide has no significant retention for n-alkanes up to C20 and a high increasing retention for long-chain n-alkanes. n alkanes from the MOSH fraction are also removed, so that losses of up to 43 % (for n-C43) can occur during purification depending on the molecular distribution of the contamination (Biedermann et al. Anal Chim Acta, 2009, 102-109).

For the selective epoxidation of squalene or carotenoids there are different methods in the relevant literature (according to Biedermann; with m-CPBA dissolved in DCM or according to Nestola; m-CPBA dissolved in ethanol). According to our comparative laboratory results, both approaches give the same result.

It is important to use epoxidation only where matrix components interfere with the analysis of the MOAH fraction. Due to the reasons mentioned above, this also applies to the clean-up of the MOSH fraction with aluminium oxide. Epoxidation removes up to 30 % of the MOAH contained in the MOSH fraction. Since m-CPBA also attacks the internal standards 2-methylnaphthalene and 1-methylnaphthalene, it is important to evaluate the epoxidized samples via tri-tert-butylbenzene (TBB).

If necessary, a further characterisation of the samples is carried out using **GCxGC-TOF-MS**. By coupling two-dimensional gas chromatography with a mass spectrometer, subgroups can be characterised, false positive results avoided and marker substances for the origin of MOH can be identified (see section GCxGC and Fig. 3).

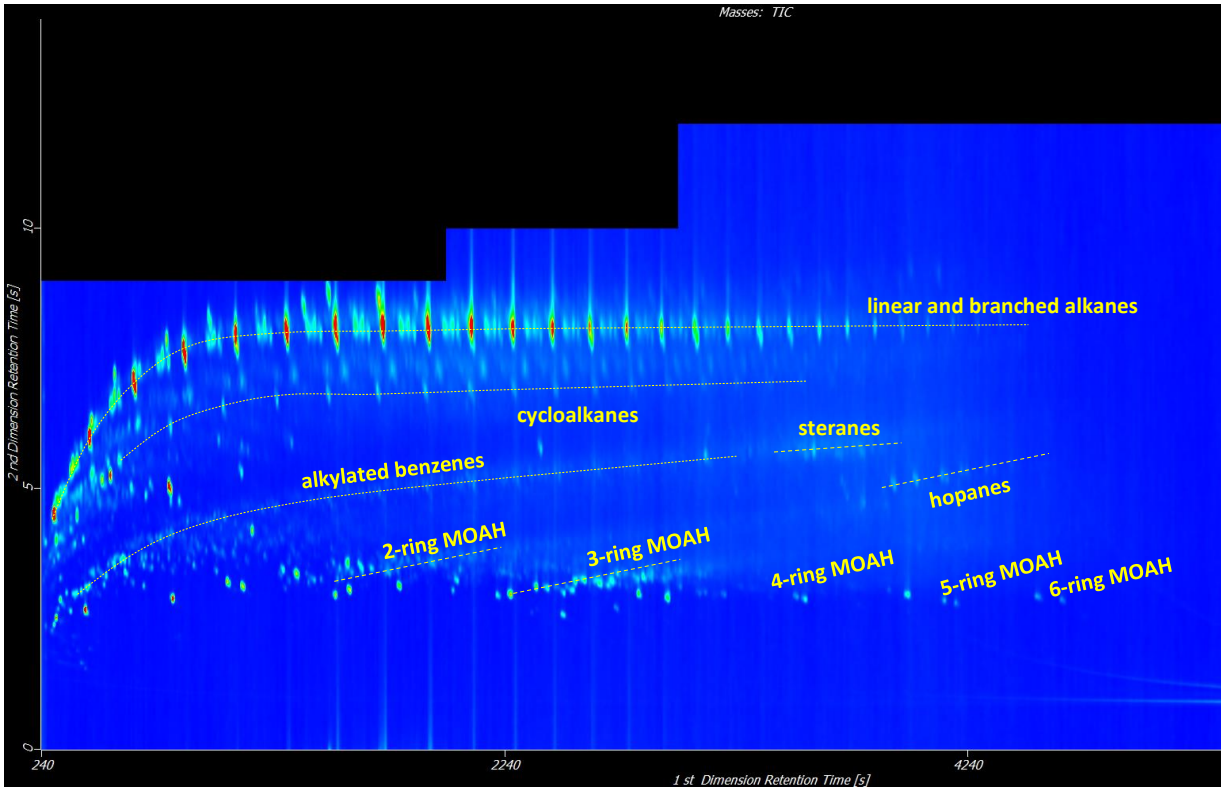
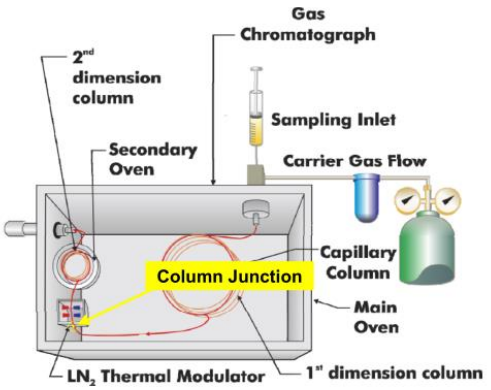



Fig.3: GCxGC-TOF-MS contour plot of a MOH; in addition to the respective subgroups of the MOSH and MOAH fraction, typical markers for the fossil origin (e.g. hopanes and steranes) can be identified.

**GCxGC-Characterization**

The GCxGC-TOF-MS is a two-dimensional (2D) chromatographic measuring method. It requires the use of two analytical columns with orthogonal properties (e.g.: non-polar/polar). This increases the separation performance and thus the peak capacity of the analytical system enormously, which allows the analysis of complex mixtures such as MKW.




The most common column setup for the analysis of HC is the so-called reversed phase (RP) arrangement, where a long polar and in the second a short non-polar column is used. In this way, the complex mixtures of hydrocarbons to be analysed are first separated according to their polarity and then according to boiling points. In the reversed and so-called NP (normal phase) setup, a non-polar stationary phase is used in the first dimension and a polar one in the second dimension.

The analysis can be described as "comprehensive" because the analytes pass from the first separation column directly to the second column without losses. Between the columns there is a unit called a modulator, which "cuts" the eluate from the first column at fixed intervals by cryofocusing and transfers it to the second column.

The sample molecules are refocused by "freezing" and then transferred to the second column by heating. On the one hand, the focussing results in an advantageous increase of the signal intensity, on the other hand, the resulting "partial peaks" become very narrow, which makes the use of a very fast detector necessary to achieve a sufficient number of data points per peak. The TOF (time of flight) mass spectrometer is ideally suited for this purpose, as it is able to record up to 500 full-scan spectra (in the set mass range) per second and thus also image peaks whose peak width is only in the two-digit millisecond range.

As a result of a GCxGC analysis, a contour plot is produced which shows the separation of the MHC mixtures over the polar and non-polar column, with the abscissa corresponding to the polar dimension and the ordinate to the non-polar dimension (Figure 4).

Figure 4 shows the isolated MOAH fraction of a lubricating oil (MOAH content about 26%) chromatographed by online HPLC-GC-FID and GCXGC-TOF-MS. The complex mixture of aromatic hydrocarbons and the internal standards are clearly visible in both chromatograms.

Due to the different chemical properties of the different possible substance classes, structured two-dimensional chromatograms with specific bands are obtained. By filtering according to substance class-specific masses (m/z) and matching them with the spectra database, these bands can then be assigned to the various compound classes (see Fig. 3). In this way it can also be determined whether a sample contains monoaromatic, diaromatic or triaromatic compounds. However, a complete chromatographic resolution of the sample into individual compounds is also not possible using GCxGC-TOF-MS. Since the response in the mass spectrometer is substance dependent, humps can only be characterised but not quantified.

One strength of the technique is the detection of oligomers of PP or adhesive resins. Due to the characteristic distribution in the contour plot, the oligomers can be identified even when superimposed with MOSH but not quantified.

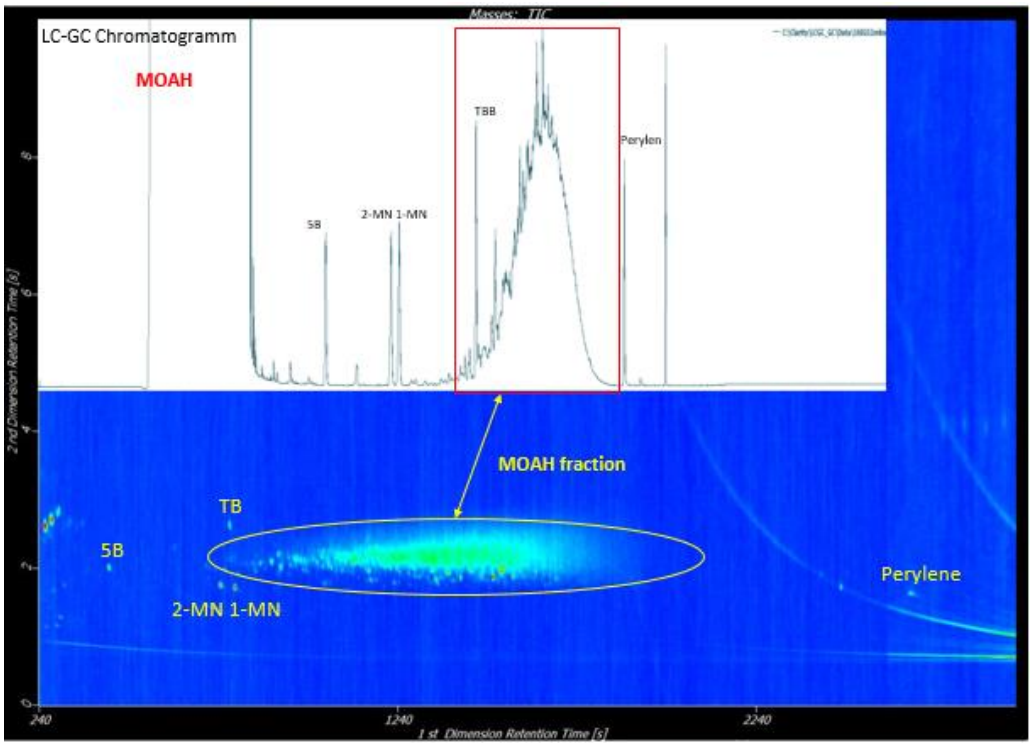



Fig. 4: GCxGC contour plot of the MOAH fraction of a lubricating oil. Comparison with LC-GC chromatogram. *(Legend internal standards: 5B-pentylbenzene, 2-MN: 2-methylnaphthlaine, 1-MN: 1-methylnaphthalene, TBB: tritertbutylbenzene)*

Marker substances are an aid in identifying the source of contamination. With the help of the GCxGC-TOF-MS markers in the MOSH and MOAH fraction can be identified. Markers in the MOSH fraction are cylcoalkanes, steranes and hopanes (markers for the fossil origin of the MOH). The following marker substances can be used in the MOAH fraction: Diisopropylnaphthalenes (DIPN; markers for recycled cardboard, dithiophenes (markers for transport in jute bag and for fossil origin of the MOAH, but easily epoxidized) and biphenyls.

In some matrices (e.g. rapeseed oil, palm oil), despite epoxidation, non-epoxidized carotenoids or sterenes may remain in the MOAH fraction. This can lead to a false positive evaluation of samples. An additional characterisation by means of GCxGC-TOF-MS clearly identifies interferences and false positive results are excluded. Serious financial damage is thus avoided.

**Results**

At the Kirchhoff Institute in Berlin, samples of various food matrices are analysed, including many packaging materials. For different matrices, stage controls and projects to minimize MOSH and MOAH were supported with our expertise. In packaged foods up to 60 mg/kg mineral oil hydrocarbons were determined and in many foods a basic contamination with mineral oil hydrocarbons could be determined. In general, however, the trend for MOSH and MOAH levels in foodstuffs is downward. The sources of contamination with mineral oil hydrocarbons are often multifactorial (e.g. raw materials, production process, transport and packaging).

The successful participation in interlaboratory comparisons of different matrices (DRRR), cooperation in method interlaboratory comparisons (e.g. ISO 17780, CEN/TC 275 N 1069, Proof ACS), in §64 working groups as well as the performance of comparative laboratory tests are components of the quality assurance of the results in our company.