Regulatory Toxicology and Pharmacology 132 (2022) 105193

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Comparison of PAC and MOAH for understanding the carcinogenic and developmental toxicity potential of mineral oils

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ARTICLE INFO

Handling Editor: Dr. Lesa Aylward

Keywords:
MOAH
PAC
Mineral oil
Alkylated aromatics
IP346
Modified Ames test
Chromatography
White mineral oil

ABSTRACT

The carcinogenicity and developmental toxicity of unrefined mineral oil is related to its 3–7 ring polycyclic aromatic compounds (PAC) content. Therefore, refining operations focus on the targeted removal PAC from mineral oil that may contain aromatics of low toxicological concern. There are thus, two types of aromatic substances in mineral oil: hazardous and non-hazardous. The first type consists of 3–7 ring PAC which may be naked (unsubstituted) or lowly alkylated. The second type or non-hazardous consists of 1–7 ring aromatics with high degree of alkylation or lack of bay or fjord regions. Although these are toxicologically different, they may both elute in the same fraction when using chromatography. To understand how these two aromatic types are related we have assessed the entire mineral oil refinement process by measuring total mineral oil aromatic hydrocarbons (MOAH) content by chromatography next to regulatory hazard tests which focus on 3–7 ring PAC. MOAH content is positively correlated to its molecular weight resulting in aromatic content bias for high viscosity substances. Hazard to 3–7 ring PAC is best controlled by the validated IP346 or modified Ames test. We explain the concept of high vs low alkylation by shortly reviewing new data on alkylated PAC.

1. Background on MOAH –historical developments

Mineral oils (also known as base oils, mineral base oils or lubricant base oils[1] are complex substances with variable proportions of straight and branched-chain paraffinic, naphthenic (cycloparaffinic), and aromatic hydrocarbons with boiling points in the range of ~300–600 °C (CONCAWE 2017; IARC 2012). After several refining steps, the potentially hazardous polycyclic aromatic compounds (PAC) are removed to meet safety thresholds, assuring that the refined mineral oil is non-carcinogenic (Carrillo et al., 2019). It has been shown that 3–7 ring PAC with no or limited degree of alkylation are the potentially hazardous constituents associated with carcinogenic, mutagenic and developmental toxicity of mineral oils (Agarwal et al. 1985, 1988; Gray et al., 2013; Kamelia et al., 2019b; Mackerer et al., 2003). Hence mineral oil refinement targets the removal of these type of aromatics. For clarity, PAC include polycyclic aromatic hydrocarbons (PAH) and heteroatom ring systems that contain S, N and O; with no (unsubstituted) or low degree of alkylation (Achten and Andersson 2015; Andersson 2009).

Because of the physical-chemical properties of mineral oils, which are tailored through the manufacturing process, they are versatile substances that enable their use in an array of industrial and consumer applications including greases and lubricants, metal working fluids, in thermoplastic elastomers, adhesives, printing inks and cosmetics formulations, pharmaceuticals and vaccine adjuvants, and in agriculture as dust suppressing agents and spray oils (EFSA 2012).

These uses require mineral oils of different physical chemical properties such as viscosity and aromatic content. The latter is an essential element of the type of mineral oil that is selected for the intended use. For example, a mineral oil in printing ink requires a certain level of aromaticity to aid solvency of other components in the formulation,

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[1] Also referred to as “other lubricating base oils” under CONCAWE petroleum substance categories.

https://doi.org/10.1016/j.yrtph.2022.105193
Received 26 November 2021; Received in revised form 31 March 2022; Accepted 19 May 2022
Available online 23 May 2022

while sensitive applications such as pharmaceutical, cosmetics or food contact applications require a mineral oil of high purity (e.g. pharmacopeia grade) that has very low levels of aromatics. These very pure mineral oils are often called white mineral oils or liquid paraffins, which are part of the "Highly Refined Base Oil (HRBO)" category as per CONCAWE petroleum substance categories.

It should be emphasized that the reference to "aromatics" present in non-carcinogenic mineral oils is a broad term and does not refer to the 3–7 ring PAC, associated with hazardous properties. Rather, "aromatics" in refined mineral oils (i.e. lubricating base oils and white mineral oils) include those non-carcinogenic constituents consisting of aromatic structures with multiple long alkyl side chain substituents and in the case of white oils mostly highly alkylated 1 or two ring aromatics (Carrillo et al., 2019). The lack of distinction between the general term *aromatics* and the more precise *hazardous 3–7 ring PAC* has led to the popular misconception that all "aromatics" found in a mineral oil are somehow hazardous and similarly toxic, followed by the erroneous conclusion that a mineral oil containing aromatics is not safe, even if it is refined.

This assumption has been core of the "MOAH" issue over the last decade. Mineral oil aromatic hydrocarbons – MOAH – was a term coined in 2009 to name the aromatic fraction obtained from the chromatographic analysis of sunflower (and other) oils contaminated with mineral oil (Biedermann et al., 2009; Biedermann and Grob 2009a, 2009b). Given its complex composition and lack of distinction between aromatic sub-classes, it was assumed that some of the MOAH might be of carcinogenic concern.

Thus, the scope of this paper is to clarify the concept of MOAH from the industry perspective and to review the current knowledge of its toxicological properties. This will aid the understanding of what mineral oils are and how their safety is ensured.

2. Manufacturing of mineral oil

Petroleum substances, including mineral oils, consist of thousands of different types of hydrocarbons (alkanes and aromatics) and are described as complex substances of 'unknown or variable composition, complex reaction products or biological materials', or shortly "UVCBs" under EU REACH regulation. In the EU, by law, "mineral oils" are UVCBs and not mixtures (EU 2008; Rasmussen et al., 1999). In this sense, a mineral oil substance would for example, be considered a mixture when a mineral oil is intentionally mixed with additives to produce for instance an engine oil or a printing ink. Thus, a mineral oil is rather a matrix in which hydrocarbon constituents follow a physical chemical pattern varying according to crude oil and controlled manufacturing specifications, which results in a single entity with its own intrinsic properties behaving as a (complex) substance (CONCAWE 2017).

Mineral oils are thus petroleum derived substances, produced by refining crude oil via distillation processes (Fig. 1). Firstly, the atmospheric distillation yields a "long residue" which is then further processed by vacuum distillation at temperatures between ~300 °C and ~700 °C. Vacuum distillation is necessary to prevent cracking of the long residue at temperatures above 300 °C which is the feedstock necessary for the manufacturing of "mineral oils". Because the feedstock for mineral oil production contains unwanted hazardous 3–7 ring PAC, these must be removed by further, specific refining processes. There are different refining techniques, used solely or in combination, designed to eliminate these unwanted constituents from mineral oils. The most common processes for their elimination are solvent extraction and catalytic hydrotreatment. These techniques are based on the principles of either removing (extraction) or saturating (hydrotreatment) the potentially hazardous 3–7 ring PAC with no or limited alkylation that are associated with carcinogenicity, mutagenicity, systemic and developmental toxicity (Agarwal et al. 1985, 1988; Carrillo et al., 2019; Feuston et al., 1994; Gray et al., 2013; Kamelia et al., 2019b; Mackerer et al., 2003). When undergoing the solvent extraction process, the extracted hazardous 3–7 ring PAC are concentrated into a separate stream called "aromatic extract" which is carcinogenic (Doak et al., 1985). The solvent extracted (or hydrotreated) long residue yields a "waxy raffinate" which is the refined feedstock for "paraffin wax" and "mineral oil" production. Its main hydrocarbon constituents include normal, iso- and cyclo-alkanes (normal paraffins, iso-paraffins and naphthenics, respectively).

Normal paraffins in a mineral oil are undesirable constituents because they affect technical performance at low temperature, therefore de-waxing (removing of n-alkanes) of the waxy-raffinate is necessary. This can be achieved either through use of solvent or catalytic processes. Solvent de-waxing yields two refinery streams: a "slack-wax" and a "lubricant base oil" (LBO) often referred to as "base oil" or "mineral oil". The former is the feedstock used to produce paraffin waxes.[2] Whilst they are of mineral origin, paraffin waxes consist primarily of n-alkanes, which are solid at room temperature and thus are not oils.

Lubricating base (mineral) oils differ in their composition from paraffin waxes in that the main alkane constituents are iso and cycloalkanes because n-alkanes have been significantly eliminated by dewaxing. They may still contain aromatics often causing confusion in their hazard interpretation. If the hazardous 3–7 ring PAC were effectively removed during refinement, the mineral oil will pass the required carcinogenicity regulatory test, IP346, the oil is not classified as carcinogenic in the EU under CLP note L (CONCAWE and Ellison, 1994; CONCAWE 2016). The non-carcinogenic mineral oil may still include some 1–7 aromatic ring systems (Dalbey et al., 2014; McKee et al., 2013) but these aromatic structures are highly alkylated and as explained further on, of low toxicological concern (Wang et al., 2020; Wang et al.; Wang et al., 2021). If base oils are further processed by for example, hydrogenation, the remaining aromatics are converted into cycloalkanes (naphthenics) so that in the resulting highly refined base oil (white oils) the remaining aromatic structures are predominantly 1–2 ring highly alkylated rings with only trace levels of PAC. Highly refined base oils are considered of "medicinal grade" if the PAC levels meet pharmacopeia purity requirements, typically in ppb levels (EDQM 2019; FDA, 2022a).

In summary, the generic term "mineral oil" may refer to the liquid oil fractions obtained at temperatures between ~300 °C and ~700 °C from vacuum distillation without specific information of refining processes, purity or health hazards. There are about 40 substances (each with its own identifier or

CAS number) which could be regarded as members of the mineral oil family, all differing in their physical chemical properties (e.g. viscosity) and chemical composition, including aromatic content. Aromatic constituents in mineral oils can be either hazardous or non-hazardous. Because of the mineral oil boiling point of >300 °C, the potentially hazardous aromatic constituents consist of 3–7 polycyclic aromatic compounds (PAC) with naked or limited degree of alkylation, while the non-hazardous encompass 1–7 ring aromatics with high degree of alkyl substitution or lack of bay or fjord regions. Benzene or naphthalene are not present because of their lower boiling points (BP = 80 °C and 218 °C, respectively) and are, therefore, excluded by the manufacturing conditions. Under these conditions the initial BP of 300 °C dictates that the first 3 ring PAC that may be present is phenanthrene (BP = 340 °C). All types of aromatics that may be present in mineral oil are often referred to as MOAH (Biedermann and Grob 2009a; Warentest 2015), without any distinction on their ring number, hazard profile or refinement history. The most reliable way to assess the hazardous properties of "MOAH" is by testing the DMSO extract of a mineral oil in either the IP346 or the modified Ames test. It has been clearly shown experimentally that a DMSO will selectively extract the potentially hazardous 3–7 ring PAC (Carrillo et al., 2019; CONCAWE and Ellison, 1994;

[2] Catalytic de-waxing aims at isomerizing n-paraffins into iso-paraffins and results in increased base oil production. It does however not yield any slack-wax stream.

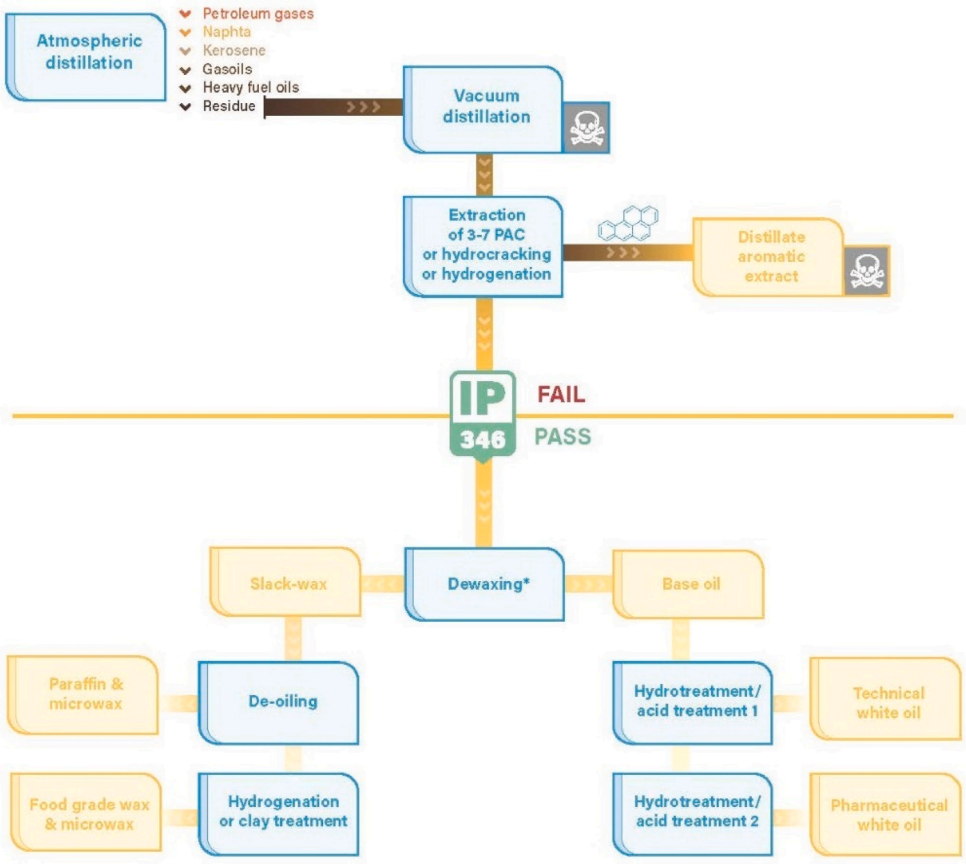

Fig. 1. Manufacturing process of mineral oils and paraffin waxes (CONCAWE 2017). The vacuum distillation "long residue" is carcinogenic and therefore the potentially carcinogenic 3–7 ring polycyclic aromatic compounds (PAC) need to be lowered to such a level that it can pass the IP346 test which indicates that the feedstock is safe and can be further process into oils and waxes. PAC include PAH and heteroatom multi-ring systems. * Dewaxing is not necessary for manufacture of naphthenic base oils and/-white oil oils.

CONCAWE 2016). For the purposes of this paper and in the context discussed so far, the following terms will be used:

- **Mineral oil:** generic term that encompasses "lubricating base oils" or "highly refined base oils".
- **Lubricating base oil (LBO):** refined and dewaxed mineral oil, which in the EU complies with IP346 (i.e. DMSO extract is <3% w/w).
- **IP346 test:** a gravimetric method in which an oil sample is dissolved in cyclohexane and extracted twice with DMSO. The combined extracts are then used to determine the total polycyclic aromatic compounds (PAC) content in the sample gravimetrically, including 3- to 7-ring PAC and other polar extractable material. The result is expressed as the weight percentage (% w/w) of DMSO extractables PAC in an oil sample. A good correlation exists between % DMSO extract by IP346 and tumor incidences obtained in the mouse skin painting carcinogenicity study of mineral oils (CONCAWE and Ellison, 1994; 2016). Hence, % DMSO extract by IP346 method can be used for the prediction of the carcinogenic potential for mineral oils. The samples are considered non-carcinogenic if % DMSO extract as measured by IP346 is less than 3% w/w (i.e. gravimetric cut-off value of <3% w/w).
- **Highly refined base oils (HRBO):** also known as "white oils" are obtained from a base oil after generally one (for technical grade) or two (for medicinal grade) additional refinement steps by either hydrogen or acid treatment. Technical white oils comply with FDA § 178.3620(b) (FDA, 2022c). Medicinal grade white mineral oils have lower aromatic levels than technical white oils and must therefore comply with EU pharmacopeia purity standards (EDQM 2016) or US FDA 21 CFR §172.878 for direct food contact use (FDA, 2022a), or §178.3620(a) for indirect food contact (FDA, 2022b).
- **PAC:** polycyclic aromatic compounds which include heteroatom ring structures and polycyclic aromatic hydrocarbons (PAH). An equivalent term is polycyclic aromatics (PCA). Both are used interchangeably in literature.
- **3–7 ring PAC:** potentially hazardous 3–7 ring PAC with limited or no alkyl substituents which can be selectively extracted from a mineral oil by a polar solvent such as furfural, NMP or DMSO. Not all the DMSO extracted 3–7 ring PAC are necessarily hazardous, but they all show high affinity towards DMSO and a high correlation to carcinogenicity in the IP346 and modified Ames test (Blackburn et al., 1986; CONCAWE 2016; IP 1996). They have also been shown to correlate well to systemic and developmental toxicity (Feuston et al., 1994)
- **Non-hazardous PAC:** highly alkylated 1–7 ring PAC which are found in the refined oil after removal of the hazardous 3–7 ring PAC. These show low or no affinity towards DMSO (Carrillo et al., 2019).
- **DMSO extract: DMSO** selectively extracts >3 ring aromatics with no or limited number of substituents per molecule. Substituents have few carbon numbers (short chains). Heteroatom PAC are also found in the DMSO extract as well as other polar material (Carrillo et al., 2019).
- **MOAH:** mineral oil aromatic hydrocarbons (MOAH) give an indication of the total aromatic content of a sample by encompassing a chromatography fraction any type of PAC or alkylated 1–2 ring aromatics after its separation from the mineral oil saturated hydrocarbon (MOSH) chromatographic fraction. Consequently MOAH is not a substance on its own, but rather an unresolved chromatography hump with hardly any signal on top obtained by the instrumentation method. (Biedermann and Grob 2012). Its composition may be elucidated by two dimensional chromatography (GCxGC) (Biedermann and Grob 2009a). Therefore, no default hazard interpretation can be attributed to a measured MOAH fraction.

3. Screening tests for carcinogenicity

3.1. IP346

The gold standard for the assessment of the carcinogenic potential of mineral oils is the *in vivo* mouse skin painting study. This mouse skin reproduces the same type of skin tumors as seen in humans when exposed to carcinogenic oils (Cruickshank and Squire 1950; Henry 1946; Smith et al., 1951). For the hazard assessment of all possible exposure routes, dermal testing in the mouse model is ideal and is regarded as worse-case scenario. Mouse skin is a more sensitive model than models with other routes of exposure to 3–7 ring PAC- mediated carcinogenicity due to higher CYP1 expression but lower epoxide hydrolase activity (Oesch et al., 2014), providing maximum bioactivation and minimal elimination of reactive metabolites. This bioassay is time consuming (>2 years), expensive and uses a high number of animals. Therefore, a rapid screening method, IP346, with high predictivity for carcinogenicity was developed by the industry and adopted in the European and Australian regulation (Carrillo et al., 2019; IP 1996; NICNAS 2020). The IP346 is a rapid gravimetric DMSO extraction method which is highly selective towards the 3–7 ring PAC. Over 100 mouse skin painting studies using different mineral oils were performed with concomitant IP346 tests on the same oils. The correlation between the results of the two assays (tumor incidence caused by the oil in a mouse skin painting carcinogenicity study vs. the oil's gravimetric determination of the DMSO extract) showed the predictability of the carcinogenic potential of a mineral oil with 94% accuracy using a pass/fail assessment based 3% DMSO extract by the IP346 assay (CONCAWE 2016). Although the carcinogenicity data base behind the IP346 method includes DMSO extract/mouse skin painting study pairs of several vacuum distillate substances other than lubricating base oils, the 3% cut off value of IP346 is only applicable for virgin lubricating base oils (IP 1996). The IP346 method is legally binding in the EU for classification and labelling of lubricating base oils (EU 2008) and is currently the only analytical method ever developed for the prediction of the carcinogenic potential of mineral oils. The established gravimetric cut of value of <3% w/w DMSO extractables to consider a non-carcinogenic oil has also recently been independently evaluated and considered adequate (BfR 2018).

In practice the IP346 test can be applied in situ to determine whether the established refinement process to decrease the levels of 3–7 ring PAC is adequate to deliver non-carcinogenic mineral oils and related products (see Fig. 1).

3.2. Modified Ames test

Like the IP346, the modified Ames test is also based on a DMSO extraction step that concentrates the potentially hazardous 3–7 ring PAC. The term "modified" refers to the modifications to the standard Ames test to render a more sensitive test towards PAC. Testing petroleum substances in the standard Ames test resulted in false negative and to avoid negative results for substances, which were clearly carcinogenic in mouse skin painting studies, the modified Ames test was developed (Blackburn et al., 1986). These modifications included 1.) the use of only the TA98 salmonella typhimurium strain because it is more specific and selective towards PAC, 2.) the use of hamster instead of rat liver S9 for metabolic activation in combination with an increased concentration of activation cofactor, NADP (from 4 mM to 8 mM) that together would significantly increase the bioactivation of PAC; and, most importantly, 3.) the use of DMSO to extract and concentrate the PAC from the test sample. The DMSO extract will ensure that the bacteria in an aqueous environment are exposed to the mutagenic constituents of the oil. When this extraction step is omitted by testing the neat oil, the hydrophobic petroleum substance will not mix with the hydrophilic culture media and result in no exposure to the bacteria. This is why oils test false negative when assessed in the standard OECD 471 Ames test protocol.

Additional to IP346, screening for carcinogenicity can be performed using Modified Ames test for two petroleum categories, namely Lubricant Base Oils and Residual Aromatic Extracts. For these two categories, relationships between Modified Ames test and *in vivo* mouse painting studies have been established. For the substances of these categories, the calculated mutagenicity index (MI) is not only indicative of mutagenic activity but also of carcinogenic potency as it is correlated to the tumor incidence and latency from mouse skin painting study data base (Blackburn et al., 1986). A MI of $\geq$1 is indicative of mutagenic and carcinogenic potential of the tested samples (Mackerer et al., 2003; Roy et al., 1988). This modified Ames test is the standardized industry mutagenicity method for base oils designated as ASTM E 1687 (ASTM 1995). Its applicability domain has been extended to residual aromatic extracts (RAE) with a lower MI cut off, where an MI > 0.4 is indicative of carcinogenic potential (CONCAWE et al., 2012). For other petroleum categories, like UATO and DAE, Modified Ames test can only predict the mutagenicity potential of the substances and the MI does not screen for carcinogenicity.

It has been demonstrated that the PAC composition of a DMSO extract from petroleum substances with final boiling points >340 °C can be used to reliably predict (94% accuracy; 90% sensitivity and 100% specificity) the outcome of a modified Ames test, which indicates that the aromatic constituents responsible for mutagenic effects of mineral oil are encompassed in its DMSO extract (McKee et al., 2013).

In summary, the DMSO extract of an oil assessed in the IP346 and the modified Ames test is an indirect way of assessing its carcinogenic potential. It may be argued that the non-DMSO extractable aromatics may be of concern because it is not assessed in either test. However, it must be emphasized that it is not the overall measurement of aromatics which shows good correlation to *in vivo* data but the DMSO extract because it concentrates the 3–7 PAC which were contained in the oil when it was tested in neat form in carcinogenicity studies. The corollary is that those aromatics not extractable in DMSO which do remain in the oil are directly assessed when the oil is tested *in vivo*. If the oil shows no carcinogenic activity *in vivo*, it is an indication that its aromatic constituents are of low concern (discussed further in this paper).

4. Type of aromatics found in mineral oil – PAC vs total aromatics in MOAH measurements

As indicated earlier, there are two types of aromatic constituents found throughout the process of mineral oil refining. Firstly, the 3–7 ring PAC linked to toxicity and secondly, those aromatics that remain in the oil after the former have been removed. The remaining aromatics are expected to be predominantly highly alkylated PAC. Those are metabolized by side chain oxidation eliminating their mutagenic potential (see section 6). It has been demonstrated that DMSO can discriminate between these two aromatic types (Carrillo et al., 2019). The assessment of "aromatics" in a mineral oil must, therefore, distinguish between these two aromatic types so that health hazard assessments and screening tests are tailored to selectively target the aromatics of concern (i.e., hazardous 3–7 ring PAC). For this purpose, the DMSO oil extract is used in the IP346 assay and the modified Ames test. Hence, an assessment which is based on "aromatics" may be taken out of context and lead to wrongful assumptions about the hazardous properties of mineral oil.

For example, the chromatography method, originally developed to analyze sunflower oil contaminated with mineral oil, identifies two fractions: mineral oil saturated hydrocarbons (MOSH) and the aromatic fraction MOAH (Biedermann et al., 2009; Biedermann and Grob 2009a, 2009b). Following this method, the MOAH content of the mineral oil contaminating the vegetable oil could be up to 25% (Biedermann and Grob 2009a).

However, for the interpretation of MOAH and as shown in Fig. 2, there should be a qualitative difference between the MOAH found in a lubricating base oil (Fig. 2A) and the MOAH found in a distillate aromatic extract – DAE (Fig. 2B) (Biedermann and Grob 2009a). While both contain "MOAH", the aromatics found in lubricating base oil (~26%)

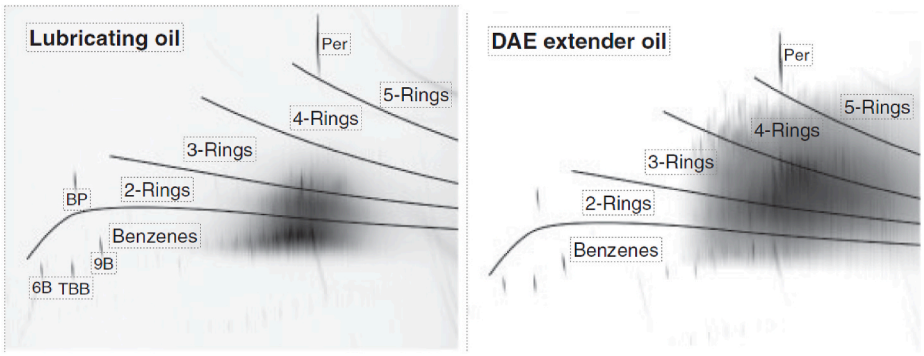

Fig. 2. GCxGC-FID plots of MOAH LC fractions A) The lubricating base oil contained an estimated 26% MOAH consisting mostly of alkylated benzenes and 2-ring aromatic compounds. **B)** The distillate aromatic extract DAE oil shows a broader MOAH composition of 1–5 rings, with 85% MOAH content. Internal standards shown are hexyl benzene (6B), nonyl benzene (9B), perylene (Per), 1,3,5-tri-tert. Butyl benzene (TBB) and biphenyl (BP). Adapted from (Biedermann and Grob 2009a).

consist predominantly of alkylated 1–2 aromatics because the 3–7 ring PAC were removed by refinement and subsequently concentrated in the DAE (discussed previously in section 2 and shown in Fig. 1) which has a MOAH content of ~85%. Apart from the clear difference of "low"/"-high" MOAH content of base oil and DAE, respectively, base oils that pass the IP346 or the modified Ames test are not carcinogenic even if the MOAH content is, for example, 26%. DAE is carcinogenic not because of "high MOAH" content but because it contains the 3–7 ring PAC extracted from the base oil feedstock, which constitute the bulk of the MOAH fraction. In fact, when the DAE was hydro-treated to such an extent that its 3–7 ring PAC content is significantly decreased, it can be converted to a non-carcinogenic extender oil with still very high (74%) aromatic content (Doak et al., 1985), which is fundamental for the intended use in e.g. tires. Hence, MOAH content *per se* is not indicative of hazard but rather a measure of total aromatic content, which may or may not contain hazardous PAC depending on the oil's refinement level assessed by IP346 or modified Ames Test.

For example, the GCxGC-FID plots of the MOAH LC fraction in Fig. 2 provide a qualitative compositional analysis of the MOAH present in two oil samples, but because we don't have the IP346, or MI values of the samples visualized herein we don't know how to interpret MOAH in relationship to these hazard indicators. This prompted us to investigate how refinement affects MOAH content compared to its corresponding IP346 and modified Ames values. We have thus assessed the entire mineral oil refinement process using these parameters. This process is presented and discussed in the next section.

5. PAC and MOAH levels at different manufacturing steps

Information on refining history gives insights into the interpretation of MOAH values as this knowledge allows a better understanding whether the measured aromatics are of concern or not.

This is exemplified by a series of measurements, where each intermediate refinement step starting from a vacuum distillate aimed to become SN600[3] base oil and further refined into a medicinal white oil was tracked for MOAH content by LC-GC-FID and with the corresponding IP346 value and/or MI by the modified Ames test where applicable. The values obtained from UVCB substances are not to be taken as absolute and may slightly vary based on the precision of individual assays but are nonetheless highly indicative of typical results and directional relation (Fig. 3).

The SN 600 vacuum distillate from which oils (and waxes) will be derived has not yet been cleared from the potentially hazardous 3–7 ring PAC. This substance belongs to the category of Unrefined/acid treated oils (UATO) which is classified as carcinogenic due to positive findings in mouse skin painting studies. Yet the mutagenic potential of UATO has been extensively studied *in vivo* and *in vitro* (API 1976; ARCO 1987; Blackburn et al., 1986; Blackburn et al., 1984; Przygoda et al., 1999) and based on the data, UATO is not considered to be a germ cell mutagens (CONCAWE 2021). MOAH content of the SN 600 vacuum distillate is about 35% and The MI value is < 1 (0.83 with a 95% confidence interval between 0.64 and 1.03, p-value < 0.001) which is in line with the classification and labelling of the category of unrefined/acid treated oils to which the SN600 distillate belongs (non-mutagenic).

After extraction of the 3–7 ring PAC from the vacuum distillate, these aromatics become concentrated in the distillate aromatic extract (DAE) which shows a concomitant MOAH increase up to 60%. No accompanying IP346 or modified Ames test was conducted as DAE are not in the applicability domain of IP346 and empirically are known to be carcinogenic and mutagenic due to the refinery process of concentrating 3–7 ring PAC (Doak et al., 1985).

While the extraction of PAC from the SN600 distillate into the DAE resulted in a significant increase in MOAH content of the DAE, the resulting waxy raffinate shows a reduction in aromatic content of MOAH (11%.) The MOAH in this product is not of concern because both the IP346 test and the modified Ames assay results are below the respective cut off levels of 3% and 1.0, respectively. Dewaxing (removal of the wax constituents from the waxy raffinate to produce base oil) may explain a slight increase in the MOAH content of the base oil (MOAH = 13%), because of a "concentration" effect of all aromatics encompassed in this fraction. This "increase" in MOAH does not have any effect on IP346 or MI values because these measurements are a reflection of only those PAC that can be extracted by DMSO in contrast to MOAH which is a "catch all" measurement. We observe a significant decrease in MOAH level after a first hydrogenation step (acid treatment samples were not tested, but expected to be similar) of the base oil to produce a technical white oil (MOAH = 3%), the accompanying IP346 and MI values remain equivalent to those of the base oil despite the 4-fold decrease of the MOAH content. Technical grade white oil will typically comply with the US FDA 21 CFR § 178.3620 (b) requirements containing low levels of PAH measured by UV absorption (FDA, 2022c), but not low enough to be regarded as of "medicinal grade". A second hydrogenation step (or acid treatment) delivers a medicinal grade white oil, which have a MOAH content of 0.05%. The IP346 method was not performed on the medicinal grade white oil because at this level of oil refinement the DMSO extractable material is negligible and thus at the limit of the

[3] *"Solvent Neutral" relates to categories of lubricating base oils (mineral oils) further defined by their viscosity at 100°F in Second Saybolt Universal (SSU) unit. SN600 relates to a base oil having a viscosity ~600 SSU at 100°F, which means in the range of ~110 to* 120 mm^2/s *at 40°C. The term is further extended to intermediate refinery cuts (distillates, raffinates) used to produce base oils of the designed viscosity.*

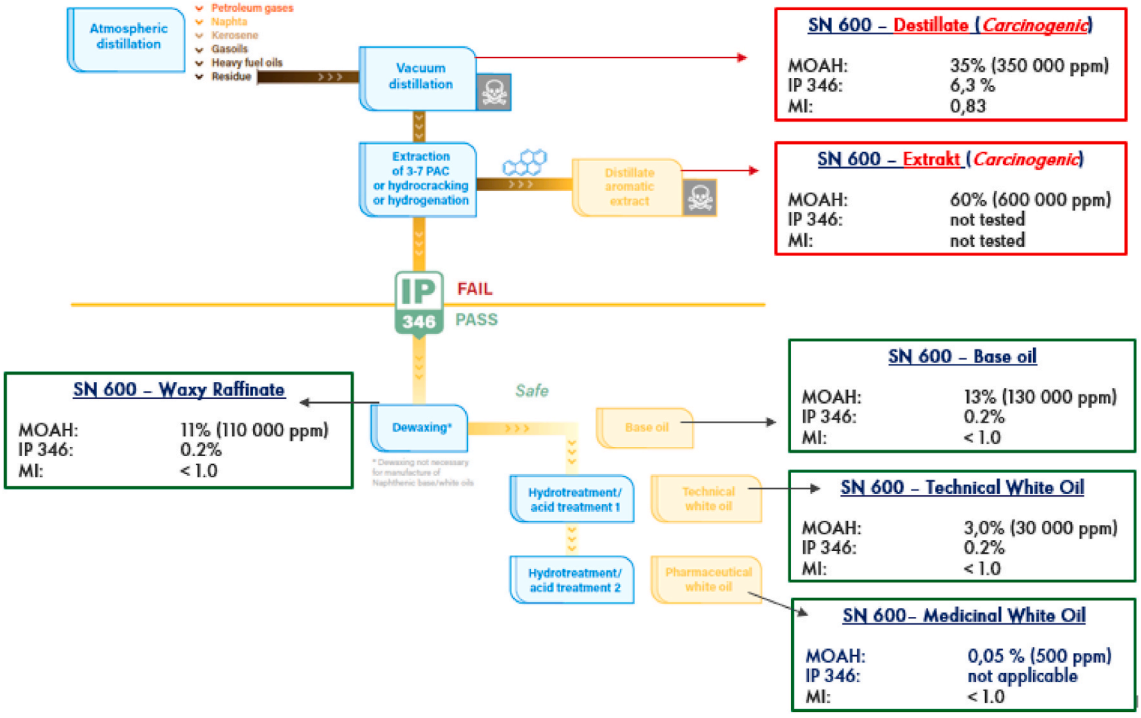

Fig. 3. SN600 mineral oil cut production line evaluated for mineral oil aromatic hydrocarbons (MOAH) content with corresponding IP346 and mutagenicity index (MI) using the modified Ames Test.

method applicability; literature values for IP346 in these type of oils indicate values close to zero (CONCAWE 2016). Accordingly, the obtained MI indices are also practically zero. These highly refined oils are regulated according to their PAC content measured using UV-DMSO methods (Haenni et al., 1962) as stipulated in the EU pharmacopeia monograph (EDQM 2019) or FDA (FDA, 2022a). Although these purification steps do decrease MOAH levels, 1–7 ring aromatics may still be present at ppm levels even if the 3–7 ring PAC have been decreased to negligible ppb levels.

For comparison, lower viscosity SN100 refinery products were also evaluated at different refinement steps, focusing primarily on MOAH and IP346. Modified Ames test was only done for the base oil, and subsequent technical and white oils because these were, at that time, the products of interest (Table 1). However, MI values for a similar viscosity products (SSU 130) distillate, DAE, Waxy raffinate have been reported as 5.1; 9.3; and 0.2 respectively (Dalbey et al., 2014). In our SN100 samples, MOAH values vary according to the refining steps as observed for in the SN600 case. Removal of 3–7 ring PAC delivers oils that pass IP346 and have mutagenicity indices < 1.0, despite having up to 10% MOAH. It was observed that at equivalent refinement level, MOAH in SN100 products tended to be lower than the SN600 counterpart. The effect of oil viscosity on MOAH is discussed in the next section.

Table 1
Comparison of MOAH % content in two different refinery cuts for mineral oil production at each stage of the production process. Corresponding IP346 and MI values are presented where applicable or available. Viscosity at 100 °C in cSt (ASTM D445) for SN600 and SN100 determined as 11 and 4.1, respectively.

Type of mineral oil or wax	SN 600	SN 100	SN 600	SN 100	SN 600	SN 100
	MOAH (%)[a]		IP346 (%)		MI	
Distillate	35	30	6.3	6.6	0.83	n.t.
DAE	60	60	n.t.	n.t.	n.t.	n.t.
Waxy raffinate	11	8	0.2	0.5	0.03	n.t.
Slack wax	5	1	n.a.	n.a.	n.a.	n.a.
Base oil	13	10	0.2	0.4	0.00	0.1
Technical White oil	3	2	0.2	0.2	0.1	0.06
Medicinal White oil	0.05 (500 mg/kg)	0.025 (250 mg/kg)	n.a.	n.a.	0.00	0.02

n.t. – not tested.
na. – not applicable.
[a] MOAH by LC-GC-FID.

6. Influence of viscosity over MOAH

As shown before, MOAH content in mineral oils changes at defined refining stages. However, because of mass-based calibration of the method, the molecular weight of the mineral oil aromatic constituents has a direct influence on the MOAH result. Because the bulk of the MOAH fraction consists of highly alkylated aromatics which are calibrated to similar molecular weight MOSH constituents, higher viscosity oils tend to give higher MOAH values when compared to lower viscosity oils with the same refinement level. This is because high viscosity reflects high molecular weight oil constituents and thus necessarily longer alkyl side chain substitution on the aromatic rings. For this reason and as shown in Table 1, MOAH values for SN100 oil are lower than for the heavier cut SN600.

Given the importance of viscosity when interpreting MOAH levels in an oil, we conducted an array of parallel measurements to characterize the different types of aromatics present in oils with different viscosities. Three additional European medicinal grade mineral white oils (EDQM 2019) with different viscosities and distillation profile were assessed. Based on their kinematic viscosity (at 100 °C) and JECFA designation (JECFA, 2002) these were Class 1, 2, and 3 mineral oils represented specifically by P90H, N70H, and P20H oils, respectively. The nomenclature of these oils indicates firstly their Paraffinic (P) or Naphthenic (N) nature of crude oil origin, then their approximate kinematic viscosity measured in centistokes (cSt) at 40 °C, and lastly the last processing step they underwent, which for these white oils is hydrogenation (H) (Smith et al., 1996). The aromatic assessment included: 1.) MOAH carbon number blocks by LC-GC-FID, 2.) their characterization by comprehensive two dimensional GCxGC-TOF/MS 3.) aromatic proton

content by ^{1}H-NMR spectroscopy, and 4.) total aromatic content by UV, and 5.) measurement of the eight PAHs in consumer articles as controlled in the EU.[4] These medicinal grade mineral oils are compared to a gas to liquid (GTL) oil, which is of synthetic origin and has equivalent properties but virtually no aromatics serving as negative control and a low melting point (LMP) microcrystalline wax which was also included to emphasize the role of high molecular weight petroleum products in MOAH assessment.

Higher viscosity is correlated with higher molecular weight and longer carbon numbers of the alkyl substituents on the aromatic structures (Table 2). Therefore, viscosity is also reflected in the molecular weight of MOAH constituents found in a mineral oil. For example, in the P90H oil, at 5% distillation point the carbon number is C_{28}, so that the MOAH content measured at the C_{24} carbon number is below the detection limit, indicating that there is virtually no MOAH $\leq C_{24}$. There is, however, MOAH in the upper carbon number ranges C_{24}– C_{35} and $> C_{35}$ consistent with the oil's carbon number at the 5% distillation point. Even though the highest carbon number block is indicated as $> C_{35}$ - $\leq C_{50}$, this does not necessarily mean that there will be MOAH across this entire range; this will depend on the oil's upper distillation point such as in the case of other heavier Class I oils, where MOAH may be present up to C_{45}. On the contrary, due to its lower viscosity, the P20H oil contains a relatively higher level of MOAH constituents below C_{24}. This is consistent with its C_{20} carbon number at 5% distillation point and thus also reflected in a lower average molecular weight and shorter alkyl chain substituents of its MOAH structures. As consequence of its lower molecular weight, it also shows a 10-fold lower amount of $> C_{35}$ MOAH constituents compared to class I oils. Finally, Class II oils are in between regarding viscosity, carbon number and MOAH carbon number distribution. Although we see a clear trend between higher molecular weight correlated with higher MOAH values and vice versa, the MOAH value of the N70H oil, which has viscosity, average molecular weight, and initial carbon between the P90H and P20H oils, is not an intermediate value but lower than the less viscous P20H oil. This is not likely due to an incomplete separation between the MOSH and MOAH fractions. Naphthenic constituents (which are proportionally higher in a naphthenic oil) elute last in the MOSH fraction and should not co-elute with the MOAH fraction when the MOSH tailing is minimized by LC pre-separation of MOSH as applied in this analysis. (Biedermann and Grob 2009a). Thus, we conclude that the lower MOAH content of the naphthenic oil is not likely due to incorrect fractionation but rather the specific manufacturing conditions of this particular N70H sample compared to the paraffinic oils. Some of the identified MOAH molecules in all mineral oils included alkylated 1–2 aromatic ring systems some of which were partially hydrogenated derivatives of former 3 ring systems such as octahydro anthracene/phenanthrene, indicating that aromatic ring number and total ring number should not be confused.

A GTL oil included as negative MOAH control, has similar physical-chemical characteristics but is virtually devoid of MOAH since it is manufactured by chemical synthesis and thus its PAC and alkylated aromatics levels are below the detection limit or absent.

Still, the effect of molecular weight on MOAH content is best exemplified with the microcrystalline wax, that has high viscosity, molecular weight and carbon numbers that result in a MOAH content of ~5% consisting entirely of $> C_{35}$ aromatic molecules. This "high" MOAH value is better interpreted with the help of aromatic content analysis by H^{1}-NMR which indicates that only 0.4% of the MOAH molecules are aromatic protons. This is confirmed by GCxGC analysis which shows that the MOAH fraction consists predominantly of 1 ring aromatic and partially saturated 2–3 ring structures with long alkyl chains in the C_{35}–C_{50} range (e.g. alkylated benzene and tetrahydro naphthalene; alkylated tetrahydro and octahydro anthracene/phenanthrene). These MOAH molecules must necessarily have "high" alkyl chains (i.e. highly alkylated) in order to be present in a substance with such a high overall molecular weight and C_{36} carbon number at 5% distillation point. The EU 8 PAH content on the other hand, given the refinery history, is only at trace ppb levels. It has been shown that a DMSO extract of refined mineral oils analyzed for aromatic ring class (ARC) profile (wt% of each aromatic ring number) shows minimal amounts of 3–7 PAC (Dalbey et al., 2014; McKee et al., 2013), which indicates that, based on GCxGC data, the aromatic bulk is 1–2 ring aromatics including partially hydrogenated ring systems.

Therefore, the MOAH measured in highly refined mineral oil and wax is not PAC, let alone 3–7 ring PAC, but as evidenced by GCxGC, highly alkylated aromatics mostly with 1 and 2 rings, which are integral part of the UVCB substance and influenced by the carbon range distribution and molecular weight of the alkane constituents in the oil or wax. Thus, to make this distinction, the pharmacopeia UV method uses a DMSO extraction step which concentrates the 3–7 ring PAC (EDQM 2019), so that the measurement reflects the toxicologically relevant aromatic fraction and independent of MOAH molecular weight.

7. Hazard of alkylated vs non-alkylated aromatics

7.1. Carcinogenicity and mutagenicity

PAHs acknowledged by the US-EPA and European regulators to represent well-known and extensively studied group of PAHs (listed in Table 3) in relation to their mutagenicity and carcinogenicity potential as identified by IARC, for which some are known carcinogens based on animal studies (IARC 2010; WHO 1998). According to IARC, only 5-ring benzo [a]pyrene (BaP) is classified in group 1 (carcinogenic to humans), and some other PAHs including benz [a]anthracene (BaA), chrysene, dibenz [a,h]anthracene, benzo [b]fluoranthene, benzo [j]fluoranthene, benzo [k]fluoranthene, are classified in group 2A/B (IARC 2010) (see Table 3).

Some of these PAH may be present as part of an oil's DMSO PAC extract (Mehrotra et al., 1987), which encompasses aromatic structures, whose structures and alkylation degrees modulate their toxicity potency (Lavoie et al., 1985; LaVoie et al., 1981).

A great deal of knowledge has been gathered to highlight the importance of genotoxicity as a function of PAH chemical structure (Luch 2009). The configuration of naked PAH ring system to form so called "bay" and "fjord" regions play crucial role in determining the potency of substance when these are bioactivated by CYP enzymes (e.g. CYP1A1 and 1B1) to form diol-epoxides which are the ultimate carcinogenic species that directly react with genetic macromolecules forming DNA adducts.

On the contrary, limited toxicological knowledge is available on the less common PAHs, including their alkylated PAHs analogues. This is of relevance since most aromatics present in MOAH fractions of refined oils are, in fact, highly alkylated and, depending on the degree of refinement, they may also be partially hydrogenated as reported earlier. Published studies on mutagenicity and carcinogenicity potency of unsubstituted and alkylated 3–5 ring PAHs showed that alkyl substitution on the aromatic ring may change the toxicity potency of the respective parent PAHs (Baird et al., 2007; Iyer et al., 1980; LaVoie et al., 1983; Santella et al., 1982; Utesch et al., 1987). BaPs with two methyl substitutions (7,8-dimethyl-BaP and 7,10-dimethyl-BaP) were also less active when compared to the parent BaP.

Depending on the position of the alkyl substitution, some monomethylated BaPs are more mutagenic than their parent (unsubstituted) BaP in the Ames test that included S9 metabolic enzymes (Santella et al., 1982). Of all methylated BaP tested, 6-methyl-BaP is the most mutagenic on TA100 *Salmonella typhimurium* strains, followed by 11-methyl-BaP and BaP. Lower mutagenic activity was observed when the methyl group was on the 7-, 8-, 9-, 10 position of the BaP ring system.

As explained earlier, mutagenic activity of PAH requires that the multi-ring system forms a bay or fjord region-like motif as target for the

[4] EC 1907/2006 Annex XVII, Entry 50.

Table 2
Comparison of different white oil and microwax properties and aromatic content.

JECFA classification	Class I – P90H	Class II – N70H	Class III – P20H	GTL oil (no JECFA classification)	Microwax
Origin	Paraffinic	Naphthenic	Paraffinic	Synthetic	Paraffinic
Viscosity at 100°C in cSt	11	7.9	4.0	4.1	12–17
Viscosity at 40°C in cSt	90	70	20	17	–
Av. Molecular weight g/mol	530	415	360	370	700
Carbon number at 5% distillation point	C_{28}	C_{23}	C_{20}	C_{25}	C_{36}
MOAH (LC-GC-FID)					
≤ C_{24}	<10 mg/kg[a]	30 mg/kg	110 mg/kg	<10 mg/kg[a]	<10 mg/kg[a]
> C_{24} - ≤ C_{35}	150 mg/kg	220 mg/kg	195 mg/kg	<10 mg/kg[a]	<10 mg/kg[a]
> C_{35} - ≤ C_{50}	480 mg/kg	41 mg/kg	49 mg/kg	<10 mg/kg[a]	49 000 mg/kg
SUM MOAH	630 mg/kg	291 mg/kg	354 mg/kg	<10 mg/kg[a]	49 000 mg/kg
MOAH characterization by GCxGC TOF/MS	alkylated tetrahydro naphthalene; alkylated tetrahydro anthracene/ chrysene	alkylated tetrahydro naphthalene; alkylated tetrahydro anthracene/ phenanthrene; alkylated octahydro anthracene/ phenanthrene	alkylated tetrahydro anthracene/phenanthrene; alkylated octahydro anthracene/phenanthrene	–	alkylated benzene; alkylated tetrahydro naphthalene; alkylated biphenyle diphenylmethane or biphenyle
H^{b} -NMR - aromatic proton content	<10 mg/kg[a]	<10 mg/kg[a]	<10 mg/kg[a]	<10 mg/kg[a]	0.4%
UV (SMS 2728-8) total aromatic content	600 mg/kg	70 mg/kg	<5 mg/kg[a]	<5 mg/kg[a]	Not applicable
EU 8 PAH – total ppb (EN16143)[c]	<0.1	<0.27	<0.15	<0.1	<2.1

[a] Below limit of detection <10 mg/kg or < 5 mg/kg. No signals of aromatic protons were detected in the.

[b] H spectra of these samples. To estimate the detection limit two samples comprising mixtures of ethyl benzene and decane were prepared. Concentration of ethyl benzene was 100 and 10 mg/kg.1H spectra of these two samples were acquired. The signals of aromatic signals of ethyl benzene were still visible in the1H spectrum of 10 mg/kg sample. Hence, it was estimated that the aromatic content of base oils is below 10 mg/kg as no signals were detected.

[c] EU 8 Polycyclic-aromatic hydrocarbons (PAH) concerned: (1) Benzo [a]pyrene (BaP) (CAS No 50-32-8); (2) Benzo [e]pyrene (BeP) (CAS No 192-97-2); (3) Benzo [a]anthracene (BaA) (CAS No 56-55-3); (4) Chrysen (CHR) (CAS No 218-01-9); (5) Benzo [b]fluoranthene (BbFA) (CAS No 205-99-2); (6) Benzo [j]fluoranthene (BjFA) (CAS No 205-82-3); (7) Benzo [k]fluoranthene (BkFA) (CAS No 207-08-9); (8) Dibenzo [a,h]anthracene (DBAhA) (CAS No 53-70-3).

Table 3
IARC classification for PAHs acknowledged by US-EPA and EU.

Substance	Ring number	IARC classification[a]
Naphthalene (EPA)	2	2B
Acenaphthene (EPA)	2	3
Acenaphthylene (EPA)	2	No IARC classification
Fluorene (EPA)	2	3
Anthracene (EPA)	3	3
Phenanthrene (EPA)	3	3
Fluoranthene (EPA)	3	3
Benzo [c]fluorene (EU)	3	3
Pyrene (EPA)	4	3
Chrysene (EPA + EU)	4	2B
5-methylchrysene (EU)	4	2B
Benz [a]anthracene (EPA + EU)	4	2B
Benzo [b]fluoranthene (EPA + EU)	4	2B
Benzo [j]fluoranthene (EU)	4	2B
Benzo [k]fluoranthene (EPA + EU)	4	2B
Cyclopenta [cd]pyrene (EU)	4	2A
Benzo [a]pyrene (EPA + EU)	5	1
Dibenzo [a,h]anthracene (EPA + EU)	5	2A
Indeno [1,2,3-cd]pyrene (EPA + EU)	5	2B
Benzo [ghi]perylene (EPA + EU)	6	3
Dibenzo [a,e]pyrene (EU)	6	3
Dibenzo [a,h]pyrene (EU)	6	2B
Dibenzo [a,i]pyrene (EU)	6	2B
Dibenzo [a,l]pyrene (EU)	6	2A

[a] IARC classification group 1: carcinogenic to humans, group 2A: probably carcinogenic to humans, group 2B: possibly carcinogenic to humans, and group 3: not classifiable.

metabolic activation (via oxidative pathway). A bay region or bay-region like motif is the region produced by the angular addition of a benzene ring to a linear portion of a PAH, as illustrated with BaP and 6-Methyl-BaP shown in Fig. 4. Using the study by Santella et al. (1982) as an example, the addition of a methyl group to a different position on the aromatic ring system of BaP, in particular the 6th and 11th position, creates an extra bay region-like motif, resulting in an increased mutagenic response compared to their parent BaP possibly due to a higher formation of reactive metabolites. Methyl substitution, however at some other positions of BaP may act in the opposite direction by inhibiting metabolic activation due to steric hindrance (i.e. stereospecific bio-(de) activation) hampering the substrate's interaction with cytochrome P450 enzymes (e.g. CYP 1A1, 1B1) and thus decreasing their mutagenic potencies. This is supported by a comparison of BaP to a series of mono-methylated BaP (i.e. from position 1 to 12) in their tumor-initiating activities in mouse skin. Results showed that 1- and 11-methyl substitution enhance the tumor initiating effect of BaP naked ring, where methyl substitution in positions 7, 8, 9 and 10 eliminates the tumor-initiating ability of BaP. Also, the tumor-initiating activities of 3-, 4-, and 12-methyl BaP were comparable to that of BaP, whereas 2-, 5-, and 6-methyl BaP were all less active than its parent BaP (Iyer et al., 1980). Due to their limited alkylation in a multiple ring system, these types of aromatics (i.e. monomethylated PAC) are readily extractable by DMSO and are jointly assessed for mutagenicity and carcinogenicity prediction in the IP346 and Modified Ames test (Blackburn et al., 1986; Carrillo et al., 2019; IP 1996).

On the other hand, refined oils (compliant to IP346 or Modified Ames test) containing mostly 1 and 2 highly alkylated aromatics (Table 2) have been used as negative controls in repeated dose oral studies (McKee et al., 1987) and are not carcinogenic by dermal or oral routes (Shoda et al., 1997; Trimmer et al., 2004) at doses as high as 1200 mg/kg bw day. This implies that high alkylation of aromatics may play an important role in decreasing toxicity effects.

Recent *in vitro* studies demonstrated that the alkylation of PAHs

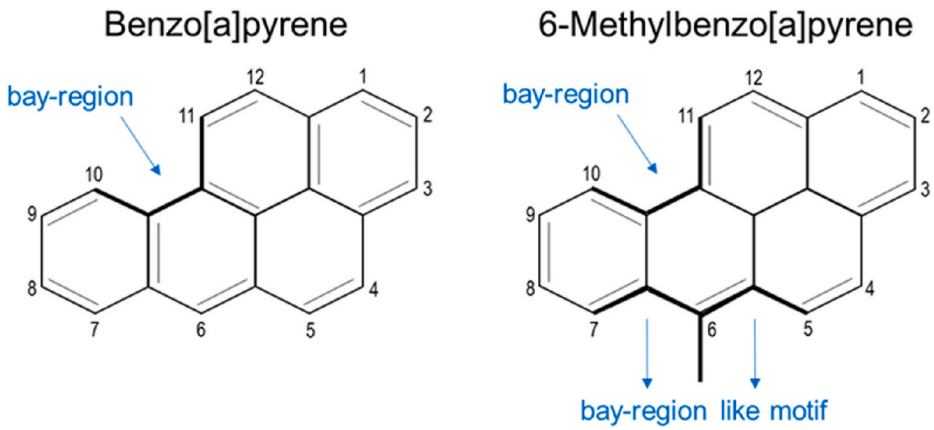

Fig. 4. Bay region or bay-region like motif in BaP and 6-methyl-BaP.

shifts the oxidative metabolism from the aromatic ring to the alkyl side chain, thus facilitating detoxification and excretion of these substances. Longer chain alkyl substitution almost completely inhibits oxidative metabolism, probably by steric hindrance of the receptor site of the cytochromes (Wang et al., 2020; Wang et al.; Wang et al., 2021). These *in vitro* studies were carried out with rat and human liver microsomes, where the effect of alkylation on CYP450-mediated metabolism of the PAHs 2-ring naphthalene, 3-ring phenanthrene, 5-ring BaP and their alkylated congeners (from C_1 methyl to C_{12} dodecyl) was investigated. The authors reported that alkyl side-chain oxidation is preferred over aromatic ring oxidation during alkyl-PAHs metabolism. To add, metabolism of alkylated PAHs becomes less efficient with elongation of the alkyl chain, starting from the alkyl side chain having 3 or more carbon atoms ($\geq C_3$), and the overall metabolism was greatly reduced with n-hexyl (C_6) and completely absent with a n-dodecyl (C_{12}) substitution (Wang et al., 2020; Wang et al.; Wang et al., 2021). The shift of the oxidative metabolism from the aromatic ring to the alkyl side chain may facilitate the detoxification and excretion of formed metabolites (Höke and Zellerhoff 1998); moreover, the configuration of the bulky alkyl substitution on the aromatic moiety may also prevent the intercalation of activated derivatives (and metabolites) into DNA. In other words, high alkylation on PAC will reduce the chance of formation of intermediate toxic and/or DNA-reactive metabolites making it a chemical differentiator from the hazardous 3–7 ring PAC which include naked and lowly alkylated polyaromatics. These structural considerations together with physical parameters such as viscosity help understand why refined mineral oils are not carcinogenic *in vivo*, even if MOAH is present up to 25%.

In summary, it can be concluded that alkyl substitution of parent PAHs may either increase, decrease or eliminate their mutagenicity and/or carcinogenicity potencies. From the data discussed we can reliably say that high alkylation of MOAH constituents present in all refined mineral oils (base and white oils) is thus of low carcinogenic concern if the oils comply with either IP346 or the modified Ames test.

7.2. Systemic, fertility, reproductive and developmental toxicity

Apart from carcinogenicity, systemic, reproductive fertility and developmental toxicity associated with PAC should also be considered.

Petroleum streams rich in 3–7 ring PAC have been shown to be systemically toxic affecting hematology parameters, increased liver, thymus and bone marrow to rats and affecting development of the fetus when applied dermally (Cruzan et al., 1986; Feuston et al., 1996) as well as orally (Feuston and Mackerer 1996). Although these streams produced developmental effects, histopathological findings and effects on reproductive parameters (e.g. sperm count, corpora lutea) and organs are not observed (Cruzan et al., 1986; Hoberman et al., 1995). Thus, systemic and developmental toxicity of petroleum substances are associated primarily with the presence of both individually or grouped 3–7 ring PAC in these substances (Feuston et al., 1994). Removal of 3–7 ring PAC is consistent with absence of similar toxicity in refined mineral oils (Dalbey et al., 2014).

Only a few 3–7 ring (un)substituted PAC, including BaP and 7,12-dimethylbenz [a]anthracene (DMBA), have been studied for effect on fertility and prenatal development in experimental animals (Bui et al., 1986; Nebert et al., 1977; Ramesh et al., 2004). Exposure to BaP increases embryo lethality, decreases fetal body weight and increases the incidence of resorptions in the offspring of pregnant rats (Archibong et al., 2002; Bui et al., 1986; Ketelslegers et al., 2018). Embryotoxicity of individual PAC has been further investigated *in vitro* where the ability of some of these substances to induce *in vitro* developmental toxicity was studied using alternative test models, such as zebrafish *(Danio rerio)* and Japanese medaka *(Oryzias latipes)* embryos (Chlebowski et al., 2017; Cunha et al., 2020; Geier et al., 2018; Lin et al., 2015; Rhodes et al., 2005; Turcotte et al., 2011; Wincent et al., 2015). Using dechorionated zebrafish embryos, the developmental toxicity potency of 123 PAC (i.e. 33 parent, 22 nitrated, 17 oxygenated, 19 hydroxylated, 14 methylated, 16 heterocyclic, and 2 aminated PAC) was evaluated. It was demonstrated that high-molecular-weight PAC ($\geq$ 3-ring) have significantly more developmental toxicity than the low-molecular-weight PAC. None of the mono-methylated 2–4 ring PAHs tested (i.e. methyl-naphthalene, -phenanthrene, -anthracene, -chrysene) cause any developmental-related effects in zebrafish embryos (Geier et al., 2018). For the other types of substituted PAC, particularly oxygenated- and nitrated-PAC, it was shown that some may induce more developmental aberrations in zebrafish embryos than their unsubstituted parent PAHs (Chlebowski et al., 2017; Cunha et al., 2020; Geier et al., 2018; Wincent et al., 2015).

Similar to the above-mentioned findings for mutagenicity and carcinogenicity, the position, type of substituents, and degree of the substitution on the aromatic ring also influences the developmental toxicity potency of the 3–7 ring unsubstituted parent PAC. This was investigated by assessing the role of methyl substitution on the developmental toxicity potency of a series of 4- to 5-ring unsubstituted (i.e. BaA, BaP, and dibenz [a,h]anthracene (DB [a,h]A)) and their mono-methylated analogues (i.e. 4-, 8- or 9-methyl BaA and 3-, 7- or 8-methyl BaP) using the zebrafish embryotoxicity test (ZET) (Fang et al., 2022). It was demonstrated that substitution, i.e. presence of a single methyl group, has an influence on the developmental toxicity potency of the respective parent PAHs, as quantified in the ZET, but this also depends on at which position the methyl substitution takes place (Jing et al., 2021). Of all mono-methylated BaP tested, only 8-methyl BaP showed a substantial developmental retardation in zebrafish embryos at non-lethal concentrations. Exposure to 3-methyl BaP disturbs the general development of

zebrafish embryos (e.g. unhatched embryos and unemptied yolk extension) without inducing embryo lethality, whereas embryos exposed to increasing concentrations of 7-methyl BaP only caused failure to hatch at the highest concentration tested. For mono-methylated BaA effects on zebrafish embryos, only 9-methyl BaA showed a concentration-dependent embryotoxicity (at non-lethal concentrations), whilst the predominant effect induced by 4- and 8-methyl BaA was embryo lethality (Jing et al., 2021). It is worth mentioning that both 9-methyl BaA and 8-methyl BaP have a similar chemical structure due to the same position of methyl substitution on the aromatic ring (Fig. 5), thus supporting the notion that the degree of the observed developmental toxicity by some alkylated PAHs would also depend on the position where the alkyl substituent is present. On the other hand, it was observed that exposure of zebrafish embryos to parent PAHs; BaA and BaP mainly induced edemas and embryo lethality, whereas DB [a,h]An induced developmental retardation (i.e. absence of movement and circulation, yolk extension not empty, and failure to hatch) without causing embryo lethality (Jing et al., 2021). Similar findings, including yolk sac edema, pericardial edema, absence of swim bladder, and craniofacial deformity, were also observed in Japanese medaka embryos exposed to unsubstituted parent and alkylated 3- and 4-ring PAHs (Lin et al., 2015; Rhodes et al., 2005; Turcotte et al., 2011).

7.3. Bioactivation

While PAC/PAHs need to be first bioactivated to exert their mutagenic and carcinogenic effects, metabolism does not seem to be a prerequisite for the observed developmental toxicity induced by some individual PAHs and PAH-containing materials ((Kamelia et al., 2019a; Kamelia et al. 2019b; Kamelia et al. 2020; Kamelia et al. 2018; Kamelia et al. 2017). BaP needs to be bioactivated first to show its *in vitro* embryotoxic effects in the mouse embryonic stem cell test (mEST), whilst another 5-ring PAH, DB [a,h]A, induced a concentration-dependent developmental toxicity in the absence of bioactivation (Kamelia et al., 2020). Moreover, the developmental toxicity potency of the DMSO-extracts of PAH-containing materials (i.e. petroleum UVCB substances), as quantified in the mEST, does not substantially change following bioactivation, indicating that metabolism may not play a crucial role for these complex substances to induce the (*in vitro*) embryotoxicity effects (Kamelia et al., 2020). This demonstrates that although some PAHs require bioactivation to induce developmental toxicity, others do not, and the latter appears to hold true for most PAC constituents present in mineral oils and other petroleum UVCBs substances (Kamelia et al., 2017; 2019b, 2020). This may be linked to the fact that the underlying modes of action may require non-covalent receptor or enzyme interactions rather than the chemical (DNA) reactivity which is necessary to exert the mutagenic and carcinogenic effect of these substances. Corroborating this, published studies (both *in vivo* and *in vitro*) have reported the potential role of various nuclear receptors, in particular aryl hydrocarbon receptor (AhR), estrogen receptor alpha (ERα), retinoic acid receptor (RAR), and peroxisome proliferator-activated receptor (PPAR), in the reproductive and developmental toxicity of chemical substances including PAC (Alharthy et al., 2017; Beníšek et al., 2008; Billiard et al., 2006; Goodale et al., 2013; Kamelia et al., 2018; Ketelslegers et al., 2018; Machala et al., 2001; Pieterse et al., 2013; Vondráček et al., 2017; Wincent et al., 2015). For example, exposing pregnant AhR knock-out mice (AhR−/−, AhR-KO) to BaP did not cause any embryotoxicity in comparison to the developmental toxicity observed in the offspring of pregnant wild-type Sprague-Dawley rats exposed to BaP (Ketelslegers et al., 2018).

Although some conclusions can be made on naked and lightly alkylated PAC, no data - neither *in vivo* nor *in vitro* - is available to date that is related to the developmental toxicity potency of individually tested highly alkylated PAC (i.e. >C3 substituents), thus direct comparison to the effects of light and unsubstituted PAC cannot be made. But generally, light substitution may either decrease or increase the toxicity potency, i.e. developmental toxicity potency, of its parent 3–7 ring PAC. The most likely explanation for this observation is that substitution (e.g. methyl substituents) at different positions on the aromatic rings of parent PAC change their size and molecular configuration (shape), which result in different interactions with biotransformation enzymes and various nuclear receptors and may consequently lead to different severities of toxicity (Jing et al., 2021; Lin et al., 2015). In the context of MOAH that could be analyzed in refined mineral oil products, it must be emphasized that the potential for developmental toxicity is substantially reduced with the extraction/elimination of the 3–7 ring PAC fraction. Unrefined petroleum substances which contain this fraction shows clear developmental effects in animal dermal studies (Feuston et al., 1996), and while refined mineral oils do not raise this concern (Dalbey et al., 2014).

8. Discussion

Since MOAH measurements were introduced (Biedermann and Grob 2009a, 2009b) the “MOAH” chromatography term has been taken out of context leading to the general assumption that all mineral oil aromatics that may be present in a range of consumer products are of concern (Warentest 2015). Consequently, no differentiation is made between the toxicologically relevant 3–7 ring PAC that are removed during refining and other aromatic constituents measured as MOAH in refined mineral oils (lubricating base and white oils). EFSA does recognize that it is the 3–7 ring MOAH which are of concern (EFSA 2012), which we interpret as the 3–7 ring PAC which are without or low alkylation. Thus, when assessing aromatics in mineral oils, two types of aromatics should be distinguished. The first type and most relevant for hazard assessment are the 3–7 ring PAC that encompass PAH and heterocyclic ring systems with no or low alkylation, the second type consists of so called “highly alkylated” 1–7 ring aromatics that may be found in refined mineral oils. Although both are measured as “MOAH”, only the first group is of toxicological relevance as some may induce carcinogenicity, mutagenicity, or developmental toxicity. Thus, because unrefined or poorly refined oils containing a substantial level of 3–7 ring PAC these oils are carcinogenic in the 2-year mouse skin painting bioassay (Chasey and

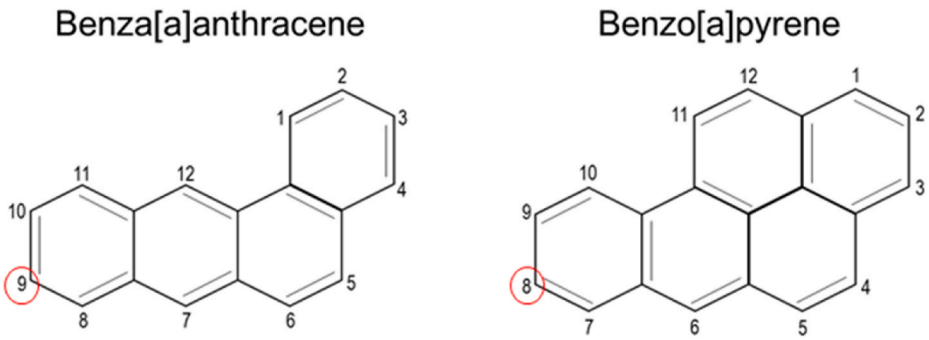

Fig. 5. 9-Methyl BaA and 8-methyl BaP share a similar chemical structure due to the same position of methyl substitution on the aromatic ring.

McKee 1993; Mackerer et al., 2003; Pirow et al., 2020), mutagenic in the modified Ames test (Blackburn et al., 1986) and may also induce systemic and developmental toxicity (Cruzan et al., 1986; Dalbey et al., 2014; Feuston et al., 1996). Reproductive toxicity however, does not seem to be adversely affected (Hoberman et al., 1995). Elimination of the 3–7 ring aromatics from oils renders those that are of low carcinogenic, mutagenic and developmental toxicity concern.

Toxicity of mineral oils is best studied by the holistic assessment of its 3–7 ring PAC content through a DMSO extraction because DMSO selectively extracts the naked ring and lowly alkylated 3–7 ring PAC. For oils with atmospheric boiling point of 300 °C at 5% recovered sample IP346 is applicable to assess carcinogenicity (Carrillo et al., 2019; IP 1996), whereas for other petroleum streams the modified Ames test is applicable (Blackburn et al., 1986; CONCAWE et al., 2012). Hence DMSO extraction is a powerful tool that allows experimental distinction between "low" vs "highly" alkylated mineral oil constituents which are collectively referred to as MOAH, a concept that does not make this distinction.

The concept of what "low" vs "highly" alkylated entails will depend on the number and the chain length of the alkyl substituents (i.e. lowly vs highly alkylated) as well as their position on the poly-ring system which will determine the accessibility of the CYP enzymes towards either the ring or an alkyl chain for detoxification. This leads to the necessity to discriminate between "lowly" vs "highly" alkylated aromatics, which will determine the toxicity of a mineral oil as well as to clarify the concept of the two types of MOAH found in an oil. It has been empirically demonstrated from a series of experiments that DMSO can selectively extract the naked and "lowly alkylated "aromatics from a carcinogenic mineral oil such that a reliable correlation exists between the DMSO extract and the outcome of a 2-year carcinogenicity mouse skin painting study, which was not the case when such comparison was done using a chromatography method (Carrillo et al., 2019). Analysis of the content of the DMSO extract showed the selectiveness of this solvent towards the 3–7 ring PAC which encompassed naked ring and "lowly alkylated" PAC. The unextracted aromatics, those which due to the length, number and position of alkyl substituents do not partition to DMSO, remain in the oil and do not cause tumors when the oil is tested in the mouse bioassay. While no actual numerical value can be given to specify what constitutes "high" vs "low" PAC alkylation, this becomes irrelevant when considering that this is intrinsically covered with the established relationship between the DMSO extract of a mineral oil and the oil's carcinogenicity tested in the mouse bioassay for either IP346 or the modified Ames test (CONCAWE 2016; Mackerer et al., 2003).

However, not all the aromatic materials in a DMSO extract are equally hazardous. Published studies showed that some lowly alkylated 3–7 ring PAC/PAHs may be less or more mutagenic compared to their parent naked ring structures, indicating that the relative potency of alkylated PAC is determined by the chain length and position of the alkyl substituents (Baird et al., 2007; Iyer et al., 1980; LaVoie et al., 1983; Santella et al., 1982; Utesch et al., 1987). Similar potency effects of PAC alkyl substituent configuration have also been observed in developmental toxicity studies (Geier et al., 2018; Jing et al., 2021; Lin et al., 2015; Rhodes et al., 2005). What has been shown is that increasing the length of the alkyl chain substituent decreases the toxicological concern of PAC, since elongation of the alkyl chain reduced overall metabolism of alkylated PAHs (≥C3) and its bioavailability (Wang et al. 2020, 2021), and the configuration of bulky ring substitution also prevents the intercalation of activated derivatives (and metabolites) into DNA. To add, presence of alkyl substituents shifts the oxidative metabolism from the aromatic ring to the alkyl side chain thus facilitating them to the non-toxifying or detoxification/excretion metabolic pathway (Wang et al., 2020). In DAE, the degree of alkylation is reflected in yields from a DMSO extraction, where lower or no alkylation results in higher DMSO extraction and inversely, increasing alkylation degree results in less DMSO extracted material (Carrillo et al., 2019). These extracts may be then tested directly *in vitro* (Blackburn et al., 1986; Kamelia et al., 2020) or correlated to results from animal data were these oils were tested (CONCAWE 2016; Feuston et al., 1996; Roth et al., 2013). The correlation between the DMSO extract of an oil and the oil's carcinogenic potential tested *in vivo* has resulted in IP346, a test included in EU and Australian regulations indicating that a mineral oil with a DMSO extract of <3% by weight is not carcinogenic. Similarly, the mineral oil is not considered mutagenic if the DMSO extract tested in the modified Ames assay result in a mutagenicity index of <1.0. Therefore, based on their refinement history, for refined mineral oils that meet IP346 < 3% by weight., or meet UV-DMSO absorbance test limits defined in pharmacopeia and/or FDA 178.3620, no carcinogenic effects are expected (ASTM 1995; BfR 2018; Dalbey et al., 2014; FDA, 2022c; Pirow et al., 2020), even if MOAH is present. Furthermore, because the systemic and developmental toxicity of mineral oils is associated with the levels of 3–7 ring PAC, none of these effects are expected for oils that are adequately refined (Feuston et al., 1994). Highly refined oils containing highly alkylated 1–2 ring aromatics are thus used as negative control substances in reproductive and systemic toxicity oral assessment of substances with high content of 3–7 ring PAC (McKee et al., 1987).

In this paper we have shown that the level of 3–7 ring PAC and MOAH in mineral oils (by IP346 and LC-GC-FID respectively) varies depending on the refining steps to produce lubricating base oils or white oils. By measuring the aromatic content of two refinery streams SN600 and SN100 by different techniques along with IP346 and modified Ames data, the MOAH measurements are put into context. While the levels of total aromatics do decrease with increased levels of refinement, the sole indicator of hazard are the levels of 3–7 ring PAC assessed via a DMSO extract in the IP346 or modified Ames test. Already at the waxy raffinate level (the streams resulting after 3–7 ring PAC elimination), both the IP346 and MI values were below the safety cut-offs (3% by weight and 1.0, respectively), and remained so during the entire downstream production as shown in Fig. 3. It must be pointed out that even though in our samples the IP346 or MI values were all close to zero, higher values in other commercially available mineral oils which are still below the respective safety cut off should not be judged as "less safe". In this "pass/fail" dichotomy there is no ambiguity, and its adequacy and validity has been justified in other publications (CONCAWE 2016; Mackerer et al., 2003; McKee et al., 2013; Pirow et al., 2020). Although no analogous test such as the IP346 exists for systemic and developmental toxicity, it has been shown that these endpoints are associated with the level of 3–7 ring PAC present in the oil (Feuston et al., 1994; Murray et al., 2013), and shown no adverse effects when refined mineral oil devoid of this fraction is tested *in vivo* by the dermal and oral route (Dalbey et al., 2014; McKee et al., 1987).

The MOAH values on the other hand vary according to the refinement stage and should be contextualized. For example, in our assessment the highest MOAH value corresponds to distillate aromatic extract (DAE, furfural extracted) which contained up to 60% MOAH. This hazard profile of this value is meaningless if no information on its 3–7 ring PAC content is available. Because DAE is the result of solvent extraction and thus a concentrate of extracted 3–7 ring PAC, it is considered carcinogenic and, therefore, no accompanying IP346 or MI values were determined. From literature, however, it is reported that DAE of varying viscosities have IP346 values ranging from 18 to 30 inversely proportional to kinematic viscosity (Carrillo et al., 2019). However, what is important to recognize is that DAE is not carcinogenic, mutagenic or developmentally toxic due to relative higher MOAH values compared to other oils, but because of their 3–7 ring PAC content which can be decreased (e.g. by saturating these species with hydrogenation), so that the refined DAE may still have very high aromatic content (74% mass) but rendered non-carcinogenic (Doak et al., 1985). Low systemic and developmental toxicity concern would also be expected although no *in vivo* data is yet available. Therefore, it is not unusual to have refined base oils with "high" MOAH content next to IP346 values < 3%, and MI < 1.0 such as the 13% and 10% values obtained from SN600 and SN100 base oils respectively.

The higher MOAH values obtained for higher viscosity SN600 products compared to SN100 is by and large due to their kinematic viscosity. Viscosity has a "blow up" effect on the MOAH content in products whose alkane constituents have a high molecular weight. As MOAH is an associated chromatographic fraction of the saturated hydrocarbon alkane fraction (MOSH), the high molecular weight of the alkane constituents is also reflected in the accompanying aromatics which must have long alkyl chain substituents to be present at the specified distillation point and average molecular weight (see Table 2). This is best exemplified by a microwax, which is not a mineral oil but given its physical chemical characteristics serves well to illustrate this point. The combined analysis showed that MOAH can be up to 5%, even though the 3–7 ring PAC by IP346 are <3% and the 8 EU-PAH are ppb trace levels. Other analysis indicates that the MOAH present are in fact 1 and 2 ring aromatics with very long alkyl chains which make up > 90% of the entire molecule. Compared to oils, which have "lower" MOAH levels due to shorter carbon numbers it becomes obvious that the longer the alkyl carbon chains, the higher molecular weight and thus, the higher the MOAH value. This observation leads to the "MOAH paradox" where the more aliphatic the MOAH structures become, the higher the MOAH content in the chromatography fraction. Therefore, the safety of the apparent "high" MOAH fraction content of refined products should be interpreted considering the physical chemical properties, such as viscosity and refining history which will determine toxicity.

Based on the previous points the following "rule of thumb" observation is postulated: as mineral oil viscosity increases the amount of DMSO extractable material decreases because an increase in viscosity reflects higher alkylation degree of aromatic constituents. Higher alkylation decreases the polarity of the molecules and thus affinity towards DMSO. Increase in alkylation decreases toxicity associated with aromatic hydrocarbon species. Consequently, a mineral oil with relatively low DMSO extract, can still have high amounts on highly alkylated aromatic material, for which low toxicity would be expected (Fig. 6).

Finally, it should be emphasized that the basics of mineral oil manufacturing allow selection of the molecules from the crude oil in a controlled manner to set the final chemical composition (and properties) of the mineral oil. In other words, the refinement process ensures targeted removal of undesirable molecules (including 3–7 ring PAC with or without light alkylation) to meet legally mandated test thresholds (e.g. IP346) and product specifications such as viscosity by only keeping molecules that ensure performance without safety concerns in occupational settings and for consumers. As a result, and shown in our data 3–7 ring PAC levels in marketed base oils and pharmaceutical/medicinal/food grade white oils are well controlled and thus do not possess any carcinogenic, mutagenic or reprotoxic risk. In addition, highly alkylated aromatics, measured as MOAH (Biedermann et al., 2009; Biedermann and Grob 2009a), if present, are not biologically active, thus present low toxicological concern (Wang et al. 2020, 2021).

9. Conclusions

To conclude, we advocate that the term "MOAH" should be used and interpreted carefully because two types of aromatics (hazardous 3–7 ring PAC and non-hazardous PAC) are measured when a chromatography analysis is used. Sufficiently refined mineral oils (IP346 < 3%), which may contain MOAH fractions due to their petroleum origin, possess low toxicological concern due to the lack of hazardous 3–7 ring PAC. The MOAH content is not a determinant of toxicity of mineral oils, biased towards molecular weight and thus a poor predictor of toxicity. Therefore, the regulatory focus should neither focus on "high in aromatic content" nor on "MOAH content" but rather to what is toxicologically relevant; namely controlling the levels of 3–7 ring PAC which ensures the safety of mineral oil and petroleum compounds intentionally used in consumer products.

10. Material and methods

10.1. Oils and wax

SN600 and SN100 samples, white mineral oils, and microwax were obtained from Shell Deutschland GmbH Grasbrook Lubricants Centre, Hamburg, Germany, SN 100 Technical White Oil - Distillates (petroleum), hydrotreated light paraffinic – CAS 64742-55-8; SN 100 Medicinal White Oil - White mineral oil (petroleum) – CAS 8042-47-5; SN 600 Distillate - Distillates (petroleum), heavy paraffinic – CAS 64741-51-1; SN 600 Waxy Raffinate - Distillates (petroleum), solvent-refined heavy paraffinic - CAS 64741-88-4;

SN 600 Base Oil - Distillates (petroleum), solvent-dewaxed heavy paraffinic – CAS 64742-65-0; SN 600 Technical White Oil - Distillates (petroleum), hydrotreated heavy paraffinic – CAS 64742-54-7; SN 600 Medicinal White Oil - White mineral oil (petroleum) – CAS 8042-47-5; Shell microwax LMP - Hydrocarbon waxes (petroleum), hydrotreated microcrystine - 64742-60-5. GTL synthetic oil was of medicinal purity and also obtained from the same provider; Distillates (Fischer-Tropsch), heavy, C18-50-branched and linear -CAS 1262661-88-0.

10.2. Methods

PAC determination by DMSO extract by IP346 was carried out using the standard industry method (IP 1996). The modified Ames test was carried out according to ASTM protocol (ASTM 1995), where the undiluted DMSO extract (60 μl/plate) as well as dilutions containing 52.5, 45, 30, 15 and 7.5 μl extract/plate were tested in the *Salmonella typhimurium* tester strain TA98 obtained from Trinova Biochem GmbH, Germany. The test was performed in the presence of S9-mix (hamster liver S9 induced by Aroclor 1254). The positive control was Distillates (petroleum), heavy naphthenic CAS 64741-53-3.

Viscosity of oils and microwax was measured according to ASTM D

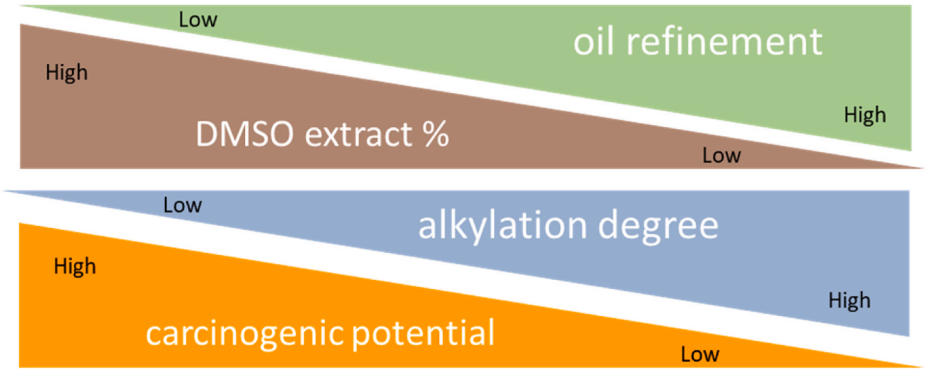

Fig. 6. Toxicity of a mineral oil is directly proportional to its PAC content by DMSO extract, which is inversely proportional to viscosity and MOAH alkylation degree.

445 and ASTM D 3236 respectively. Determination of aromatic hydrogen by high resolution nuclear magnetic resonance spectroscopy method was done according to industry standard IP 392/90. Total aromatic content by UV method was carried out by Shell Method – SMS 2728-8, which describes the determination of the aromatic hydrocarbon content of hydrocarbon solvents and streams having a total aromatic content below 0.5% (m/m), down to a level of 5 mg/kg. The method is applicable to hydrocarbon solvents and streams mainly consisting of linear paraffins, isoparaffins, cycloparaffins. Analysis of 8 standard EU-PAH (see Table 2) was done by EN16143.

10.3. MOAH analysis by chromatography

The MOAH analysis was carried out at the Institut Kirchhoff Berlin GmbH (Oudenarder Straβe 16/Carrée Seestraβe, 13347 Berlin-Mitte).

10.3.1. Sample preparation

For the method we solve the sample, remove insoluble n-alkanes above n-C50 by cooling to 4 °C followed by centrifugation and removed MOSH by chromatography on silver nitrate impregnated silica gel. Finally, a keeper is added and the extract is concentrated prior to injection to LC-GC-FID.

10.3.1.1. LC-GC-FID parameters. A summary of the methodology described elsewhere is provided (Koch et al., 2020). The MOSH were measured by an on-line HPLC-GC-FID system (Axel Semrau GmbH, Sprockhövel, Germany), using a PAL CTC sampler (CTC Analytics AG, Zwingen, Switzerland) on a 1260 Infinity HPLC instrument (Agilent Technologies, Waldbronn, Germany). A silica gel column (Restek Allure Silica 5 μm, 250 mm × 2.1 mm) was connected via a Y- interface to a DANI Master GC (DANI Instruments S.p.A., Cologno Monzese, Italy) equipped with an uncoated precolumn (Restek MXT 10 m x 0.53 i.d.) followed by a steel t-piece union connecting to SVE (solvent vapour exit) and a nonpolar separation column (Restek MXT-1, 15 m × 0.25 mm i.d. X 0.25 μm). A gradient of n-hexane with dichloromethane was used with backflush after the elution of the MOAH, started at 0.3 mL/min with 100% n-hexane, reaching 35% dichloromethane after 1.5 min, backflush initiated after 6.2 min with 100% dichloromethane at 0.5 mL/min for 9 min, followed by a recondition with 100% n-hexane for 10 min at a flow rate of 0.5 mL/min and 5 min at 0.3 mL/min. The injection volume was 90 μL for the tri-/polyaromatic fraction (40 μL were dissolved to 100 μL after the GCxGC-TOF-MS injection), and 10–50 μL for mineral oil aromatic hydrocarbons (MOAH) and mono-/diaromatic fractions. Hydrogen was used as a carrier gas with 90 kPa applied during the fraction transfer from LC to GC through the Y-interface and 150 kPa after the partially concurrent solvent evaporation and closure of SVE valve. GC started at 58 °C (11 min), followed by a temperature program of 5 °C/min to 80 °C, then at 15 °C/min to 110 °C and at 25 °C/min to 370 °C (7 min), resulting in a total run time of 34 min.

10.3.1.2. GCxGC-parameters. A summary of the methodology described elsewhere is provided (Koch et al., 2020). For GCxGC-TOF-MS, a Leco Pegasus 4D (Leco Instrumente GmbH, Mönchengladbach, Germany) was used, controlled by Leco ChromaTOF acquisition software. The instrument consisted of a 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a split/splitless injector, a PAL combi XT autosampler (CTC Analytics AG, Zwingen, Switzerland), a secondary internal oven, a cryogenic consumable-free (CF) nitrogen-cooled (FC100 chiller from SP Scientific-FTS Systems, Warminster, PA, USA) jet modulator and a TOF mass spectrometer. The column configuration was of the reversed polarity type, with a 30 m × 0.25 mm i.d. X 0.15 μm DB-17HT (Agilent Technologies, Waldbronn, Germany) first dimension column connected via the ultimate union connection system (Agilent Technologies, Waldbronn, Germany) to a 1.5 m × 0.25 mm i.d. X 0.1 μm DB-5HT (Agilent Technologies, Waldbronn, Germany) second dimension column. These columns were temperature-programmed from 60 °C to 370 °C at 3 °C/min without secondary oven offset. The modulator offset was 20 °C. Helium was used as a carrier gas in constant flow mode (1 mL/min). Modulation was in staged mode, from 9 s to 14 s at the end of chromatographic separation in order to avoid the wrap-around of high boiling compounds. Spectra were collected in the m/z range from 35 to 650, with a scan rate of 50 spectra/s. The ion source was at 250 °C, the transfer-line at 340 °C; a detector voltage of 1600 V was applied after the solvent delay of 450 s. To lower the detection limit, pooled TPA fractions (2.2) were evaporated to 40 μL. Injection volumes were between 1 and 3 μL in pulsed spitless mode.

10.3.1.3. NMR spectroscopy. The samples for the ^{1}H NMR measurements were prepared by dissolving base oils in deuterated chloroform. Microwaxes and mixtures of ethyl benzene and decane were measured using deuterated dichloromethane as solvent. The ^{1}H NMR experiments were performed using an Agilent 400 MHz spectrometer equipped with 5 mm OneNMR probe. The measurements were performed at 25 °C. ^{1}H NMR spectra of microwaxes were acquired using spectral width of 4807.69 Hz, relaxation delay of 5 s, 1000 repetitions and acquisition time of 3.42 s ^{1}H NMR spectra of other oils and mixtures of ethyl benzene/decane were acquired using spectral width of 6410.25 Hz, relaxation delay of 5 s, 128 repetitions and acquisition time of 2.56 s. The ^{1}H spectra were processed using Agilent VnmrJ software and referenced to the signal of tetramethylsilane (TMS) at 0 ppm, which was an internal standard.

CRediT authorship contribution statement

Juan-Carlos Carrillo: Conceptualizsation, Methodology, Writing – review & editing. **Lenny Kamelia:** Writing – original draft. **Julija Romanuka:** Resources, Conceptualization. **Olaf Kral:** Resources, Conceptualization. **Allison Isola:** Writing – review & editing. **Helena Niemelä:** Project administration. **Anna Steneholm:** Conceptualizsation, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Juan-Carlos Carrillo, Lenny Kamelia, Julija Romanuka, Olaf Kral, Allison Isola and Anna Steneholm are employed by companies that produce mineral oil. However, they undertook this work as members of CONCAWE special task force on Mineral Hydrocarbons, an expert group of the European Petroleum Refiners Association, and were completely free in the conduct as well as the expression of their scientific opinion in this paper and did not received monetary compensation for writing this paper. The other authors declare no conflict of interest.

Acknowledgements

This paper is the result of the activities of CONCAWE special task force on Mineral Hydrocarbons whose members and companies are represented by the following individuals:

Dirk Danneels (European Wax Federation – Chairman of the task force); Juan-Carlos Carrillo and Lenny Kamelia (Shell); Trinidad Espinosa Castilla (CEPSA); Daniela Heber and Bjorn Heuer (H&R); Allison Isola, Anna Tancorra and Laurent Jouanneau (ExxonMobil); Paula Karjalainen (Neste); Olaf Kretschmer (Sasol); Neslihan Aygun Kocabas, Philippe Lemaire and Cecile Libot (Total); Raul Garcia (Repsol); David Marin (Siwaxgroup); Anna Steneholm (Nynas); Anja Mannaerts and Helena Niemela (CONCAWE); Alain Mathuren and Marine Teixidor (Fuels Europe).

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