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Lack of human-relevant adversity of MOSH retained in tissues: Analysis of adversity and implications for regulatory assessment

A.L. Isola [a,e,**], J.C. Carrillo [b,e], P. Lemaire [c,e], H. Niemelä [e,*], A. Steneholm [d,e]

[a] ExxonMobil Biomedical Sciences, Inc., 1545 US Highway 22 East, Annandale, NJ, 08801-3059, USA
[b] Shell Global Solutions International B.V., PO Box 162, 2051, AN, The Hague, the Netherlands
[c] Total Fluides, 24 Cours Michelet-La Défense 10, F-92069, Paris La Défense Cedex, France
[d] Nynas AB, PO Box 10 700, SE-121 29, Stockholm, Sweden
[e] CONCAWE Mineral Hydrocarbon Task Force Member, Boulevard du Souverain 165, B-1160, Brussels, Belgium

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ABSTRACT

Mineral oils (food grade white oil or liquid paraffin) have historically been safely used in a number of sensitive end-uses, including pharmaceutical, cosmetic and food. Recent concern that certain mineral hydrocarbons (branched and cyclo-alkanes) may accumulate in human tissues has prevented European Food Safety Authority (EFSA) from deriving guidance values for food exposures. Analysis of human and animal tissue indicate that an unresolved cloud of mostly highly branched alkanes and alkylated cycloalkanes within the C20–C35 range is consistently present in all tissues. This critical review thoroughly assesses the retention of "mineral oil saturated hydrocarbons" (MOSH) in human and animal tissues and evaluates if the presence of MOSH is considered adverse and appropriate to use for risk assessment, generation of guidance values for food exposure and/or generation of derivation of health-based guidance values. An adversity framework was utilized to perform an in-depth weight of the evidence analysis, and it was concluded that mere presence of MOSH does not translate to hazard identification, and is not considered adverse. In light of this conclusion, it would not be appropriate to utilize this endpoint as the point of departure to calculate a health guidance value.

1. Introduction

Petroleum based mineral oils are manufactured from vacuum distillation streams of petroleum refineries. In order to produce a mineral oil, crude oil is subjected to distillation at atmospheric pressure where light fractions with low boiling ranges are separated and undergo additional processing steps to meet the specifications for lower boiling products like gasoline and kerosene. The remaining atmospheric residuum is subsequently distilled under vacuum to separate these constituents further by boiling point into various distillate fractions. They then undergo further processing to meet the specifications for higher boiling petroleum products such as mineral oils and waxes (Fig. 1).

Immediately after vacuum distillation and prior to further processing, the vacuum distillate fractions contain undesirable components, such as heterocyclic aromatics and polycyclic aromatic compounds (PAC), which are potentially carcinogenic and can negatively affect the performance of the downstream waxes and oils that will be produced after further processing. These distillate fractions may then undergo solvent extraction, which selectively extracts aromatic compounds. In essence, most PACs are transferred from the insufficiently refined distillates to what is called a Distillate Aromatic Extract (DAE), leaving sufficiently refined waxy raffinates with low levels of PACs. Alternatively, the distillate fractions can undergo hydrocracking or hydrogen treatment that decrease hydrocarbon chain length and also reduce the levels of PACs under appropriate processing conditions, usually by ring opening and saturation.

The waxy raffinate is then typically solvent-dewaxed to remove long chain paraffins from the product intended to become a lube base oil, separating out hydrocarbon waxes into a slack wax. Dewaxing can also be achieved through a catalytic process; in this case, long normal alkane chains are transformed into iso-paraffins, and no slack-wax is generated. Also, dewaxing is not necessary for manufacture of naphthenic base oils, originating from naphthenic crudes (see Fig. 1). To be sold as non-

* Corresponding author.
** Corresponding author. ExxonMobil Biomedical Sciences, Inc., 1545 US Highway 22 East, Annandale, NJ, 08801-3059, USA.
E-mail addresses: allison.l.isola@exxonmobil.com (A.L. Isola), product_stewardship@concawe.eu (H. Niemelä).

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carcinogenic substances in Europe and Australia, the Lubricant Base Oils (LBO) must pass a quality control check to ascertain that the 3–7 membered ring PACs are reduced to levels low enough to render non-carcinogenic waxes and oils. The legally (EU-CLP & AU-NICNAS) required test that was developed for this purpose measures the total amount of material extractable in dimethyl sulfoxide (DMSO) using the Institute of Petroleum's IP346 as an index of the PAC content (Carrillo et al., 2019; CONCAWE, 2016; IP, 1996). Sufficiently refined LBO streams meet the IP346 cut-off of less than 3% gravimetric DMSO extract and indirectly encompass the slack wax. De-oiling and further refining of the slack wax will result in hydrocarbon waxes (also called paraffin waxes or Fully Refined Waxes) and the corresponding dewaxed hydrocarbon oils (Dalbey et al., 2014).

These LBOs can be either used in various industrial and consumer applications, including lubricating base stocks, to formulate lubricants and greases, metal working fluids, thermoplastic elastomers, adhesives, printing inks, or are further refined to become white mineral oils:

- Technical white oils: colorless oils, derived from non-carcinogenic LBO and further refined by hydrogenation or acid treatment to achieve very low levels of aromatics which improves stability and purity (meets paragraphs C or B of FDA 21CFR 178.3620) (FDA, 21CFR178.3620(b)). However, technical white oils do not comply with the purity levels stipulated by pharmacopoeia monographs.
- Pharmaceutical white oils: colorless oils, derived from technical white oils, which are highly refined in a second hydrogenation or acid treatment to achieve extremely low levels (ppb) of aromatics and polycyclic aromatic hydrocarbons to ensure they comply with the levels and specifications stipulated by international pharmacopoeia monographs and food contact regulations (meets paragraph A of FDA 21CFR 172.878) (EDQM, 2019; FDA, 21CFR172.878).

These highly refined oils are referred to as a number of different names, including white mineral oils, pharmaceutical/medicinal/food-grade white oils, liquid paraffins, and are also sometimes just called "mineral oils". The term "mineral oil" can be misleading since lubricating base oils can also be called mineral oils in some documents (IARC, 1984). In this manuscript, the term "mineral oil" will refer to the highly refined white mineral oils.

Mineral oils have historically been safely used in a number of sensitive end-uses, including food, pharmaceutical and cosmetic. Concerns about safety were raised when sub-chronic toxicity feeding studies in Fisher 344 (F344) rats observed epithelioid granulomas in the liver and lesions in the mesenteric lymph nodes (histiocytosis) as a result of mineral oil exposure (Baldwin et al., 1992; Smith et al., 1996). This phenomenon has been studied rather extensively in other species including Sprague-Dawley and Long-Evans rats (Firriolo et al., 1995; Smith et al., 1995), dogs (Smith et al., 1995), and in human tissue samples (Barp et al., 2014; Boitnott and Margolis, 1970b; Concin et al., 2008, 2011; Cruickshank and Thomas, 1984; Dincsoy et al., 1982; Noti et al., 2003; Wanless and Geddie, 1985). Granulomas in the liver and lesions in the mesenteric lymph nodes were absent in all other strains and species studied to date, suggesting that the observed effects may be unique to the F344 rats. Although pathologists who analyzed the previous studies indicated that the F344 rat granulomas may not be relevant endpoints to determine safety for humans (Carlton et al., 2001; Fleming et al., 1998), the European Union (EU) Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established acceptable daily intake (ADI) values for different classes of mineral oils and waxes based on the observed effects in the F344 rat (JECFA, 1995; JECFA, 2002; SCF, 1995).

1.1. Regulatory interest

In 2009 it was reported that imported sunflower oil was contaminated with mineral oil (Biedermann and Grob, 2009b), which led to the development of a chromatography method that separated mineral oil into two fractions: mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) (Biedermann et al., 2009; Biedermann and Grob, 2009a). This method was then extended to other sources of potential mineral oil contamination (Biedermann and Grob, 2010) that led to several regulatory actions to minimize MOSH and MOAH exposure.

In its most recent draft of the German Mineral Oil Ordinance, the German Federal Ministry of Food and Agriculture (BMEL) requires a functional barrier to prevent the migration into food of aromatic hydrocarbons from recycled paper used to manufacture food contact materials. An exemption would be made if the manufacturer can show migration above 0.5 mg aromatic hydrocarbons per kilogram of food does not occur (BMEL, 2020). Previous versions of this draft ordinance contained a specific migration limit (SML) for saturated hydrocarbons from mineral oils in recycled paper based on the opinion that MOSH can accumulate and cause adverse effects in the liver. However, this SML was removed in the 2017 draft ordinance (BMEL, 2017).

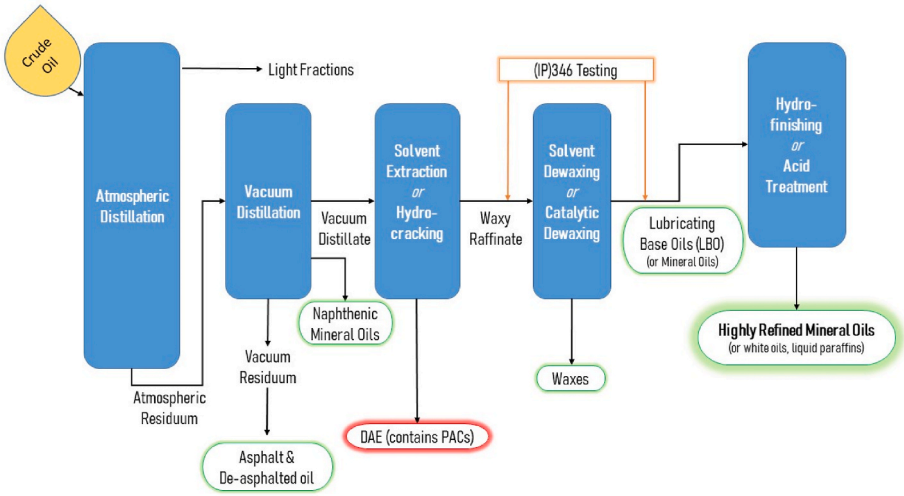

Fig. 1. Schematic of Mineral Oil Production. Green circles represent mineral hydrocarbon products, red circle represents where Distillate Aromatic Extract (DAE) is extracted, which contains hazardous heterocyclic aromatics and polycyclic aromatic compounds (PAC) and orange squares represent the time point in production where IP346 testing may be performed to confirm absence of carcinogenic potential of downstream waxes, Lubricating Base Oils (LBOs) and mineral oils.

In 2017, the European Commission published the Commission Recommendation (EU) on the monitoring of mineral oil hydrocarbons in food and in materials and articles intended to come into contact with food with the intent to understand the presence of MOSH and MOAH in food. Guidance materials, "Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials" were published in 2019 to outline specific procedures for sampling and analysis of the mineral oil components in food (Bratinova and Hoekstra, 2019).

In 2013 and 2017, CONCAWE (European fuel manufacturers' association for environmental science) convened Mineral Oil Cross Industry Issues (MOCRINIS) I & II workshops that included both industry and regulatory stakeholders (CONCAWE, 2013; CONCAWE, 2017a). Discussion on the hazard and potential risk of oral exposure to mineral hydrocarbons identified key areas of mutual interest that needed additional clarification; one of which included the question of whether progressive bioaccumulation in human liver over long periods may be associated with the potential for developing microgranulomas similar to those described in F344 rats.

All the previously mentioned activities were largely triggered by The European Food Safety Authority (EFSA) CONTAM panel 2012 Scientific Opinion on Mineral Oil Hydrocarbons in Food. In their review, EFSA concluded that, although they considered the liver microgranulomas in F344 rats the critical adverse effect of mineral hydrocarbon exposure, certain mineral oil saturated hydrocarbons - MOSH (mostly branched and cyclo-alkanes) - may accumulate in human tissues. These include liver, spleen, lymph nodes and adipose tissue (EFSA, 2012). EFSA did not derive guidance values for food exposures due to the concern for insufficient data on accumulation. Since then, numerous scientific studies have been published with the attempt to address this concern (Barp et al., 2014, 2017a; McKee et al., 2012; Nygaard et al., 2019), including an opinion from the German Federal Institute for Risk Assessment (BfR) (Pirow et al., 2019). This scientific review article assessed the accumulation potential and associated histopathological effects of MOSH, and concluded that there is likely a "low risk for adverse hepatic lesions that may arise from the retention of MOSH in the liver" (Pirow et al., 2019).

In 2020, the European Commission requested an updated scientific opinion on mineral oil hydrocarbons by EFSA, taking into account the new scientific data available since their last opinion was published in 2012. The intent of this manuscript is to thoroughly assess retention of "mineral oil saturated hydrocarbons" (MOSH) and evaluate if this effect is considered adverse and appropriate to use for risk assessment and for derivation of health-based guidance values. Currently, the EFSA CONTAM Panel is evaluating mineral oil hydrocarbons (MOH) including MOSH (EFSA-Q-2020-00664), which is the next milestone in the evaluation of MOSH and might be followed by possible regulatory consequences in Europe.

1.2. Composition of mineral oils

The composition of mineral oil hydrocarbons is highly dependent on the manufacturing process. As discussed earlier, various steps throughout the manufacturing and refining processes extract specific fractions of hydrocarbons. The level of total aromatics present in mineral oils varies depending on their refinement level; specifically, the level of total aromatics decreases with increasing refinement (Carrillo et al., 2022). While refinement decreases the level of total aromatics, it is the 3–7 ring aromatics which are the target of mineral oil refinement because these are the chemical species which have been shown to be of toxicological concern. Hence, mineral oils after refinement consist of primarily of alkanes (normal, iso and cyclo) and varying levels of total aromatics. Highly refined mineral oils, which comply with medicinal grade specifications consist virtually of only alkane sub-types.

When the on line HPLC-GC-FID chromatography methodology, developed by Biedermann et al. (2009) in 2009, is used to detect the presence of mineral oil in biological tissues, two types of hydrocarbon fractions are obtained; one consisting of mineral oil saturated hydrocarbons (MOSH) and the associated mineral oil aromatic hydrocarbons (MOAH). MOAH, which encompasses the total of aromatics in the oil, may or may not include also the 3–7 ring PAC, (Biedermann et al., 2009; EFSA, 2012), which, as indicated earlier, are targeted for removal during manufacturing process food-grade mineral oils to levels typically less than 0.1% (Carrillo et al., 2022; CONCAWE, 2017b). As in the case of MOAH, the term MOSH it is a poorly defined generic term because the one-dimensional analysis does not specify the origin or the type of alkanes it encompasses as it covers all three possible types of alkane subgroups: linear, branched and cyclic even if one of these are absent in the fraction. In other words, the chromatography results expressed under the term MOSH may refer to either a fraction that is 100% n-alkanes, or one that consists of 100% cycloalkanes (naphthenics). In either case, it is always considered MOSH. This lack of precision is a stumbling block in the interpretation of toxicological data where this distinction is key to assign biological effects to a specific alkane sub-class. One example is the confusion between mineral oils and waxes, which in the EFSA opinion of 2012 were regarded as MOSH (EFSA, 2012), even though compositionally they are different.

Normal (n)-alkanes (also called linear or n-paraffins) are mostly absent in mineral oils; the n-alkanes are intentionally removed during the de-waxing of the waxy raffinate. The mono- and multi-branched alkane group (also called iso-paraffins) consist of linear alkanes carrying often one or more methyl substituents. Whether a given mono- or multi-branched alkane will be present in an oil is dependent on the position of the methyl substituent on the carbon chain (CONCAWE, 2017b). Mono-branched alkanes with the methyl substituent near the end of the chain will partition to waxes and be removed during the de-waxing process, and those with the substituents in more internal positions will reside in the oil. Similar to the branched alkane group, cycloalkanes (also called naphthenes) with a saturated ring located at an end of a carbon chain will likely partition with hydrocarbon waxes, and if the ring is methylated, or the carbon chain contains internal branching, the cycloalkane will become a constituent of the hydrocarbon oil. Petroleum based oils contain one alkane structural class that is typical of mineral oil; Polycyclic alkanes (or polycondensed cycloalkanes). These are typically hydrocarbon constituents with more than one 6-membered ring. In oils, these are mostly fused rings with decreasing occurrence as the number of fused rings increases. They are further substituted with several, mostly methyl but also other short side chains over the ring system. Hydrotreatment of an oil increases the proportion of naphthenics by converting MOAH structures into saturated ring structures.

Of some of the thousands of possible isomers of the different structures described above, the differences between them are in the number, position or distribution over the backbone of a limited number of short, mostly methyl side chains of chemically inert molecules. What distinguishes petroleum waxes from oils is that the linearity of their backbone and the position near the end of it, of a methyl, cyclohexyl or cyclopentyl substituent provides sufficient regularity for these molecules to fit a crystalline matrix and render them solid at room temperature. Mineral oils on the other hand, never crystallize at room temperature and are thus liquid with different viscosity grades defined by the molecular weight and structure of its alkane constituents. The combination of the molecular weight range, the presence of specific saturated hydrocarbon types and the isomers present in mineral oil drive the majority of the chemical or toxicological properties of the substance, including MOSH retention in exposed tissues.

The aim of this critical review is thus to thoroughly review the latest scientific data, and assesses whether the presence of MOSH in human and animal tissues should be considered adverse and appropriate for its use in risk assessment, generation of guidance values for food exposure and/or derivation of health-based guidance values.

2. Mineral oil saturated hydrocarbons, "MOSH"

As mentioned above, the term "mineral oil saturated hydrocarbons" or "MOSH" is defined with poor specificity in the literature, and may broadly refer to alkanes encompassed by a chromatography fraction of mineral oil origin which is constituted by three main subgroups: linear, branched and cyclic saturated hydrocarbons. The European Branch of the International Life Science Institute (ILSI Europe) broadly described the composition of MOSH as "composed by different subclasses, i.e. linear, branched and alkyl-substituted cyclo-alkanes (naphthenes)" (Hochegger et al., 2021). Despite a great deal of public and regulatory interest with regard to this extremely broad fraction of mineral oils, a specific, harmonized definition of the characteristics of the fraction encompassed by this term has not been developed. The use of this term in the literature has been focused on potential toxicological hazards with exposure to substances or articles that contain saturated hydrocarbons. The focus of this manuscript is to outline the most useful definition of MOSH and address the hazard concerns of MOSH exposure and retention in human tissues. Mineral oil saturated hydrocarbons (MOSH) is a chromatographic fraction from mineral oil origin. Consequently, MOSH is not a substance on its own, but rather an unresolved chromatography hump obtained by the applied instrumentation method (Biedermann and Grob, 2012). Its composition, consisting mostly of iso and poly-condensed cycloalkanes, may be elucidated by two-dimensional chromatography (GCxGC) (Biedermann and Grob, 2009a). Neither n-alkanes nor wax origin alkanes are considered MOSH, nor can a default hazard interpretation can be attributed to a measured MOSH fraction. The composition of MOSH in human liver versus F344 rat liver is presented in Table 1.

2.1. Body absorption of MOSH

Hydrocarbon chain length is the major determinant in the rate of absorption of alkanes by the body and by extension of those alkanes encompassed by MOSH. Studies on individual hydrocarbons have shown that absorption potential is inversely proportional to hydrocarbon chain length; low molecular weight hydrocarbons are more efficiently absorbed by the gastrointestinal tract (60% for C14) when compared to high molecular weight hydrocarbons (5% for C28) (Albro and Fishbein, 1970). No marked difference in bioavailability of the different alkane types (linear, branched and cyclic) with similar hydrocarbon numbers was observed in rats (Albro and Fishbein, 1970; Tulliez and Bories, 1975), interestingly, as the hydrocarbon number has been shown to be more relevant to mineral oil hydrocarbon uptake than the increasing complexity of the structures (Albro and Fishbein, 1970). Based on this, it is biologically plausible to assume that there exists a certain hydrocarbon chain length threshold above which is impenetrable to the cells that line the gastrointestinal tract. Studies in both animals and humans have supported this hypothesis, where a key study specifically assessing MOSH in human tissues indicated that the upper limit for saturated hydrocarbons detected in human liver and spleen was about C40 with a peak at about C27, and in other tissues was approximately C35 (Barp et al., 2014; Biedermann et al., 2015). The authors concluded that these higher molecular weight hydrocarbons are likely too large to pass through membranes in humans and thus not absorbed. Noti et al. (2003) demonstrated that the upper range of aliphatic hydrocarbons found in breast milk were a hydrocarbon length of C33. From both of these studies, the presence of hydrocarbons > C35 in humans occurred at very low levels and virtually negligible at > C40. Therefore, experimental and human data assessing individual alkanes and MOSH support the conclusion that hydrocarbons > C35 are poorly absorbed, and are not likely to pose a concern with regards to potential for accumulation in human tissues (Albro and Fishbein, 1970; Barp et al., 2014; Biedermann et al., 2015; Scotter et al., 2003). This concept of a molecular weight threshold for absorption is supported by regulatory perspectives on substances that have a molecular mass above 1000 Da (Da). The European Food Safety Authority (EFSA) states that these substances are not likely to be absorbed and are not considered to be of toxicological concern, and considers substances below 1000 Da to be toxicologically relevant (EFSA, 2008).

Longer chain alkanes are excreted by the feces, which was observed in the two most common rat models for mineral oil toxicity, F344 and Sprague-Dawley rats (Halladay et al., 2002) and for different types of mineral oil tested (Scotter et al., 2003). The remaining dose was likely absorbed through the GI and corresponded to lower molecular weight alkanes, mostly < C35. Once absorbed in the body, the fate of the absorbed alkanes is dependent on the uptake by the liver and subsequent metabolism. When the body absorbs the mineral oil constituents at a rate faster than it can be metabolized or excreted, the alkanes are then partitioned to other tissues and retained.

Table 1
Liver Absorption of Hydrocarbon Types by Test Subject. Eliminated or limited absorption (−); Retained (+).

	F344		Humans	
<C20	-	(Barp et al., 2017b; McKee et al., 2012; Scotter et al., 2003)	-	(Barp et al., 2014; Biedermann et al., 2015)
C20–C25				
n-alkanes	±	(Barp et al., 2017b; Scotter et al., 2003)	-	Biedermann et al. (2015)
iso-alkanes	+	(Barp et al., 2017a; Scotter et al., 2003)	+	Biedermann et al. (2015)
cyclo-alkanes	+	Barp et al. (2017a)	+	Biedermann et al. (2015)
C25–C35				
n-alkanes	+	(Barp et al., 2017b; Scotter et al., 2003)	-	Biedermann et al. (2015)
iso-alkanes	+	(Barp et al., 2017a, 2017b; Scotter et al., 2003)	+	Biedermann et al. (2015)
cyclo-alkanes	+	Barp et al. (2017a)	+	Biedermann et al. (2015)
>C35	-	(Barp et al., 2017a, 2017b; McKee et al., 2012; Scotter et al., 2003)	-	Biedermann et al. (2015)

2.2. Retention of MOSH

In its 2012 Scientific Opinion on Mineral Oil Hydrocarbons in Food, EFSA stated that certain mineral hydrocarbons (branched and cycloalkanes) may accumulate in human tissues. These include liver, spleen, lymph nodes and adipose tissue. The focus on the presence of mineral oil hydrocarbons in tissues is on those within the C16–C35 range (EFSA 2012). Mineral oil hydrocarbons within this range are not homogenous; differences in molecular weight (i.e. hydrocarbon chain length) and structure are present. These differences between hydrocarbon types have an impact on the potential for deposition in tissues of the body.

2.2.1. Aromatic hydrocarbons

Food grade mineral oils undergo severe refining processes to minimize the aromatic hydrocarbon (MOAH) fraction to negligible concentrations (Carrillo et al., 2022). Barp et al. (2014) analyzed human tissue samples for the presence of MOAH by GC-FID and found no evidence of MOAH in the subcutaneous abdominal fat tissue, mesenteric lymph nodes (MLN), spleen, liver or lung, concluding that MOAH does not deposit in these tissues. The available data leads to the conclusion that either MOAH are metabolized and excreted efficiently in humans, or lack of exposure due to regulatory controls on food-contact materials (or both) are responsible for the absence of MOAH found in human tissues.

2.2.2. Aliphatic hydrocarbons

A thorough review of the distribution and excretion of aliphatic hydrocarbons was published by Pirow et al. (2019). Briefly, ingested

alkanes may be metabolized prior to absorption by the intestinal mucosa into fatty acids. Post absorption, alkanes are transported to the lymph towards systemic circulation. The alkanes may be transported with plasma lipoproteins through the lymph and blood to the liver, where it is metabolized and distributed to other tissues (preferably the adipose tissue). The main excretion process for n-alkanes is through metabolism, as other methods of excretion have not been shown to contain n-alkanes that were not metabolized. Metabolism occurs by different oxidative pathways, and the pathways are likely dependent on the type of alkanes (Pirow et al., 2019). If the saturated hydrocarbons are absorbed, but not completely recovered by way of excretion pathways, it can be assumed that they are retained to some extent in the tissues.

Evidence of aliphatic hydrocarbons in human tissues have been identified in the literature (Barp et al., 2014; Boitnott and Margolis, 1970a; Concin et al., 2008, 2011; Cruickshank and Thomas, 1984; Dincsoy et al., 1982; Noti et al., 2003; Wanless and Geddie, 1985). Table 1 summarizes the distribution of MOSH in the liver and compares the animal model (F344 rat) to human tissues. An associated histological finding, non-inflammatory lipogranuloma, have been observed in the liver of humans, however after thorough analysis by pathologists, these findings were deemed not associated with any adverse clinical effects (Carlton et al., 2001; Cruickshank and Thomas, 1984; EFSA, 2012; Wanless and Geddie, 1985).

Analysis of tissues from rats fed a diet with high concentrations of mineral oil demonstrated that the corresponding carbon number ranges of MOSH retained in the tissues are narrow (narrower than those found after dosage with waxes) and are proportionate to viscosity (Scotter et al., 2003) and similar to the fraction retained in humans. Differences in the structure and size of the hydrocarbons play a role in their retention in the body.

2.2.2.1. Aliphatic hydrocarbon in tissues based on hydrocarbon number. Differences in tissue deposition are related to the structure and molecular weight of the alkane molecule and thus by extension of the MOSH fraction. Barp et al. (2014) concluded that the concentrations of saturated hydrocarbons below C20 are virtually absent in the human liver and spleen. Also in humans, Noti et al. (2003) analyzed the carbon ranges of saturated hydrocarbons found in breast milk and determined that the hydrocarbons detected were ≥C23. Animal studies with rats fed saturated hydrocarbons of different molecular weight also reported the detection of negligible or low concentrations of hydrocarbons below C20 in liver and spleen (Barp et al., 2014, 2017a; Cravedi et al., 2017; McKee et al., 2012; Scotter et al., 2003).

Although a common understanding that low molecular weight hydrocarbons are more efficiently absorbed in the gastrointestinal tract when compared to high molecular weight hydrocarbons (Albro and Fishbein, 1970), the data above suggest that those lower molecular weight species are present at low to negligible concentrations in human and rat livers exposed to mineral hydrocarbons. This suggests that lower molecular weight hydrocarbons (<C20) are efficiently eliminated from the rat at a rate that prevents the potential for deposition in the body (McKee et al., 2012). This reasoning has also been deemed applicable to humans (Barp et al., 2014). Based on the available studies and analysis of animal and human data, it is reasonable to conclude that saturated hydrocarbons with a hydrocarbon chain length shorter than C20 are not retained in human hepatic tissues due to the likely rapid metabolism after absorption through the intestinal wall.

In order for a saturated hydrocarbon to be retained in tissues, it must first be absorbed. Multiple studies on human tissues show limited absorption or low presence of hydrocarbons greater than C35 in tissues (Albro and Fishbein, 1970; Barp et al., 2014, 2017a; Noti et al., 2003). This results in the conclusion (as discussed in section 2.1) that these large hydrocarbons are not able to cross cell membranes and not absorbed by the gastrointestinal system to a significant degree, thus, not able to be retained in tissues.

The remaining alkane hydrocarbon number range (>C20 – C35) found in mineral oils is absorbed across the intestinal wall more efficiently than the higher carbon number range fraction (>C35), but less efficiently than the smaller fraction (<C20). Due to the rate of metabolism, however, this alkane fraction is most able to be retained in the compartment with the highest potential for tissue accumulation. Results from a number of different studies involving both humans and animals support this conclusion. Retention of alkanes greater than C20 in F344 rats were detected in Barp et al. (2017a) where the authors observed retention of C20–C25, however, at lower concentrations than those from C26–C30. Barp et al. (2014) identified the C20–C29 range as the preferentially retained fraction in the human liver tissues studied, where the chromatographic data indicated presence of hydrocarbons in the C20–C45 range in the liver and spleen centered around C27, while adipose tissue data indicated presence of hydrocarbons in the C16–C36 range, centered on the C23 peak. Later, Barp et al. (2017a) determined that the distribution of the retained saturated hydrocarbons in the liver and spleen of rats show a maximum at C29. Concin et al. (2008) and Noti et al. (2003) reported similar findings at C23–C33. Of the hydrocarbons identified, virtually no n-alkanes were detectable in the human liver, however present in the MLN and adipose tissue (Barp et al., 2014; Noti et al., 2003). and the authors speculated that due to selective uptake, elimination and metabolic degradation, n-alkanes have low retention potential in the liver.

The composition of MOSH tends to differ dependent on the tissue location. Similarities in structure type and carbon number can be found between adipose tissue and lymph nodes, however liver and spleen contain a slightly different composition of MOSH analyzed by comprehensive two-dimensional GC with flame ionization detection (GC × GC-FID) (Barp et al., 2017a; Biedermann et al., 2015). A number of animal studies with high exposures to mineral oils identified mineral oil component concentrations within tissues that were greater than controls (Baldwin et al., 1992; Cravedi et al., 2017; Firriolo et al., 1995; McKee et al., 2012; Smith et al., 1996; Trimmer et al., 2004). The liver is the primary tissue type that is studied for MOSH retention; the highest concentrations in F344 rats exposed to high doses of mineral oil in the feed are in the liver, the second highest concentration can be found in the spleen, and then followed by the adipose tissue (Barp et al., 2017a). F344 rats (especially females) had a higher mineral oil hydrocarbon concentration in the liver than that of Sprague-Dawley rats (Firriolo et al., 1995) or than in humans (Barp et al., 2014).

An animal study was performed by Trimmer et al. (2004) with F344 rats chronically exposed to up to a high dose of two different paraffinic white oils, P70H and P100H. The hydrocarbon ranges for P70H and P100H were C27–C43 and C28–C45, respectively. The study evaluated the concentrations of mineral oil hydrocarbon in the animal tissues at various time points during the study. A maximum hepatic concentration of retained was reached in 3 months in the P70H group and 18 months in the P100H group, respectively. This delay in reaching maximum hepatic concentration in the P100H group containing a hydrocarbon range slightly lower than the P70H group, thus a higher concentration of saturated hydrocarbons with less than C30. This supports that the uptake of alkane subtypes in the >C20–C35 hydrocarbon range have higher absorption and lower rates of metabolism, as the P70H oil contained a higher concentration of alkanes with this hydrocarbon range than the P100H oil, thus reaching a steady state faster than the P100H, which contains more alkane structures that would likely not be absorbed (>C35).

2.2.3. MOSH - Alkane types in human tissues

Mineral oils contain various types of saturated hydrocarbons; predominantly branched alkanes and cycloalkanes; n-alkanes may be present in low viscosity oils but at low concentrations. The alkanes with carbon numbers > C20–C35 are the most likely to be retained and it is important to understand what types of alkanes within that hydrocarbon range are likely to be found in tissues.

There are a number of studies that identified mineral oils present in human tissues (Barp et al., 2014; Concin et al., 2008, 2011; Cruickshank and Thomas, 1984; Dincsoy et al., 1982; Noti et al., 2003; Wanless and Geddie, 1985). The specific fraction retained in tissues has been explored initially by Boitnott and Margolis (1970b), who used a molecular sieve to determine that the predominant saturated hydrocarbon structures present in the examined human tissue samples were branched chain and cyclic alkanes. Mass spectrometric analysis supported this conclusion by indicating that the retained hydrocarbon fraction is a complex alkane mixture with high concentrations of polycycloalkanes (Boitnott and Margolis, 1970b). n-Alkanes are likely either more efficiently eliminated or not retained to any appreciable level when compared to cyclic or branched alkanes; tissue samples that contained the higher proportions of n-alkanes contained the lowest concentration of total saturated hydrocarbons (Boitnott and Margolis, 1970b).

The ability of detection and identification of alkane subtypes in human tissues has increased dramatically in the last decade. Specifically, analysis of tissues has been performed by on-line normal phase high performance liquid chromatography (HPLC)-GC-flame ionization detection (FID) (Biedermann et al., 2009; Biedermann and Grob, 2012). Barp et al. (2014) speculated that structures such as cyclo- and branched hydrocarbons would be metabolized at a slower rate than linear alkanes and thus were predominantly retained in tissues. Barp et al. (2017a) demonstrated that a series of dominant branched open chain alkanes resisted elimination, however, it was concluded that cycloalkanes (also called naphthenes) are primarily retained in tissues (Biedermann et al., 2015). More specifically, branched alkylated (poly)naphthenes are predominantly accumulated (Barp et al., 2017a). As discussed above, studies on human tissues identified a range of saturated hydrocarbons in the size range of C20–C35 (Barp et al., 2014; Concin et al., 2008; Noti et al., 2003).

Human liver tissues are nearly depleted of n-alkanes, and Biedermann et al. (2015) identified a "gray cloud" in the GCxGC FID plots of human tissues that was consistently present in all human tissues analyzed, as well as in samples of 'paraffin oil'. Patterns of hydrocarbons were similar within the liver and spleen, while slightly shifted to higher in hydrocarbon number when compared to the mesenteric lymph nodes (MLN) and fat tissue. The authors concluded that this cloud represented highly isomerized hydrocarbons, and is likely due to exposure to paraffin oil (Biedermann et al., 2015). Based on this data, this gray cloud in the C20–C35 range consisting of mostly highly branched and alkylated cycloalkanes represents the fraction of retained mineral oil constituents. Thus, in this paper we focus on analyzing adversity of MOSH retained in human tissues, which is the C20–C35 range and consists of mostly highly branched and alkylated cycloalkanes.

3. Biological significance of MOSH retention

As mentioned above, the focus on mineral oils in food-grade application began with the discovery of liver microgranulomas occurring uniquely in F344 rats fed high concentrations of mineral oils, however the strain-specificity of this effect has become increasingly clear (Adenuga et al., 2017; Carrillo et al., 2021), and the focus has shifted to the accumulation of mineral oils fractions in tissues (i.e. MOSH). These more recent concerns arose from a series of papers evaluating saturated hydrocarbons present in human breast milk and fat tissues (Concin et al., 2008, 2011). Concin et al. (2011) found that a predominant factor of saturated hydrocarbon accumulation in human fat tissue was age and concluded that, if the source of the mineral hydrocarbons were from food, that there was a potential for progressive accumulation. Additionally, it has been proposed that setting a health-based guidance value for mineral oils should be based on hydrocarbon accumulation, as opposed to the liver granulomas found in F344 rats (Barp et al., 2014; Grob, 2018).

Evidence for retention in human tissues has been presented by a number of different studies, which show the presence of similar saturated hydrocarbon structures in different tissues of humans (Barp et al., 2014; Concin et al., 2008, 2011; Cruickshank and Thomas, 1984; Dincsoy et al., 1982; Noti et al., 2003; Wanless and Geddie, 1985). The presence of a narrow MOSH fraction, seen as a "gray cloud" in two dimensional GC analysis in human tissues, especially the liver, and the unknown potential for accumulation has been considered by others as an endpoint of more relevance than liver granulomas.

The term 'accumulation' is often used interchangeably with 'bioaccumulation'; the Organisation for Economic Co-operation and Development (OECD) defines both accumulation and bioaccumulation as "Increase of the amount of a substance over time within tissues (usually fatty tissues, following repeated exposure); if the input of a substance into the body is greater than the rate at which it is eliminated, the organism accumulates the substance and toxic concentrations of a substance might be achieved" (OECD, 2010). Bioaccumulation is also defined as a "progressive increase in the amount of a substance in an organism or part of an organism which occurs because the rate of intake exceeds the organism's ability to remove the substance from the body" (IUPAC, 2007).

Human exposure to environmental substances can be at the level of multiple physiological compartments; these individual compartments may differ in the concentrations of the xenobiotic substance of interest. In this case the individual compartments are considered discrete organs, and it is important to note that the kinetics of eliminations may differ within each of the different compartments (organs). Tissue burden within the individual compartments, in some cases, increases initially over time, reaching a steady-state tissue concentration where the rate of substance uptake is equal to substance redistribution/elimination rates. At this steady state, the tissue concentration measured is considered the maximum tissue burden, even at constant exposure. If the concentration of a substance reaches a steady state in the tissue, then this is no longer considered accumulation according to the definition outlined above, as accumulation is defined as a progressive increase. This section will outline the available evidence that MOSH concentrations reach a steady state in some animal and human tissues, specifically the key organ for risk assessment, the liver, and thus should not be considered 'accumulation', rather 'retention'.

3.1. Steady state

A number of repeated dose animal studies monitored the concentrations of mineral oil residues found in tissues and suggested that a steady state of tissue retention can be reached. Trimmer et al. (2004) measured hepatic concentrations of saturated hydrocarbons in F344 rats throughout a two-year dietary study with P70(H) and P100(H) mineral oils (mineral oils from paraffinic crudes of ~70 cSt and 100 cSt viscosity at 40 °C, hydrofinished). The study concluded that maximum hepatic concentrations of saturated hydrocarbons were reached for both oils, however the time to reach a steady state differed. The F344 rats fed 1200 mg/kg P70H oil reached a maximum hepatic concentration after 3 months of treatment, while the rats treated with 1200 mg/kg P100H reached a steady state after 18 months of exposure. As mentioned earlier, the P70H oil (C27–C43) contained a higher concentration of alkanes in the saturated hydrocarbon range than the P100H oil (C28–C45), thus reaching a steady state faster than the P100H, which contains more alkane structures that would likely not be absorbed (>C35) (Trimmer et al., 2004). In the study by Barp et al. (2017a), F344 rats were treated with two different mineral oil fractions (an oil fraction largely below C25 (S–C25) and a fraction largely above C25 (L-C25) at doses of 400, 1000 and 4000 mg/kg were administered in the feed during a treatment period of 120 days. MOSH in the rat liver increased in the first 30 days of exposure, and slowed to reach a plateau between 90 and 120 days (Barp et al., 2017a). This is supported by an additional study with a wide range mixture of mineral oils that demonstrated a levelling off of MOSH concentration after 90 days, showing no difference between day 90 and 120 at 4000 mg/kg feed (Barp et al., 2017b).

A thorough study on F344 rats using a broad MOSH mixture (Cravedi et al., 2017), concluded that a steady state in the liver had not been reached after 120 days of exposure, however for the highest dose group (4000 mg/kg), an apparent MOSH concentration plateau had been reached between 90 and 120 days of exposure, indicating a threshold for liver MOSH retention.

A similar steady state can be observed in humans with respect to age. If no steady state occurred in humans, assuming constant exposure throughout life, a continual increase in hydrocarbon deposition would be observed as humans age. Wanless and Geddie (1985) found that liver lipogranulomata (cluster of oil droplets as a result of saturated hydrocarbon retention) occurrence correlated with an increase in age from 465 human liver samples, but reached a plateau at around age 50, after which, no increase in lipogranulomata was observed. If accumulation of saturated hydrocarbons through dietary exposure were likely to occur in humans, increased tissue levels would be expected to be a function of age throughout an entire lifetime.

A study with a smaller sample size (142) evaluating levels of hydrocarbons in subcutaneous fat in women undergoing elective Cesarean section reported no correlation between saturated hydrocarbon levels in the tissues and nutritional habits of the women. However, multivariate analysis showed that other parameters, including age, body mass index (BMI), country of residence, number of previous childbirths and use of cosmetics all were significant predictors of saturated hydrocarbon levels in fat (Concin et al., 2011). This correlation with age is important to note, however, the group of women in this cohort were within the ages of 19–47, below the age of the lipogranulomata plateau that was observed in the study by Wanless and Geddie (1985), and therefore would not be expected in this age group. If dietary intake of hydrocarbons throughout life would result in life-long accumulation, one would expect nutritional habits to also be correlated with the hydrocarbon levels in the diet. Additionally, it is unclear that if other underlying factors skewed the data towards a certain age bracket; a subset of these subjects had underlying diseases such as diabetes mellitus and/or hyperlipidemia, which have been associated with the presences of lipogranulomata in the liver and spleen, and are distinct from oil droplets associated with mineral oil exposure (Wanless and Geddie, 1985). Furthermore, a steady state has been identified in liver tissue in animals exposed to high concentrations of mineral oil, however, a steady state has not been identified in adipose tissues in rats (Barp et al., 2017a), nor MLNs, as discussed later (Section 3.2, Reversibility), which could be a potential contributor to the lack of steady state with respect to age in the subcutaneous fat tissues in this study. These data could suggest a potential for MOSH deposition in adipose tissues and MLNs, however, in the liver, the data suggest the presence of MOSH reaches a steady state in humans and animals. For the assessment of potential MOSH adverse effects it is not the subcutaneous fat tissues, but rather the liver that is the tissue of interest (EFSA, 2012).

Barp et al. (2014) evaluated saturated hydrocarbon levels in several different tissues (from 37 subjects) in a wider age spread (25–91 years), and concluded that there was no correlation with saturated hydrocarbon concentrations throughout various tissues in each subject. For example, the subjects with the highest concentrations of the cohort in certain tissues (spleen, MLN and lung), had less than half the concentration of mineral oil hydrocarbons of the subject with the highest concentration in the liver. This finding was inconsistent with the assumption that persistent exposure to saturated hydrocarbons in the diet would result in high saturated hydrocarbon content in tissues as has been reported in F344 rats.

Overall, the data suggests that although MOSH fractions are present in the tissues of humans, most humans are at a steady state with the environment and an increasing MOSH level in the liver is not constantly occurring. Based on this information, as mentioned above, the term ‘accumulation’ is not entirely accurate, and may be misleading, therefore the term ‘retention’ is a more accurate representation of the detection of mineral hydrocarbons present in human tissues of toxicological relevance.

3.2. Reversibility

Barp et al. (2017a) concluded that safety of high concentration of MOSH in human tissues needed to be re-evaluated, considering endpoints other than lipogranulomas. This is an important consideration if, in fact, concentrations with endpoints other than granulomas occurred, and if metabolism, and steady state in humans did not prevent retention of high concentrations of MOSH.

When evaluating the potential for retention, it is important to note that this concept assumes that the substance in question remains in the tissue, however, animal studies have shown that there is a significant reduction in the concentration of saturated hydrocarbons present in the tissues after a period of recovery post exposure. A number of studies with recovery arms (Barp et al., 2017a; Cravedi et al., 2017; Smith et al., 1996; Trimmer et al., 2004) on F344 rats with approximately 30 day recovery period show a reduction in the concentrations of saturated hydrocarbons within the liver of the rats. Barp et al. (2017a) observed a 30 day recovery period after which liver MOSH levels were reduced from 34 to 60% depending on the dose groups; the higher the exposure group, the lower the reduction after 30 days. Interestingly, in adipose tissue, the MOSH continued to increase after the recovery period, likely due to transfer from other tissues. The recovery period of just 30 days resulted in a total of 50% reduction in the retained MOSH from the rat body. Another study exposing F344 rats to a broad MOSH mixture observed a reduction in greater than 30% in the liver after 90 days of exposure, and a 30 day recovery period (Cravedi et al., 2017).

Smith et al. (1996) performed a study on multiple oil types with varying viscosities and/or hydrocarbon ranges, and observed statistically significant increases of saturated hydrocarbons in tissues with the F344 rats exposed to up to 20,000 ppm N10A (mineral oil from naphthenic crude of ~10 cSt viscosity at 40 °C, acid treated), N15H, P15H (mineral oils from paraffinic crudes of ~15 cSt viscosity at 40 °C, hydrofinished), N70A, N70H, and P70H. After a 28-day recovery period, liver saturated hydrocarbon content was reduced up to 30 and 50% in females and males, respectively. In the MLN, however, statistically significant increases of saturated hydrocarbons were observed (higher concentrations in female rats) with no apparent decrease. Similarly, in samples of adipose tissue, saturated hydrocarbon content increased after 90-day exposure, however, experienced limited reversal after the 28-day recovery period (Smith et al., 1996).

Perhaps the most impactful study, based on recovery period, was performed by Trimmer et al. (2004); this study had the longest recovery time period, and showed a decrease in hepatic levels of saturated hydrocarbons to near background levels. F344 rats were chronically exposed to up to 1200 mg/kg/d of two different white oils, P70H and P100H. The carbon ranges for P70H and P100H were C27–C43 and C28–C45, respectively. The study evaluated the hepatic concentrations of mineral oil hydrocarbons throughout the span of the study, as well as after a 12-month recovery period. A maximum hepatic concentration of saturated hydrocarbons was reached in 3 months in the P70H group and 18 months in the P100H group, respectively. The study revealed that reversibility of mineral oil residues in the liver occurs at 6 months, although incomplete, to that of background levels was seen after a 12-month recovery. Overall, the animal data supports that after a period of no mineral oil exposure, the concentration of saturated hydrocarbons retained in the tissues are metabolized and either re-distributed to other tissues and further metabolized and/or excreted.

3.3. Adversity

EFSA, in its 2012 opinion on saturated hydrocarbons in food, expressed concern that high tissue concentrations of saturated hydrocarbons are considered of potential concern. However, there is no evidence in any animal model (i.e. rats, dogs) or in humans, which tissue

concentrations of saturated hydrocarbons, specifically of the MOSH gray cloud, even in those exposed to very high doses, for long exposure periods (Trimmer et al., 2004) progress to an adverse effect.

In order to generate a health-based guidance value, a No Observed Adverse Effect Level (NOAEL) is identified in the literature from a critical study, used as a point of departure (PoD) to which uncertainty factors are applied. The crucial step is identifying the NOAEL, which is dependent on the most sensitive adverse effect seen in the study. In generating a health-based guidance value for mineral oils, the 'adverse' effect to rely on to determine the NOAEL (and the PoD) still remains debated. Ever since the liver granulomas found in F344 rats were understood to be strain-specific and n-alkane related, and therefore of questionable relevance to humans, the recently debated endpoint of interest is the 'accumulation' of mineral oil hydrocarbons in human tissues. It is important to note that an effect (such as accumulation) observed during a toxicological study may not be considered adverse, and not appropriate to use as a PoD to derive a health-based guidance value.

Pandiri et al. (2017) noted that reversibility, per se, is not sufficient to determine lack of adversity, however, this is in the context of an underlying adverse effect that would be seen at a higher dose. This is not entirely relevant in the case of MOSH retention, as at doses even higher than the limit dose show no increased retention or progression to other effects (adverse or otherwise) (Shoda et al., 1997; Trimmer et al., 2004), aside from the granulomas in F344 rats, which also have been shown to not progress in chronic, high dose studies (Shoda et al., 1997).

Adverse effects have been defined a multitude of times in the literature, and in 2011, a Health and Environmental Sciences Institute (HESI) workshop of scientific experts established considerations to guide thinking when concluding on adversity (Keller et al., 2012). The workshop was framed around the IPCS/WHO definition of an Adverse Effect: "A change in morphology, physiology, growth, development, reproduction, or life span of a cell or organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences" (IPCS, 2004). A scientific article was cited in the HESI workshop to develop this definition which proposed a framework that recommended a consistent study interpretation of the adversity of an effect (Lewis et al., 2002). This outline begins with the toxicologist differentiating the effects from control values to determine if they are treatment related or if they are chance deviations. Next, whether or not the said effects are adverse or not should be determined. To do this requires consideration of whether the effect is "adaptive; transient; the magnitude of the effect; its association with effects in other related endpoints; whether it is a precursor to a more significant effect; whether it has an effect on the overall function of the organism; whether it is a specific effect on an organ or organ system or secondary to general toxicity; or whether the effect is a predictable consequence of the experimental model." The optimal way to come to an adversity conclusion, is in combining an analysis of all of these considerations to use a weight of the evidence approach. Here, we will follow the proposed framework (Lewis et al., 2002), and evaluate the adversity, or non-adversity of the presence of mineral oil hydrocarbons present in tissues following the discriminating factors used to differentiate a non-adverse effect of treatment from an adverse effect.

3.3.1. *There is no alteration in the general function of the test organism or the organ/tissue affected*

In order to evaluate whether the general function of the test organism is affected by the presence of MOSH, specifically the liver, the data from a standard liver function panel can be used to determine the general function of the organ. Increases in certain serum enzymes present in the peripheral blood indicate disruption of the general function of the liver. They include ALP (alkaline phosphatase), ALT (alanine transaminase), AST (aspartate aminotransferase), and gamma-glutamyl transpeptidase (GGT), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), albumin, bilirubin and total protein.

A number of studies collected data on the hematology and clinical chemistry of animals treated with mineral oils. The clinical chemistry included assessments of varieties of the above listed enzymes in order to determine the general function of the liver following exposure to mineral oil hydrocarbons. Not surprisingly, slight differences based on the rat strain or species were observed. Studies using F344 rats observed slight hepatic functional disturbance (Baldwin et al., 1992; McKee et al., 2012; Smith et al., 1996), which could be related to the presence of liver granuloma, and some dependent on sex, or type/concentration of mineral oil hydrocarbons (Baldwin et al., 1992; Smith et al., 1996). However, in some cases, the clinical chemistry changes of F344 rats observed were within normal ranges (Firriolo et al., 1995; Trimmer et al., 2004). In studies with other rat strains and other species (Sprague-Dawley rats, Long Evans Rats and Beagle Dogs), no changes in clinical chemistries were observed (Firriolo et al., 1995; Hoglen et al., 1998; Smith et al., 1995).

Additionally, studies involving humans determined that the pathology of human lipogranulomas has been described as benign, with no evidence of inflammation or fibrosis, and not associated with liver dysfunction (Cruickshank and Thomas, 1984; Wanless and Geddie, 1985). Taken together, this collection of animal and human data suggest a lack in alteration in the general function of the test organism or the organ/tissue affected by the retention of MOSH.

3.3.2. *It is an adaptive response*

An adaptive response as "the process whereby a cell or organism responds to a xenobiotic so that the cell or organism will survive in the new environment that contains the xenobiotic without impairment of function" (Keller et al., 2012). Retention of saturated hydrocarbons could be considered an adaptive response using this definition.

As animals and humans are exposed to high concentrations of saturated hydrocarbons, retention occurs before and up until a steady state is reached, where the concentration of saturated hydrocarbons in the tissues remains steady as metabolism and excretion occurs (as discussed in section 3.1). A sub-chronic study in Sprague-Dawley rats with gavage administration of de-aromatized hydrocarbon solvents (hydrocarbon number range C10–C13), and although no MOSH retention was measured, moderate liver cell hypertrophy was observed (Adenuga et al., 2014). Such finding is, in the absence of any pathological alterations, considered by the CONTAM Panel to be an adaptive response and not an adverse effect (EFSA, 2012). In a 90-day study with a recovery period of 30 days, liver weight increase was observed after exposure to a mineral oil fraction largely above C25 (L-C25), up to 4000 mg/kg, which was not directly related to the retained amount of total MOSH in liver, but rather to specific chemical fractions (Nygaard et al., 2019) consisting of various multi-branched and long, less branched isoalkanes and multi-alkylated cycloalkanes (Barp et al., 2017b). Liver weights were reversible after the recovery period. Similar MOSH fractions have been found in human with no progression to pathology (Boitnott and Margolis, 1970a; Fleming et al., 1998).

The retention of absorbed saturated hydrocarbons occurs with no impairment of function, and as increased exposure continues, a steady state occurs and therefore can be considered an adaptive response.

3.3.3. *It is a transient effect*

A transient effect is one where the effect disappears during the course of treatment, such as those not directly related to the substance in question. For example, a transient effect could be a change in body weight due to the palatability of the feed as a result of the presence of the substance, or increased stress due to inhalation or gavage dosing. These transient effects usually disappear with adaptation of animal after an acclimation period. Transient effects are distinctly different than reversibility of effects, as reversibility is seen after a period without exposure (Lewis et al., 2002). Retention effects observed in animals

treated with mineral oils do not disappear after an acclimation period, and thus are not considered to be transient effects.

3.3.4. The severity is limited, below thresholds of concern

Lewis et al. (2002) addresses the importance of severity in the determination of adversity of an effect, as target organ effect severity can vary greatly. The authors intended for this consideration to take into account whether or not the severity and nature of the effects could be considered adaptive or compensatory responses. In order to be non-adverse, these responses would be below a certain threshold or not have biological significance. In certain circumstances, a clear delineation between adversity and non-adversity is established, however, often this distinction is not clearly defined and adversity is dependent on scientific judgement. Retention of MOSH in tissues is not considered a severe effect; this effect is not associated with morphological change in the organs in which it is found, nor does it cause any changes in liver function. This is with the exception of slight alterations of liver markers in the most sensitive F344 rat strain likely caused by liver granuloma, as discussed above, and in cases of extreme intoxication with mineral oil where a massive deposition can alter the liver structure (Nochomovitz et al., 1975).

Lewis et al. (2002) discussed organ weight as a threshold of adversity, however minor elevations in this endpoint that are not accompanied by morphological or functional changes are considered not adverse. For example, two long term studies observed minor changes in organ weight, however this was not accompanied by morphological or functional changes. Specifically, Shoda et al. (1997) observed an increase in liver weights of F344 rats exposed to the high dose (5% in the diet) of 3 and 4% for males and females, respectively. This was accompanied by no functional or morphological changes. Trimmer et al. (2004) observed increased spleen weights (with no dose response) and higher MLN weights. The MLN weights (relative weights increased 16%) were not statistically significant after a recovery period. Additionally, these organ weight changes were not accompanied by morphological or functional changes, except for slight increases in infiltrating histiocytes in the MLN. Taken together, the organ weight changes observed in these long term studies, according to the guidance by Lewis et al. (2002), are below thresholds of concern and are not considered adverse.

3.3.5. The effect is isolated or independent. Changes in other parameters usually associated with the effect of concern are not observed

Another consideration for the determination of adversity is if the effect of treatment occurs in isolation or not related to other endpoint effects (Lewis et al., 2002). This consideration is not observed as a result of mineral oil administration, as the MOSH levels measured in animals and human tissue samples do not show concomitant physiological changes in other organs (e.g. lymph nodes or spleen) that will raise concern.

3.3.6. The effect is not a precursor. The effect is not part of a continuum of changes known to progress with time to an established adverse effect

If the effect is not a precursor, or does not progress to adversity, an effect may be considered non-adverse (Lewis et al., 2002). Retention of MOSH has not been shown, in any species, to progress to an established adverse effect. Even taking into account the most sensitive F344 rat, liver granulomas do not progress to adverse lesions even after high dose (5% of feed) chronic exposure (Shoda et al., 1997). Although it is hypothesized that in the F344 rat model, retention of saturated hydrocarbons, specifically n-alkanes, may be responsible for the progression to granulomas (Miller et al., 1996), MOSH retention in other species (including humans) is not a precursor to any other effect. Concerns for the inflammatory granulomas observed in F344 rats prompted long term studies to determine a progression, if any, of the granulomas to an established adverse effect. Chronic studies with the most sensitive rat strain show no indication that the observed inflammatory granulomas progress to an established adverse effect, such as carcinogenicity, even at high dose levels (Shoda et al., 1997; Trimmer et al., 2004). The chronic studies were performed in order to understand the fate of the granulomas in F344 rats after time. In a chronic study (104 weeks) using a high dose of a mineral oil similar to P70H, the effect does not progress to carcinogenicity, and the granulomas in MLNs was shown to be without potential for long term progression (Shoda et al., 1997). The authors concluded that the kinetics of the retention of mineral oil in this study may have reached a plateau, and thus the possibility of developing a more severe adverse effect is not possible. Additionally, in a chronic study with F344 rats treated with chlorinated paraffins (C23 containing 43% Cl, CAS no. 63449-39-8), authors observed incidences of liver granulomas and MLN histiocytosis that did not progress to any adverse effects (NTP, 1986). Another chronic feeding study with P70H and P100H oils in F344 rats showed increased levels of saturated hydrocarbons, with marginal histopathological changes in liver and MLNs, which had effect on rat health or survival, nor were not associated with any "progressive pathological change[s]" (Trimmer et al., 2004). Furthermore, subchronic studies confirmed that the inflammatory granuloma in the liver of the F344 rats was not associated with immune responses (Nygaard et al., 2019) and that MOSH exposure did not have an impact on the immune response when challenged (Cravedi et al., 2017).

Other animals and rat strains, as mentioned earlier, have been demonstrated to retain alkanes and MOSH within tissues, however, have not been shown to progress to epithelioid granulomas as seen in the F344 rats, regardless of the viscosity of the mineral oil utilized in the study (Miller et al., 1996). The effect generally observed in F344 rats with retention of low and medium molecular weight mineral oil fractions is liver enlargement associated with granuloma formation (Baldwin et al., 1992; Firriolo et al., 1995; Smith et al., 1996), except for a study that demonstrated retention L-C25 MOSH associated with an increased, but reversible, liver weight without concomitant granuloma development (Nygaard et al., 2019). The studies with Sprague-Dawley rats or dogs did not observe an increase in liver weight (Firriolo et al., 1995; Smith et al., 1995; Trimmer et al., 2004). This lack of progression of MOSH retention, granuloma formation and subsequent liver enlargement to any other effect (adverse or otherwise) indicates that the retention of MOSH, per se, is not a precursor to another downstream established adverse effect.

3.3.7. It is secondary to other adverse effects

Observations made in toxicology studies can appear to be adverse when, in fact, they are secondary to other adverse effects. An example of this would be a systemic effect as a result of route of exposure; if an irritant is administered orally, and causes inflammation to the gastrointestinal tract, a resulting systemic effect that is secondary to the inflammation is not likely to be systemically adverse (Lewis et al., 2002). With respect to hydrocarbon retention, no primary toxicity is observed, therefore this retention could not be considered a secondary effect.

3.3.8. It is a consequence of the experimental model

MOSH retention itself is not necessarily a consequence of the experimental model. However, numerous groups have shown data to support that, although retention is seen in the models studied, the F344 model demonstrates a significantly reduced ability to metabolize and excrete when dosed with high concentrations of saturated hydrocarbons. Studies comparing rat tissue retention have elucidated that there is a difference in the concentrations of MOSH found in different rat strains, suggesting a difference in ability to metabolize the retained mineral oil fractions. A toxicokinetic study compared the metabolism of a low-viscosity mineral oil between F344 and Sprague-Dawley rats. The dose contained a radiolabeled cyclic C6 with a C20 side chain, which was a surrogate for a mineral oil saturated hydrocarbon. Fecal excretion data suggested a slightly higher alkane absorption (30% at the low dose and 12% at the high dose) for Sprague-Dawley rats than the absorption by F344 rats (24% and 8%, respectively) (Halladay et al., 2002).

Interestingly the absorption decreases with the increasing concentration of the dose.

The Sprague-Dawley rats were shown to have a higher absorption of the mineral hydrocarbons, however, toxicokinetic studies based on blood concentration-time profiles and hepatic concentrations suggested that F344 experienced a higher systemic exposure to the mineral oil hydrocarbons than did the Sprague-Dawley rats (Boogaard et al., 2012). The absorption rates and blood and liver concentrations of mineral oil hydrocarbons suggest a higher metabolic activity in the Sprague-Dawley rats than the F344. Therefore, F344 rats retain higher concentrations of alkanes in tissues compared to other rat strains and dogs.

There are differences in the retention of alkanes in animal models compared to humans. Namely, the retention of n-alkanes occurs in F344 rats (Firriolo et al., 1995; Nygaard et al., 2019), while the retention of this alkane sub-type is negligible in Sprague-Dawley rats and human tissues, especially in the liver and spleen (Barp et al., 2014; Biedermann et al., 2015; Firriolo et al., 1995; Noti et al., 2003). Nygaard et al. (2019) identified that the increased weights of the liver and spleen of the animals exposed to mineral oils were a result of iso-alkanes and (substituted) cyclo-alkanes. The study identified that the liver granuloma were induced in female F344 rats by the retention of n-alkanes with carbon numbers greater than C25, and is not dependent on the concentration of MOSH in the liver (Nygaard et al., 2019). Conversely, liver weight increase was not associated with liver granuloma when rats were fed an oil fraction virtually free of n-alkanes. This is consistent with the study of two high molecular weight oils devoid of n-alkanes (P70H and P100H), which were fed for two years to F344 where no liver weight increase was observed nor accompanying liver granuloma, indicating that liver granuloma in the F344 is solely the result of exposure to n-alkanes within a critical range of C20–C35. A critical review of the unique liver granuloma effects in the F344 due to n-alkane exposure has been recently published (Carrillo et al., 2021). This, however, does not negate the relevance of animal models when evaluating the relevance in humans. The F344 rat (Barp et al., 2017b), with the exception of the high hepatic retention of n-alkanes not relevant for humans, does retain the same MOSH gray cloud found in human tissues suggesting that exposure to this particular fraction of MOSH occurs in animals, arguably to the same qualitative extent. This demonstrated selective retention of MOSH maintains the relevance of animal models regardless of the generation of granulomas. The extent to which the F344 rats retain saturated hydrocarbons and the progression to liver granulomas compared to any other animal studied is a consequence of the experimental model.

4. Conclusion and discussion

Mineral oils are substances of petrogenic origin and contain a complex and variable combinations of saturated and aromatic hydrocarbons. The highly refined version (called white mineral oils, pharmaceutical or medicinal or food-grade white oils, liquid paraffin, or simply “mineral oils”) of white mineral oils have been safely used, for decades, in a number of sensitive end-uses, including food contact, pharmaceutical and cosmetic products. In its 2012 Scientific Opinion on Mineral Oil Hydrocarbons in Food, EFSA concluded that, although certain MOSH (mainly branched and cyclo-alkanes) may accumulate in human tissues, due to insufficient data on accumulation, guidance values for food exposures were not derived (EFSA, 2012).

Alkanes with carbon numbers below C20 are efficiently absorbed and rapidly metabolized, preventing retention. Alkanes with carbon numbers above C35 are less able to pass through cellular membranes, thus less bioavailable, preventing their significant retention. The remaining hydrocarbon chain length alkanes, >C20 - < C35, have the potential for retention in certain tissues. Recent data have shown that aromatic hydrocarbons are not found in human tissues, leading to the conclusion that they either biologically do not have the potential to be retained or are not present due to a lack of exposure attributable to regulatory controls on food-contact materials (Barp et al., 2014). The fraction of MOSH retained in human tissues, and is also present in animal tissues (highly branched alkanes and polycondensed cycloalkanes within the carbon range of C20 – C35), and can be visualized as a “gray cloud” in liver GCxGC FID plots (Barp et al., 2017b; Biedermann et al., 2015). Although this MOSH fraction is also present in animal tissues, the formation of epithelioid liver granulomas in F344 rats is not likely a consequence of the presence of this “gray cloud” fraction; hepatic epithelioid granuloma formation has been associated with the reduced metabolism and resultant retention of n-alkane structures, which is unique to the F344 and not relevant to humans (Carrillo et al., 2021; Nygaard et al., 2019).

There is no evidence to suggest that retention or accumulation of MOSH within animal or human tissues, including the liver, leads to an adverse effect in animals or humans, and the mere presence does not suggest an adverse effect in and of itself. In order to evaluate the adversity, or non-adversity of the presence of MOSH in tissues, a weight of evidence approach was followed. This approach included an evaluation of the retention effect observed in both animal models and human tissue samples which relied on the adversity framework published by Lewis et al. (2002) that contained factors for consideration to differentiate a non-adverse effect from an adverse effect.

As addressed in detail above, tissue retention of MOSH and formation of lipogranuloma in humans has been shown to be adaptive, the magnitude of the effect is minimal, it has no association with effects in other related endpoints, it has no effect on the overall function of the organism. Furthermore, the secondary and adverse effects (epithelioid liver granulomas) due to presence of n-alkanes in the liver are predictable consequences of the experimental model (F344 rats). Therefore, the adversity considerations do not apply in the case of hepatic MOSH retention. This was demonstrated following high dose chronic exposure in animals (Shoda et al., 1997; Trimmer et al., 2004).

According to the weight of the evidence, and utilization of a published adversity framework to inform that evidence, (hepatic) retention of saturated hydrocarbons is not considered an adverse effect. This has implications in a regulatory context; in particular, used of this endpoint as the point of departure to calculate a health guidance value is questionable and considered a highly precautionary approach. A health guidance value is generated based on a No Observed Adverse Effect Level (NOAEL) identified in a critical study. The NOAEL is used as a point of departure (PoD) to which uncertainty factors are applied. In generating health-based guidance value for mineral oils with broad applications, a thorough assessment of the potential adversity of all of the effects is essential. However, after a thorough assessment of the potential adversity of MOSH retention (gray cloud) in the key organ, liver, it is clearly not an ‘adverse’ effect from which a point of departure can be determined.

Mineral oil fractions can be present in both the tissues of animals and those of humans after exposure to mineral oils, which has been made clear by the increasing sensitivities and impressive advancements in the detection methods today’s technology. However, the concept of risk assessment relies on hazard and evaluating it against exposure. The exposure component of the risk assessment is clear, albeit low, based on the data showing retention of a narrow MOSH fraction of the original mineral oil substance in human tissues. Specifically, in humans, this narrow MOSH fraction (gray cloud) includes cycloalkanes, and to a lesser extent highly branched alkanes (see above sections). However, a hazard, or adverse effect, has not been identified. Although hepatic epithelioid granulomas may form in F344 rats when exposed to mineral oil substances, adversity of the effect is not evident. The human relevance of this effect is implausible based on the mechanism of granuloma formulation by way of n-alkane retention (Carrillo et al., 2021) and the lack of n-alkane retention observed in the human liver (Biedermann et al., 2015).

This manuscript thoroughly assessed the retention of MOSH in both human and animal tissues, in particular the liver as key organ, and

evaluated if the presence of MOSH is considered adverse and appropriate to use for risk assessment, generation of guidance values for food exposure and/or generation of derivation of health-based guidance values. We conclude that the mere presence of MOSH does not translate to hazard identification. Regardless of dose, animal model or sensitive rat strain, the effects identified are not considered adverse, nor do they progress to adversity. Therefore, the effect of MOSH hepatic retention is not appropriate to base a risk assessment or to generate a health-based guidance value from, as it is neither a hazard, nor an adverse effect.

CRediT authorship contribution statement

A.L. Isola: Conceptualization, Writing – review & editing. **J.C. Carrillo:** Writing – review & editing. **P. Lemaire:** Writing – review & editing. **H. Niemelä:** Project administration. **A. Steneholm:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors are employed by the companies/association as specified in the list of authors: Concawe is an association for improving environmental science for European Fuel Manufacturers. For this, we maintain REACH registration lead dossiers for 150 petroleum substances in about 4000 registrations for our 38 member companies. We also work on areas such as fuels quality and emissions, air quality, water quality, soil contamination, waste, occupational health and safety, petroleum product stewardship and cross-country pipeline performance. More information is available on our website www.concawe.eu.The work was conducted by the Concawe STF-33 on Mineral Hydrocarbons, where the authors were nominated to work as their company representatives.The open access fee for the publication will be paid by Concawe. No other financial contributions have been made.

Data availability

No data was used for the research described in the article.

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