SCIENTIFIC OPINION

Scientific Opinion on Mineral Oil Hydrocarbons in Food[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

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ABSTRACT

Consumers are exposed to a range of mineral oil hydrocarbons (MOH) via food. Mineral oil saturated hydrocarbons (MOSH) consist of linear and branched alkanes, and alkyl-substituted cyclo-alkanes, whilst mineral oil aromatic hydrocarbons (MOAH) include mainly alkyl-substituted polyaromatic hydrocarbons. Products, commonly specified according to their physico-chemical properties, may differ in chemical composition depending on the oil source. Technical grade MOH contain 15 - 35 % MOAH, which is minimised in food grade MOSH (white oils). Major sources of MOH in food are food packaging and additives, processing aids, and lubricants. Estimated MOSH exposure ranged from 0.03 to 0.3 mg/kg b.w. per day, with higher exposure in children. Specific production practices of bread and grains may provide additional MOSH exposure. Except for white oils, exposure to MOAH is about 20 % of that of MOSH. Absorption of alkanes with carbon number above C_{35} is negligible. Branched and cyclic alkanes are less efficiently oxidised than n-alkanes. MOSH from C_{16} to C_{35} may accumulate and cause microgranulomas in several tissues including lymph nodes, spleen and liver. Hepatic microgranulomas associated with inflammation in Fischer 344 rats were considered the critical effect. The no-observed-adverse-effect level for induction of liver microgranulomas by the most potent MOSH, 19 mg/kg b.w. per day, was used as a Reference Point for calculating margins of exposure (MOEs) for background MOSH exposure. MOEs ranged from 59 to 680. Hence, background exposure to MOSH via food in

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* Changes have been made to harmonise the name of the protein α_{2u}-globulin. A sentence in Section 7.2.2.1 on the occurrence of liver and mesenteric lymph node microgranulomas was corrected to better reflect the toxicological dataset for food grade mineral oil hydrocarbons. A correction in the NOAEL for low and intermediate melting point waxes was made in Section 7.5.1. An EFSA staff member was included in the acknowledgements section. The changes do not affect the overall conclusions of the opinion. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request, as is a version showing all the changes made.

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Europe was considered of potential concern. Foodborne MOAH with three or more, non- or simple-alkylated, aromatic rings may be mutagenic and carcinogenic, and therefore of potential concern. Revision of the existing acceptable daily intake for some food grade MOSH is warranted on the basis of new toxicological information.

KEY WORDS

Mineral oil hydrocarbons (MOH), alkanes, aromatic hydrocarbons, analysis, sources of MOH, human dietary exposure, toxicokinetics, toxicity, risk assessment, margin of exposure (MOE), acceptable daily intake (ADI), food contact materials.

SUMMARY

Following a request from the European Commission, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on mineral oil hydrocarbons (MOH) in Food. Mineral oil hydrocarbons occur in food both as a result of contamination and from various intentional uses in food production. In order to assess the need for possible regulatory measures as regards MOH in food, EFSA was requested to assess the risks related to their occurrence in food. More specifically, the opinion should evaluate whether new toxicity data are available and whether the current acceptable daily intakes (ADIs) are still applicable, explore whether certain classes (or subclasses) of MOH are more relevant due to their toxicity or to differences in the way they are metabolised by the human body, and identify the different background sources, other than from adulteration or misuse, of MOH occurrence in food. In addition a dietary exposure assessment was requested for the general population and for specific subgroups of the population (e.g. infants, children and people following specific diets), by taking into account the background occurrence of MOH in food. Included in the request was also to advise on MOH and food classes to be included if monitoring were to be set up for their presence in food.

The scientific literature and other sources were searched for relevant information on the subject and, for the purpose of exposure assessment, EFSA issued a call for data on the occurrence of MOH in foods. The information gathered on MOH occurrence in food was assessed and then combined with data on food consumption in European countries, taken from EFSA's comprehensive food consumption database.

Mineral oil hydrocarbons (MOH), or mineral oil products, considered in this opinion are hydrocarbons containing 10 to about 50 carbon atoms. Crude mineral oils are by far the predominant source of the MOH considered, but equivalent products can be synthesised from coal, natural gas or biomass. MOH consist of three major classes of compounds: paraffins (comprising linear and branched alkanes), naphthenes (comprising alkyl-substituted cyclo-alkanes), and aromatics (including polyaromatic hydrocarbons (PAHs), which are generally alkyl-substituted and only contain minor amounts of non-alkylated PAHs). MOH may also include minor amounts of nitrogen- and sulphur-containing compounds. Within these classes there are enormous numbers of individual components.

In this opinion, MOH have been divided into two main types, mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). MOH are derived by physical separation (such as distillation or extraction) and chemical conversion processes (cracking, hydrogenation, alkylation, isomerisation, etc.) from crude oils and/or synthetic products derived from liquefaction of coal, natural gas or biomass. Of the many commercially available products, little is known about their composition, as specifications are generally expressed in terms of physico-chemical properties (such as viscosity), related to the applications of the products. Products with the same physico-chemical specification may vary considerably in their composition, depending on the source of the oil and its processing. Food grade MOH products are treated in such a way that the MOAH content is minimised. Technical grades of MOH typically contain 15-35 % MOAH.

Because of their complexity it is not possible to resolve MOH mixtures into individual components for quantification. However, it is possible to quantify the concentration of total MOSH and MOAH fractions, as well as certain sub-classes, using methods based on gas chromatography (GC). Currently, the most efficient methods for analysis of MOSH and MOAH in food and feed comprise extraction followed by pre-separation by high performance liquid chromatography (HPLC) on-line coupled to GC with flame ionisation detection (FID). Detection limits depend on the mass distribution, the sample matrix and any prior enrichment, and can be as low as 0.1 mg/kg. Comprehensive GCxGC-FID enables a rough separation and quantification of paraffins and naphthenes in the MOSH fraction, but it is of limited practicality for routine analysis. Contamination with polyolefin oligomeric saturated hydrocarbons (POSH), e.g. from plastic bags, heat sealable layers or adhesives, may interfere with MOSH analysis. Analytical capacity to distinguish the different MOAH subclasses in food is limited. For this purpose, GCxGC appears to be the most effective method. Due to the

complexity and the variable composition of MOH mixtures, it is not possible to define certified standards of general applicability.

The CONTAM Panel identified numerous sources for the occurrence of MOH in food. Among food contact materials, sources are food packaging materials made from recycled paper and board, printing inks applied to paper and board, MOH used as additives in the manufacture of plastics, e.g. internal lubricants in polystyrene, polyolefins, adhesives used in food packaging, wax paper and board, jute or sisal bags with mineral batching oil, lubricants for can manufacture and wax coating directly applied to food. Food additives, processing aids and other uses contribute to MOSH levels, together with release agents for bakery ware and sugar products, and oils for surface treatment of foods, such as rice and confectionery. MOH are used in feeds as binders for minor additives added as powder. Paraffinic waxes are authorised for use in e.g. chewing gum and coating of certain fruits, and in pesticide formulations. Further uses of MOH are as defoamers and as anti-dusting agents for cereal grains. Environmental contamination sources are lubricating oil from engines without a catalyst (mainly diesel), unburned fuel oil, debris from tyres and road bitumen. Further sources are machinery used for harvesting (diesel oil, lubricating oil) and food processing, e.g. lubricating oils in pumps, syringe type dosing and other industrial installations. In addition, solvents consisting of individual alkanes or complex MOH mixtures containing cyclic- and open chain alkanes defined by their chromatographic co-elution with n-alkanes of carbon numbers ranging from C_{10} to C_{14}, used as cleaning agents, may contaminate food products.

Occurrence data on MOH were available only for a limited number of food groups and only from a few countries. These data partly originated from targeted sampling. Nearly all data refer to total MOSH and little information is available on specific sub-classes, such as cyclic, branched and linear alkanes. MOAH measurements were not available for the majority of the samples, but MOAH concentrations can be estimated based on the typical composition of the mineral oil product detected. For MOH found in food, the number of carbon atoms typically ranges from 12 to 40.

MOSH are present at different levels in nearly all foods. In the available dataset (except for the high values for 'Bread and rolls' and 'Grains for human consumption' (mainly represented by rice), which showed a bi-modal distribution of occurrence values) the highest mean occurrence values were found in 'Confectionery (non-chocolate)', 'Vegetable oil', 'Fish products' (canned fish), and 'Oilseeds' varying from 38-46 mg MOSH/kg), followed by 'Animal fat', 'Fish meat', 'Tree nuts' and 'Ices and desserts' varying from 14-24 mg MOSH/kg).

The food groups 'Bread and rolls' and 'Grains for human consumption' showed some high values that could be due to the use of MOSH as release agents or spraying agents, respectively. In these cases, the distribution of values was modelled using maximum likelihood log-normal fitting in order to identify a mean value for both the 'background' occurrence and the additional high levels of occurrence. The resulting mean background occurrence values for 'Bread and rolls' and 'Grains for human consumption' were 1.8 and 4.1 mg/kg, respectively. The resulting mean occurrence values for high levels of MOSH in the same food groups were 532 mg/kg and 977 mg/kg. In contrast to the background occurrence, which is of unknown composition and most likely contains MOAH, these high values arise from food grade white oils, which are virtually free of MOAH.

Occurrence data on dry foods which could be attributed to the use of recycled paperboard packaging were available from two different surveys. Mean concentrations of MOH were up to 32 mg/kg for MOSH found in creme/pudding mix and 4.5 mg/kg MOAH found in noodles. Maximum occurrence values were 100 mg/kg in semolina and 17 mg/kg in noodles, for MOSH and MOAH, respectively.

Chronic exposure was estimated for different age classes of the population based on mean occurrence values in the different food groups. These values were considered to represent background occurrence, normally expected in the respective food groups. Dietary exposure to MOSH ranged in the general population across European dietary surveys between approximately 0.03 and 0.3 mg/kg

body weight (b.w.) per day and was higher in younger consumers than in adults and the elderly. The highest exposure estimate per kg b.w. was for high consumers among children 3 to 10 years old.

The percentage contribution to background chronic exposure was calculated for the different age classes. Main food groups contributing to the exposure include 'Animal fat', 'Bread and rolls', 'Confectionery (non chocolate)', 'Fine bakery wares', 'Fish meat', 'Fish products (canned fish)', 'Ices and desserts', 'Pasta', 'Sausages', 'Vegetable oil'.

Additional exposure on top of the background was calculated for specific consumers of 'Bread and rolls' or 'Grains for human consumption' with high levels of MOSH originating from release- or spraying agents. Although it would not be appropriate to include such discrete high values in the background occurrence, for calculation of chronic exposure, it cannot be excluded that some groups of consumers (buying always from the same source or having brand loyalty) are exposed on a regular basis to food with such levels. Excluding infants, the additional exposure across European dietary surveys and age classes is in the range 0.7-6.4 mg/kg b.w. per day for the 'Bread and rolls' scenario and in the range 0.02-3.8 mg/kg b.w. per day in the 'Grains for human consumption' scenario.

For the subgroup of exclusively breast-fed infants, an exposure of roughly 0.3-0.5 mg/kg b.w. per day was estimated.

Whereas the background exposure to MOAH via food can be estimated to be roughly 20 % of the exposure to MOSH, additional high exposure to white mineral oils used as release agents for treatment of bread or for spraying of grains would not imply any increase in MOAH exposure.

Exposure to MOSH and MOAH attributed to extensive migration from recycled paper and board packaging without an internal barrier was estimated based on limited occurrence data. This indicated that toddlers and other children were the age classes of consumers potentially more exposed to MOH. Exposure to MOSH, for toddlers and other children was up to 0.04 mg/kg b.w. per day from bakery wares, 0.07 mg/kg b.w. per day from breakfast cereals and 0.11 mg/kg b.w. per day from rice. These estimates indicate that the migration from recycled paper packaging could contribute significantly to the total exposure.

Absorption of alkanes may occur through the portal and/or the lymphatic system. For n- and cyclo-alkanes the absorption varies from 90 % for C_{14}-C_{18} to 25 % for C_{26}-C_{29}. The absorption further decreases with increasing carbon number, until above C_{35} when it is negligible. Limited data suggest that cyclo-alkanes are absorbed at similar levels as n-alkanes of comparable molecular weight, whereas absorption of branched alkanes is slightly less. Alkanes are initially oxidised to the corresponding fatty alcohols by the cytochrome P450 system, subsequently biotransformed to fatty acids and in some cases subjected to the normal β-oxidation pathway. This reaction is more rapid for n-alkanes than for branched- and cyclo-alkanes. Due to low biotransformation rates, in particular for some branched- and cyclo-alkanes, MOSH having carbon number between 16 and 35 may accumulate in different tissues including adipose tissue, lymph nodes, spleen and liver. In rats, the terminal half-life of MOSH in blood (estimated from P15(H) white oils) was between 23 and 59 hours, depending on the strain. However, this reflects the elimination of the easily degraded MOSH. Although limited information exists on toxicokinetics of MOAH, the available data indicate that these compounds are well absorbed and are rapidly distributed to all organs. The data also indicate that MOAH are extensively metabolised and do not bioaccumulate. The concentration of MOSH in human tissues (mainly lymph nodes, liver, spleen and adipose tissue) demonstrates that accumulation of these compounds, mostly branched- and cyclo-alkanes, occurs in humans.

MOSH and MOAH have low acute oral toxicity and acute toxicity is not relevant in the context of the pattern of MOH exposure via food. Low molecular weight alkanes can cause α_{2u}-globulin related nephrotoxicity in male rats. This effect is known to be of no biological relevance to humans. MOSH mixtures with carbon number in the range C_{10}-C_{13} caused moderate liver cell hypertrophy, but in the absence of pathological effects the CONTAM Panel did not consider this to be an adverse effect.

In rats, bioaccumulation of MOSH can lead to formation of microgranulomas in liver and mesenteric lymph nodes (MLN). Microgranulomas in MLN are considered of low toxicological concern because they are not associated with an inflammatory response or necrosis, do not progress to adverse lesions and available studies did not show any effect on immune functions. In rat liver, microgranulomas were associated with inflammatory reactions.

In humans exposed to MOSH, microgranulomas have been observed in liver, spleen, lymph nodes and other organs, but these changes have not been associated with inflammatory reactions or other adverse consequences. There is no information on exposure levels at which these effects occur in humans.

In arthritis-prone rodent models, intradermal and intraperitoneal injections of high doses of certain MOSH can alter immune function or induce autoimmune responses. Weaker effects were observed following short term exposure through abraded skin. Whether long term oral exposure would have similar consequences is unknown although one short term study suggests this might not be the case.

All MOH are mutagenic unless they are treated specifically to remove MOAH. The mutagenicity of MOH is caused mainly by 3-7 ring MOAH, including non-alkylated PAHs. These PAHs are mainly formed by the heating of the oil, and are a minor fraction of MOAH. Some of these are covered by monitoring programmes in food. Many MOAH with three or more aromatic rings and little or no alkylation, and heterocyclic-containing analogues, can be activated by P450 enzymes into chemically reactive genotoxic carcinogens. These also form DNA adducts. MOSH are not carcinogenic, though long chain MOSH can act as tumour promoters at high doses. Some highly alkylated MOAH can also act as tumour promoters, but they are not carcinogens themselves. Some simple MOAH, such as naphthalene, are carcinogenic by a non-genotoxic mode of action, involving cytotoxicity and proliferative regeneration.

In view of the complexity and the lack of information on the composition of MOH mixtures and inability to resolve these into single compounds, it is not meaningful to establish health-based guidance values on the basis of studies on individual components. Hence, if possible, whole-mixture studies should be used for this purpose.

For MOAH mixtures there are no dose-response data on the carcinogenicity and hence it is not possible to establish a Reference Point (RP) upon which to base a margin of exposure (MOE) calculation, which would normally be the approach for the risk characterisation of MOAH mixtures.

The CONTAM Panel considered the formation of liver microgranulomas produced in Fischer 344 rats to be the critical effect of MOSH with carbon number between C16 and C35. From the available information on the different white oils and waxes tested in toxicological studies it is not possible to differentiate between subclasses (e.g. n-, branched- or cyclo-alkanes) of MOSH. Studies used to identify the respective no-observed-adverse-effect levels (NOAELs) were 90-day studies. The published data did not allow modelling of the dose-response data of the different studies. The CONTAM panel concluded that these NOAELs could potentially be used to select RPs for establishing health based guidance values.

The existing ADIs have been established for specific products intended for food use. The current classifications of food grade-MOH were set by SCF (1995), JECFA (FAO/WHO, 2002) and EFSA (2009), and are all based on toxicological studies with poorly characterised products with regard to chemical composition. Ideally, MOSH mixtures should be assessed by considering the molecular mass range and subclass composition (e.g. n-, branched- or cyclo-alkanes), rather than on physico-chemical properties such as viscosity. Based on new information about the lack of toxicological relevance for humans of the effects in MLN observed in the (sub)chronic studies in Fischer 344 rats and on newly available toxicokinetics studies, the CONTAM Panel concluded that a revision of the existing ADIs, particularly the temporary group ADI established by JECFA for medium- and low-viscosity mineral oils class II and III is warranted. With respect to microcrystalline waxes, high-

viscosity mineral oils and medium- and low-viscosity class I mineral oils, the existing ADIs are of low priority for revision, although they are based on products with a poor chemical characterisation.

With respect to background exposure to MOSH mixtures via food the distribution of carbon numbers range from C_{12} to C_{40} with centres ranging from C_{18} to C_{34} in different foods. None of the existing ADIs was considered adequate for the risk characterisation of the range of MOH present in the background exposure of humans. In the absence of toxicological studies on MOSH mixtures typical of those humans are exposed to, the CONTAM Panel considered it inappropriate to establish a health based guidance value for MOSH. Given the deficiencies in the toxicity data base, the CONTAM Panel decided to use an MOE approach and for the background exposure selected as an RP the NOAEL of 19 mg/kg b.w. per day for the most potent MOSH grades for formation of microgranulomas in the liver, the low and intermediate melting point waxes. The range of MOSH grades involved in the high exposure scenarios (MOSH used as release agents for bread and rolls and for spraying of grains) is more restricted than that for the background exposure and therefore the CONTAM Panel used the highest NOAEL below the lowest LOAEL (lowest-observed-adverse-effect level) for these grades of MOSH, 45 mg/kg b.w. per day, as an RP.

In the risk characterisation, the background exposure to MOAH and MOSH, and two high exposure scenarios for MOSH were considered. The MOAH content of MOH present in food are mostly around 20 %, but may in vegetable oil and oil seeds be up to 30- 35 % of the MOH levels. The MOAH fraction may be both mutagenic and carcinogenic, but no MOE for MOAH exposure via food could be derived. Because of its potential carcinogenic risk, the CONTAM Panel considers the exposure to MOAH through food to be of potential concern.

For MOSH background exposure from all sources, the MOEs for average consumption (based on maximum UB and minimum LB exposure across European dietary surveys, respectively) for toddlers and children and for adolescents and adults were from 100 to 290 and from 200 to 680, respectively. For high consumers of the same groups MOEs varied from 59 to 140 and from 95 to 330, respectively.

In the high exposure scenarios with regular consumption of bread and rolls with high contents of MOSH, the MOEs (based on maximum and minimum exposure across European dietary surveys, respectively) were from 16 to 55 for average consumption, and in some cases were below 10 for high level consumption of bread and rolls. For the regular mean consumers of grains, the MOEs varied greatly between different age classes and were between 35 (toddlers, maximum exposure across European dietary surveys) and 1 900 (other children, minimum exposure across European dietary surveys), and between 12 (toddlers) and 200 (elderly) for high consumers.

The CONTAM Panel took into account that the RPs were based on 90-day studies and that some of these compounds might have very long elimination half lives in humans when interpreting the obtained MOEs. In view of this, the CONTAM Panel considers that there is potential concern associated with the current background exposure to MOSH in Europe and in particular in the situation of use of white oils as release agents for bread and rolls and to some extent for spraying of grains.

The CONTAM Panel has identified MOH classes to be included if monitoring were to be set up for the presence of MOH in food. Total MOSH and MOAH should be separately determined. Among MOSH, sub-classes should be distinguished based on molecular mass ranges and structure. Two sub-classes were identified based on molecular mass: MOSH up to n-C_{16} and MOSH from n-C_{16} to n-C_{35}. Based on the MOSH structure, distinction should be made among n-alkanes, branched alkanes and cyclic alkanes. Additionally, hydrocarbons with structures similar to MOSH, such as poly alpha olefins and oligomeric polyolefins (POSH), should be distinguished from the MOSH. The total MOAH should be separately quantified. However, routine monitoring of subclasses of MOAH is presently not feasible. Improvement of the analytical methods to allow separation of MOAH in subclasses is recommended. The CONTAM Panel recommended the identification of the sources of contamination at various stages of food production, to design an effective monitoring programme.

With respect to the food classes to be included in monitoring, those food groups making a relevant contribution to the background exposure, including the particular cases related to use of white oils, should be taken into account. It is recommended to investigate whether other food groups not included in the present evaluation also make a relevant contribution to total chronic exposure.

A significant source of dietary exposure to MOH may be contamination of food by the use of recycled paperboard as packaging material. It can be effectively prevented by the inclusion of functional barriers into the packaging assembly. Other measures may include segregation of recovery fibre sources intended for recycling and the increasing of the recyclability of food packages by avoiding the use of materials and substances with MOH in the production of food packages.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

I. Historic background

The Rapid Alert System for Food and Feed (RASFF) was notified on 23 April 2008 that sunflower oil originating from Ukraine was found contaminated with high levels of mineral oil. Several shiploads of such oil had been exported to a number of Member States.

Urged by the European Commission to provide information on the origin of the contamination and on the measures taken to prevent such presence in the future, the Ukrainian authorities committed to the establishment of an appropriate control system that would ensure that all consignments of sunflower oil to be exported to the European Union are certified as not containing levels of mineral oil making the sunflower oil unfit for human consumption. Awaiting the assessment of this control and certification system, Commission Decision 2008/388/EC ensured that no exports of sunflower oil to the Community took place.

On 28 April 2008, the European Commission sent an urgent request for an assessment of the risks related to the contamination of sunflower oil with mineral oil to EFSA. Based on the limited amount of available analytical data indicating that the mineral oil present was of high viscosity and on exposure estimates, EFSA's initial considerations concluded that the exposure of sunflower oil contaminated with high viscosity mineral oil, although being undesirable for human consumption, would not be of public health concern in this case. This technical support to the Commission was followed on 27 May 2008 by a statement on the contamination of sunflower oil exported from Ukraine.

Commission Decision 2008/433/EC confirmed the system of double control laid down in the previous Decision. All shipments were tested on Ukrainian side prior to export as well as on EU side when presented for import. The favourable outcome allowed for the revision of the measures leading to a favourable vote in SCOFCAH on 28 September 2009 on a draft Decision reducing the measures to systematic testing on export combined with random testing on import.

Similar cases on a lesser scale have been notified through the RASFF system. Mineral oil has occasionally been detected in oils from other origins (at levels between 120 and 950 mg/kg) and other products such as cakes (up to 608 mg/kg). Other notification over the past years concerned the presence of diesel in red wine and oil contaminated commodities such as cocoa beans, chicken breast, fish and even oil contaminated PE-HD granules for the production of milk bottles show the possible extent of such contaminations.

II. Mineral hydrocarbons: legislative aspects

Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin classifies "Mineral hydrocarbons, low to high viscosity including microcrystalline waxes, approximately C10 – C60; aliphatic, branched aliphatic and alicyclic compounds", with the exclusion of aromatic and unsaturated compounds, as a substance for which for use in all food producing animals no MRL (Maximum Residue Limits) is required.

Under the provisions laid down in Directive 95/2/EC on food additives other than colours and sweeteners, the use of **microcrystalline wax** is permitted for surface treatment of confectionery (excluding chocolate), chewing gum, melons, papaya, mango and avocado under number E 905 following the '*quantum satis*' principle. In the scope of this directive '*quantum satis*' means that no maximum level is specified, but that the additive shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided that the consumer is not mislead.

Provisions for the use of additives which may be used in the manufacture of plastic materials and articles are laid down in Commission Regulation 10/2011EC relating to plastic materials and articles intended to come into contact with foodstuffs.

Provisions for the use of certain paraffin oils as insecticide or acaricide have been laid down in Council Directive 91/414/EC concerning the placing of plant protection products on the market.

Other uses not directly related to the food chain include human medicines and cosmetics.

III. Specific background

Mineral hydrocarbons are a heterogeneous group of substances consisting of mixtures of different-sized hydrocarbon molecules, which may include saturated and/or unsaturated hydrocarbons with a linear, branched or cyclic structure.

Mineral oil and mineral oil products consist of extremely complex mixtures of hydrocarbons varying in carbon number and structure.

Three main basic structures are distinguished:

- **Paraffins,** based on n-alkanes **and iso-alkanes**
- **Naphthenes,** based on cycloalkanes
- **Aromatic hydrocarbons**.

The paraffins/naphthenes accepted for use in food have also been classified according to viscosity:

- Medium & low viscosity: C10-C25, viscosity at 100 C 3-8.5 centiStokes, molecular weights 300-500
- High viscosity: C30, viscosity at 100 C not less than 11 centiStokes, molecular weight not less than 500
- Microcrystalline waxes: C20-C60, viscosity at 100 C 10-30 centiStokes, molecular weight 300-750+.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set several ADIs for mineral oil (2002):

- For mineral oil with high viscosity: ADI of 0-20 mg/kg body weight (b.w.)
- For mineral oil with medium and low viscosity: temporary ADIs for Class I (0-10 mg/kg b.w.) and Class II and III (0-0.01 mg/kg b.w.).

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) established an ADI of 12 mg/ kg b.w./day for high viscosity white mineral oils (2009).

In order to assess the need for regulatory measures as regards mineral hydrocarbons in food, EFSA is requested to assess the risks related to the presence of mineral hydrocarbons in food.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for a scientific opinion on the risks to human health related to the presence of mineral oil in food.

In particular, the opinion should:

- Evaluate if there are new toxicity data available and if the current ADIs are still applicable.

- Explore whether certain classes (or subclasses) are more relevant due to their toxicity or to differences in the way they are metabolised by the human body.
- **Identify the different sources of the background presence of mineral oil in food other than adulteration or misuse.**
- Contain a **dietary** exposure assessment **for the general population and specific groups of the population (e.g. infants, children, people following specific diets)** by taking into account the background presence of mineral oil in food.
- Advise on classes to be included if monitoring would be set up for the presence of mineral oil in food.

ASSESSMENT

1. Introduction

Mineral oil hydrocarbons (MOH) or mineral oil products considered in this opinion are hydrocarbons containing 10 to about 50 carbon atoms (the number of carbon atoms is defined as carbon numbers in this opinion). Crude mineral oils are by far the predominant source of the MOH considered, but equivalent products can be synthesised from coal, natural gas or biomass.

MOH comprise complex mixtures, principally of straight and branched open-chain alkanes (paraffins), largely alkylated cycloalkanes (naphthenes), collectively classified as mineral oil saturated hydrocarbons (MOSH), and mineral oil aromatic hydrocarbons (MOAH) (Biedermann et al., 2009). In this opinion, the term alkane is used to cover both paraffins and naphthenes. The term MOH excludes hydrocarbons naturally occurring in food (primarily n-alkanes of odd-numbered carbons from C_{21} to C_{35} and hydrocarbons of terpenic origin) and oligomeric hydrocarbons released from polyolefins (largely consisting of branched alkanes).

The composition of MOH products is determined by the crude mineral oil used as starting material, by the treatment during refining (such as distillation, extraction, cracking, hydrotreatment) and the addition of hydrocarbons from other sources. The composition of seemingly equivalent products may substantially differ, depending on the way they were obtained. The most important products are described in Section 2.

In the past, viscosity was the principal means of classification of mineral oil products. However, this property alone does not characterise the composition if, e.g., the carbon number distribution and the content of aromatics are unknown. See also Section 3.1.2 for further explanations.

2. Chemistry of mineral oil hydrocarbons and related products

2.1. Classes of mineral oils

MOH consist of three major classes of hydrocarbon compounds:

- alkanes, both branched and unbranched (paraffins);
- cycloalkanes, mainly cyclopentanes and cyclohexanes, alkylated and non-alkylated, mono-, di- and higher ring systems (naphthenes);
- aromatics (mono-, di- and higher ring systems), including alky-substituted.

For structural information on these compounds see Figure 1.

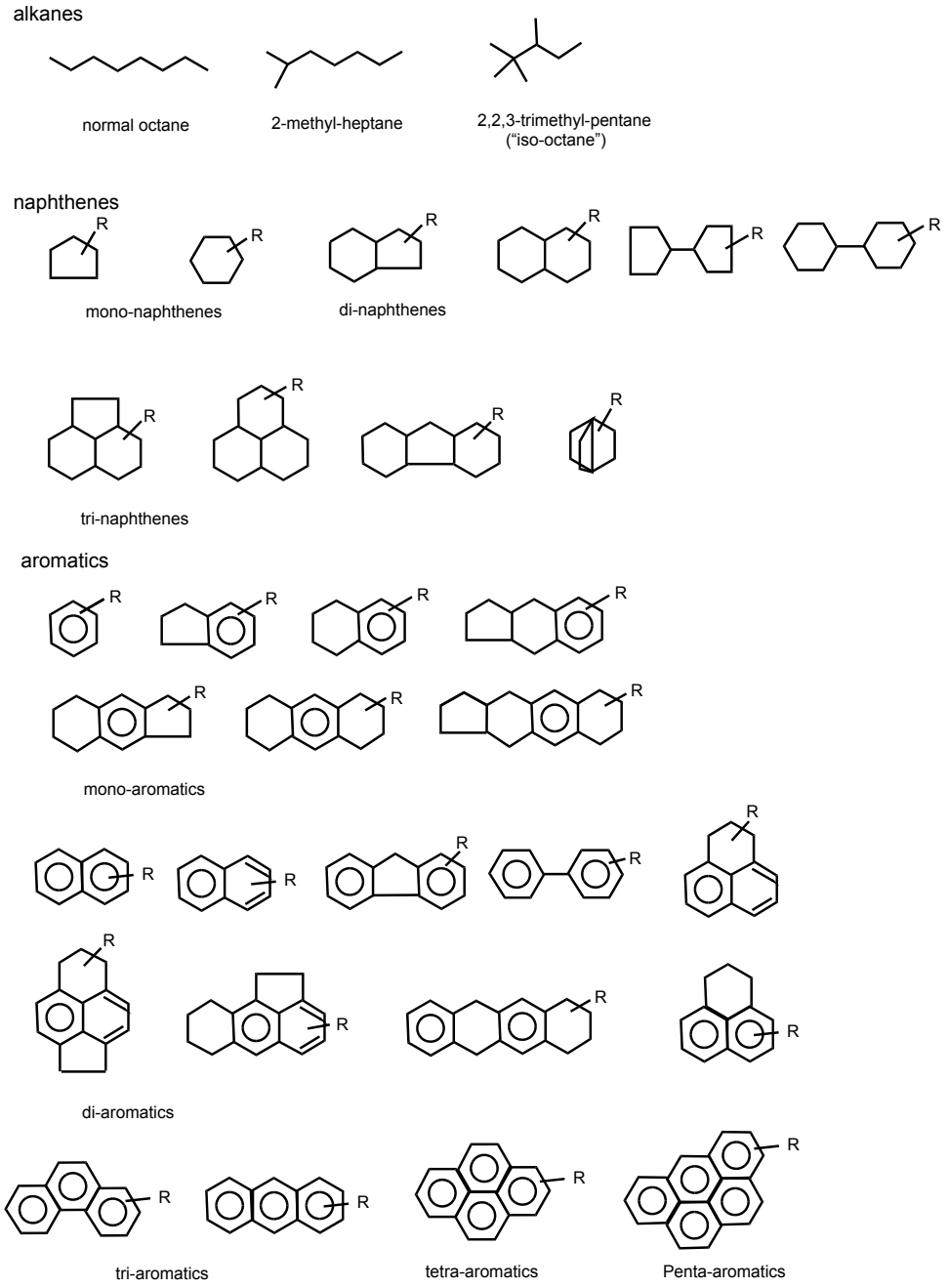

Figure 1: Examples of the different classes of hydrocarbons found in crude oil. R and R', branched or unbranched alkylgroups with 0 to > 20 C-atoms.

The possible number of hydrocarbon compounds in mineral oil products easily exceeds 100 000 for those with less than 20 carbon atoms and increases exponentially with the number of carbon atoms, as illustrated in Figure. 2. Not all possible isomers are present in every product, but the majority are (Beens, 1998).

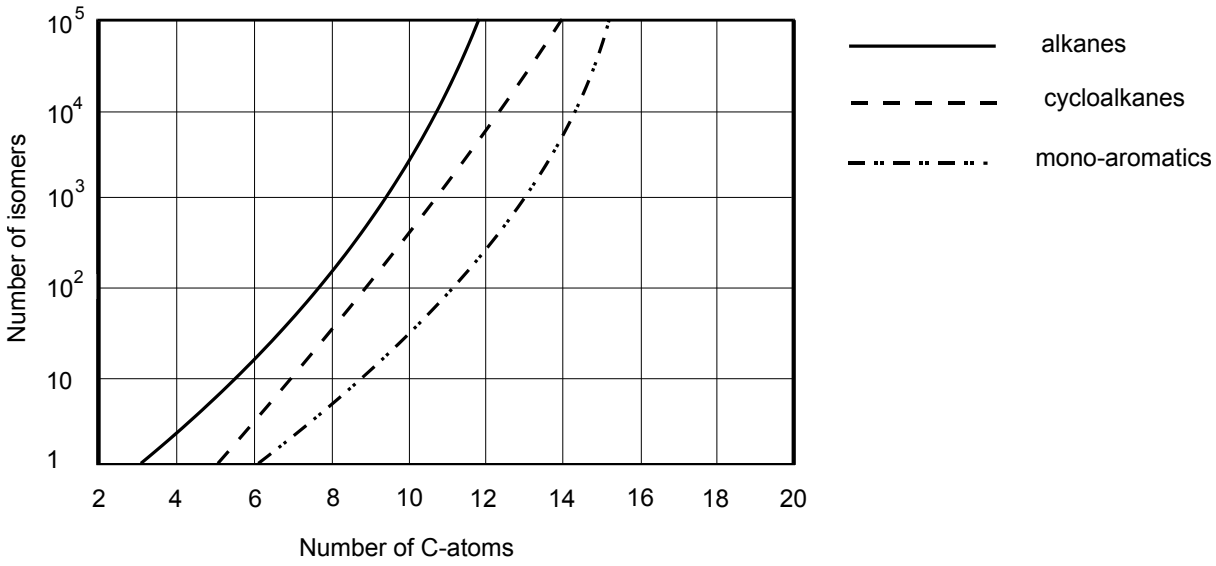

Figure 2: The figure illustrates the possible number of hydrocarbon isomers with a given number of carbon atoms.

Apart from hydrocarbons, crude oils may also contain compounds with heteroatoms, mainly sulphur and/or nitrogen. A number of examples are depicted in Figure 3. In some crude oils the sulphur containing compounds may amount to 10-15 % (w/w). The petroleum products derived from these crude oils that are used as a fuel are generally treated to reduce the sulphur content to a level of < 100 mg/kg. Other products may still contain a few percent of sulphur compounds, generally in the form of aromatic compounds, such as thiophenes, benzothiophenes, dibenzothiophenes and benzonaphthothiophenes, with dibenzothiophenes and their branched isomers being the most abundant. The amount of nitrogen compounds generally is far lower, up to a few hundred mg/kg. Crude oils may also contain oxygen- and/or metal-containing compounds, but these are removed during processing.

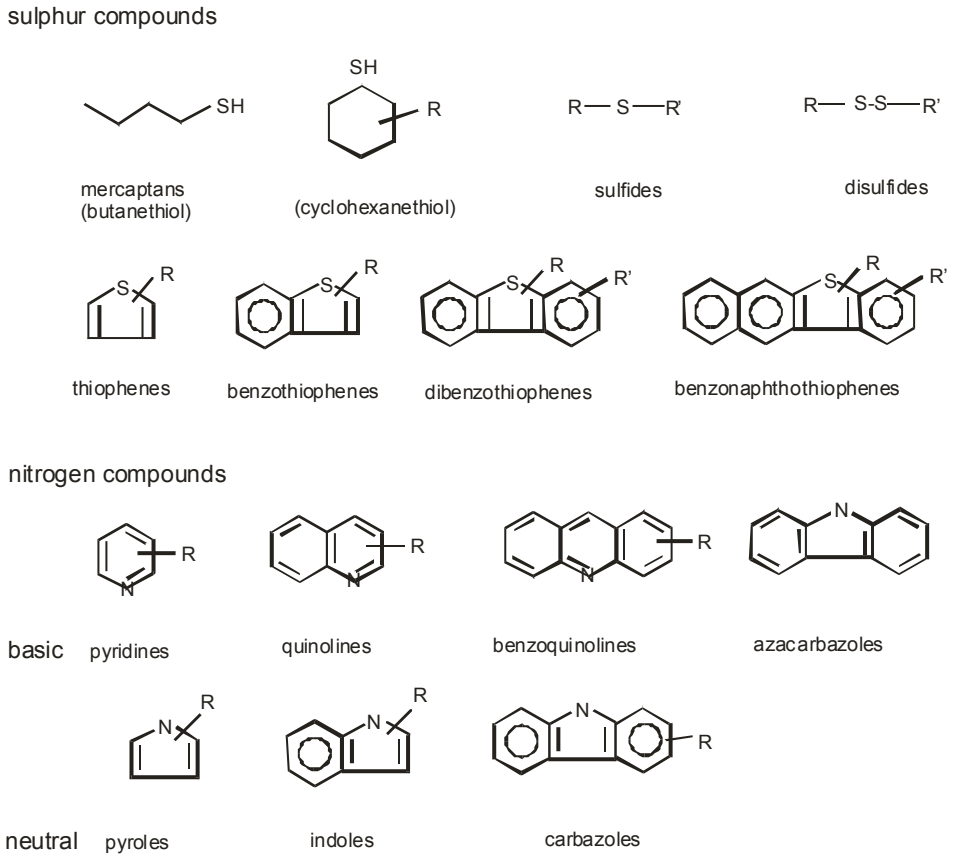

Figure 3: Examples for different classes of sulphur and nitrogen compounds in crude oil.

2.2. Crude oil processing

The composition of crude oils varies depending on the crude oil source, e.g.:

- North Sea: liquid, transparent, high concentration of paraffins and naphthenes;
- Eastern Asia (China, Indonesia): solid, high concentration of paraffins and naphthenes;
- Arabian: "liquid", high viscosity, dark brown, medium concentration of paraffins and naphthenes;
- Nigerian: "liquid", high viscosity, dark brown, high concentration of ring structures, including aromatics;
- South American: solid, black, high concentration of ring structures including aromatics.

The composition of MOH is determined by the crude oil source and the processing in the refinery, such as physical separations and chemical conversions of the substituents. In view of the large demand for crude oils, some major refineries may have to change feedstocks (starting material for the process) and adapt the refining processes accordingly.

The schematic flow diagram of a typical (integrated) oil refinery shown in Figure 4 depicts the various processes and the flow of intermediate product streams between the inlet crude oil feedstock and the final end products. The diagram depicts only one of the hundreds of different oil refinery configurations that exist.

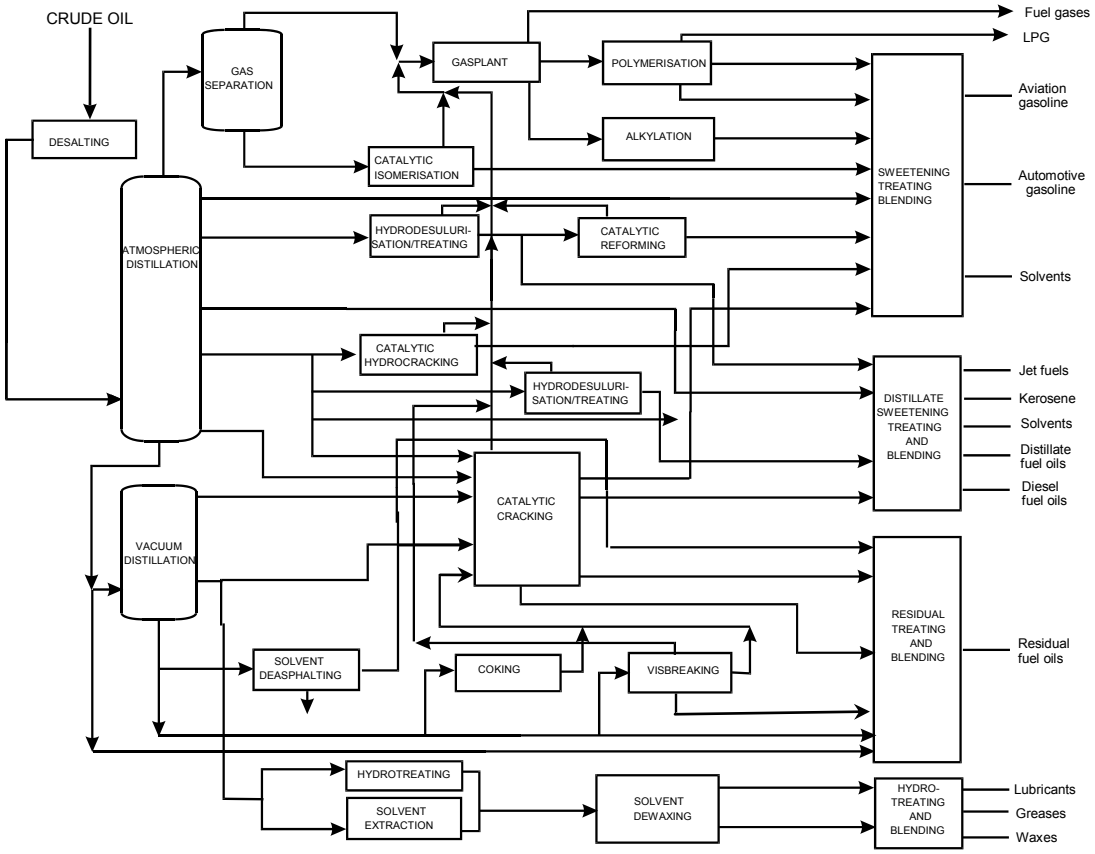

Figure 4: Schematic flow diagram of a typical integrated oil refinery.

The distillates of the crude oil are usually grouped into three categories:

- light distillates (liquefied propane gas (LPG), gasoline, naphtha);
- middle distillates (kerosene, diesel, solvents);
- heavy distillates and residues (heavy fuel oil, lubricating oils, wax,[4] asphaltic material).

A common crude oil refinery includes the following processing units (Gary and Handwerk, 1984; Leffler, 1985; Speight 2006):

Desalter unit: water washes salt from the crude oil before it enters the atmospheric distillation unit.

Atmospheric distillation unit: it distils crude oil at atmospheric pressure into several fractions, with the final fraction having boiling points up to approximately 370 °C. These may contain gases, light straight-run naphtha, heavy straight-run naphtha, straight-run kerosene, straight-run middle distillate and straight-run gas oil.

Vacuum distillation unit: it further distils the residual bottoms of the atmospheric distillation ("long residue") at a pressure of typically 0.1 bar into several fractions, such as light ends, light vacuum distillate and heavy vacuum distillate. Leaving a heavy residue ("short residue"), that contains the asphaltic material.

Naphtha hydrotreater unit: it uses hydrogen to desulphurise naphtha from atmospheric distillation. The naphtha must be hydrotreated before sending it to a catalytic reformer unit, since sulphur will poison the hydrogenation catalyst.

[4]The term wax in this opinion refers to solid paraffin waxes, comprised mainly of high molecular weight linear alkanes.

Catalytic reforming (also known as platforming) unit: it is used to convert the naphtha-boiling range molecules into higher octane reformate by dehydrogenation. Naphthenic components are dehydrogenated into aromatics over a noble catalyst (e.g. Platinum). Also some ring fusion and isomerisation of aliphatic compounds may occur. The reformate has a higher content of aromatics and cyclic hydrocarbons. An important by-product of a reformer is hydrogen, which is used in the different hydrogenation units in the refinery.

Steam reforming unit: it produces hydrogen for the hydrotreaters or hydrocracker units by the reaction of natural gas and water at high temperatures. Carbon monoxide may be converted to carbon dioxide and additional hydrogen.

Distillate hydrotreater unit: it desulphurises distillates (such as diesel and jet fuel) after atmospheric distillation by hydrogenation into hydrocarbons and hydrogen sulphide.

Fluid catalytic cracker (FCC) and thermal cracker units: they upgrade heavier fractions into lighter, more valuable products by cracking large molecules into smaller ones, i.e. the breaking of carbon-carbon bonds in the precursors. The lower (partly olefinic) molecular weight compounds are used as feedstock for, e.g., dimerisation or alkylation units.

Dimerisation unit: it converts two identical low-molecular olefins into one higher molecular compound. Generally these compounds are highly branched and produce higher-octane gasolines blending components.

Isomerisation unit: it converts short linear olefinic molecules into higher-octane branched molecules for blending into gasoline or to feed alkylation units. n-butane, n-pentane or n-hexane are converted into their respective isoparaffins of substantially higher octane number. The conversion of n-butane into iso-butane is important to provide additional feedstock for alkylation units, and the conversion of normal pentanes and hexanes into higher branched isomers for gasoline blending.

Alkylation unit: it produces highly branched, high-octane number components for gasoline blending. Alkenes from FCC are combined with iso-butane (from isomerisation) and fed to the hydrofluoric acid (HF) or catalytic alkylation reactor where they form highly-branched alkanes.

Merox unit: it treats LPG, kerosene or jet fuel to remove thiols (mercaptans) and thiophenes by oxidation into organic disulphides which are then removed by distillation.

Hydrocracker unit: it uses hydrogen to upgrade heavier fractions into lighter, more valuable products. The lower molecular weight olefinic material is hydrogenated in the process, so that the final product is olefin-free.

Desulphurisation/denitrogenation unit: it removes sulphur/nitrogen from hetero compounds by hydrogenation into hydrocarbons and hydrogen sulphide/ammonia by ring opening, if necessary of the S- or N-bearing rings. Some gasoil compounds are converted to jet fuel compounds and lighter components.

Visbreaking unit: it upgrades heavy residual oils by thermally cracking them into lighter, more valuable products of reduced viscosity. These products have to be hydrotreated in the next process, since they contain a considerable amount of unsaturated compounds.

Coking units (delayed coking, fluid coker, and flexicoker)**:** they convert very heavy residual oils into gasoline and diesel fuel, leaving petroleum coke as a residual product. The residual oil is heated to its thermal cracking temperature in a furnace with multiple parallel passes. This cracks the heavy, long chain hydrocarbon molecules into coker gasoil and petroleum coke. From the residual coke material carboneous rods are produced for the aluminium industry. The coker gasoil is further treated in hydrogenation processes.

Special processes are in use to produce highly refined, clear and transparent oils, sometimes referred to as **"white oils",** for specific applications, such as food contact materials, pharmaceutical or medicinal use. The processes involved are:

Solvent extraction unit: it physically separates the aromatic hydrocarbons and minor compounds of higher polarity. A suitable solvent, almost non-miscible with saturated hydrocarbons, preferentially extracts the aromatic hydrocarbons and polar components. The operation is usually performed in a liquid-liquid, counter-flow extraction column or rotating disc contactor with multiple contact and separation stages to improve efficiency and selectivity. The solvents used are phenol, liquid sulphur dioxide, furfural, N-methylpyrrolidone or sulfolane, the last three being most commonly used. The solvent is recovered by distillation and recycled. The raffinate obtained contains almost all saturated hydrocarbons from the initial distillate, about 1/3 of the aromatic and polar molecules, and only a few percent of the initial polycyclic aromatic hydrocarbons (PAH).

Solvent dewaxing unit: it physically removes the linear alkanes or waxes to obtain a liquid free-flowing and homogeneous at 0 °C. The warm raffinate or hydrocrackate is diluted with an anti-solvent of waxes and the blend progressively cooled. This induces the progressive and selective crystallisation of waxes. The slurry of wax crystals is separated by filtration. The filtrate is distilled to recover and recycle the solvent. Typical solvents used are light ketones, LPG, toluene and blends of these. Oil yield depends on the waxy character of the crude and the set pour point. The impact on composition is an almost complete removal of linear waxes, a reduction of little-branched isoparaffins, balanced by a slight, proportional increase of other hydrocarbon types and polar residues.

Optionally**,** the **hydrocracking unit** removes waxes by selective hydrocracking of linear molecules over a micro-porous, shape-selective catalyst at high temperature (300 to 360 °C) and under medium to high hydrogen pressure. The catalysts are aluminosilicates like mordenite or zeolites designed with controlled pore size, optionally promoted with metals like Pt. Linear and near-linear alkane molecules, to a lesser extent long, linear alkyl branches, are cut to light hydrocarbons like ethane, or re-arranged to branched paraffins. Other types of molecules are little affected. After the treatment unreacted gas and light by-products are separated by distillation or steam stripping. The key control parameters are the temperature, the hydrogen pressure and the residence time.

Dearomatisation unit: it treats the feedstock as obtained from the previous steps with an excess of sulphur trioxide or fuming sulphuric acid (oleum) in a stirred reactor. The aromatic hydrocarbons react with the sulphur trioxide or oleum to yield arylsulphonic acids; the S-, N- and O-containing molecules are decomposed, oxidised or neutralised. Once the reaction is finished, the reactants separate into a hydrocarbon layer and a sulphuric sludge layer, which contains most of the water-soluble sulphonic acids and by-products formed. These are settled by gravity or centrifugation. The oily layer with the saturated hydrocarbons and most of the oil-soluble sulphonic acids and by-products is then neutralised with an alkaline, aqueous solution and extracted with an alcohol (ethanol or propanol) to wash off sulphonates and other by-products. Although most of the aromatic hydrocarbons and impurities are removed, the process is not quantitative and the oily phase is then returned for additional oleum treatment. The process is continued until all molecules reactive with sulphuric acid are removed. After the final treatment and extraction, the oil is stripped with steam or nitrogen to remove residual moisture and alcohol, and filtered over a fixed clay bed or other suitable filtering media for a final purification to remove polar impurities.

2.3. Composition of different mineral oil products

Generally the specifications that are set for most of the commercial products do not include the composition, but physical properties, such as boiling range, density, dielectric constant, viscosity, etc. The majority of products are the result of final blending operations of different intermediate products in the refinery (see also Figure 4). These blending operations are controlled by on-line measurements of some principal physical properties other than composition. Moreover, since crude sources for the refinery may change and the process parameters may have been adjusted to market demands, the

composition may change accordingly. For these reasons, the composition of different products is only known in general terms or not known at all.

Diesel fuels: Seven grades of diesel fuel oils, suitable for various types of diesel engines. Grade No. 1-D S15; Grade No. 1-D S500; Grade No. 1-D S5000; Grade No. 2-D S15; Grade No. 2-D S500; Grade No. 2-D S5000; and Grade No. 4-D. Boiling range: (ASTM D86-10a) 200-350 °C (carbon numbers in the range C_8 - C_{22}).

Jet fuels: high paraffinic/naphthenic. Types: A, A-1, B and TS-1.

- boiling range Jet fuel A and A-1: (ASTM D86-10a) 200-300 °C (carbon numbers in the range C_8-C_{16});
- boiling range Jet fuel B: (ASTM D86-10a) 100-280 °C (carbon numbers in the range C_5-C_{15});
- boiling range TS-1: (ASTM D86-10a) 160-280 °C (carbon numbers in the range C_7-C_{15}) (Military jet fuel JP-4, JP-5 JP-8, DL-1, DI-2, DF-2).

Solvents: a large range of solvents is available, ranging from C_8 up to C_{20}, containing from 0 up to > 99 % aromatics. These products are used for different applications, from ink and paint solvents up to cleaning agents.

White oils: highly refined, low aromatic petroleum products with differing molecular mass distribution, ranging from C_8 up to C_{40}. They are used in specialty applications where a high degree of purity and chemical stability is required. Applications requiring a NSF H1/1998 USDA H-1 mineral oil (which may not be authorised in Europe):

- food processing, bottling and canning equipment;
- protective coating for raw fruits and vegetables;
- eggshell sealant;
- dust suppressant for grain or animal feed;
- drip oil for deep well water pumps;
- process oil or diluent in caulks, pharmaceuticals, cosmetics, rubber extender oils and plastics;
- textile lubricants;
- household cleaners and polishes.

Some of the white oils meet the FDA 21 CFR 172.878 and CFR 178.3620 regulations for food-related use (direct or indirect food contact) (CONCAWE, 1984, 1993, 2006; IARC, 1984).

Lubricants (or lubrication oils). The American Petroleum Institute (API) designates several types of lubricant base oil identified as (Machinery Lubrication, 2010):

- Group I – Saturates > 90 % and/or sulphur > 0.03 %, and Society of Automotive Engineers (SAE) viscosity index (VI) of 80 to 120. Manufactured by solvent extraction, solvent or catalytic dewaxing, and hydro-finishing processes. Common Group I base oil are 150SN (solvent neutral), 500SN, and 150BS (brightstock). These lubrication oils may contain sulphur compounds, not in thiophene structures, but rather as sulfides and disulfides.
- Group II – Saturates > 90 % and sulphur < 0.03 %, and SAE viscosity index of 80 to 120. Manufactured by hydrocracking and solvent or catalytic dewaxing processes. Group II base oil has superior anti-oxidation properties since virtually all hydrocarbon molecules are saturated. It has a water-white colour.
- Group III – Saturates > 90 %, sulphur < 0.03 %, and SAE viscosity index over 120. Manufactured by special processes such as isohydromerisation. Can be manufactured from base oil or slack wax from dewaxing process.
- Group IV – Poly alpha olefins (PAO).
- Group V – All others not included above, such as naphthenics, polyalkylene glycols, esters, etc.

In North America, Groups III, IV and V are now described as synthetic lubricants, with group III frequently described as synthesised hydrocarbons, or SHCs. In Europe, only Groups IV and V may be classed as synthetics.

Examples of application areas include:

- automotive: engine oils (gasoline engine oils, diesel engine oils);
- tractor (one lubricant for all systems);
- other motors;
- industrial;
- aviation;
- marine.

Extender oil, also referred to as process softening oil, is added to rubber compounds in the production process for tyres and other rubber goods to achieve an acceptable processability. The oil may also have an impact on certain performance characteristics of the final product. These oils may contain a large amount of aromatic compounds, including PAH. Alternative extender oils are under investigation by oil producers and the tyre industry is actively involved. (BLIC, 2005).

2.4. Synthetic fuels

A separate range of products is obtained from the so-called "synthetic fuels" derived from coals, natural gas or biomass through Fischer-Tropsch (FT) synthesis of carbon monoxide and hydrogen. Carbon monoxide and hydrogen is produced in the conversion unit in front of the FT unit. The composition of the final FT products is not very different from products derived from mineral oil sources.

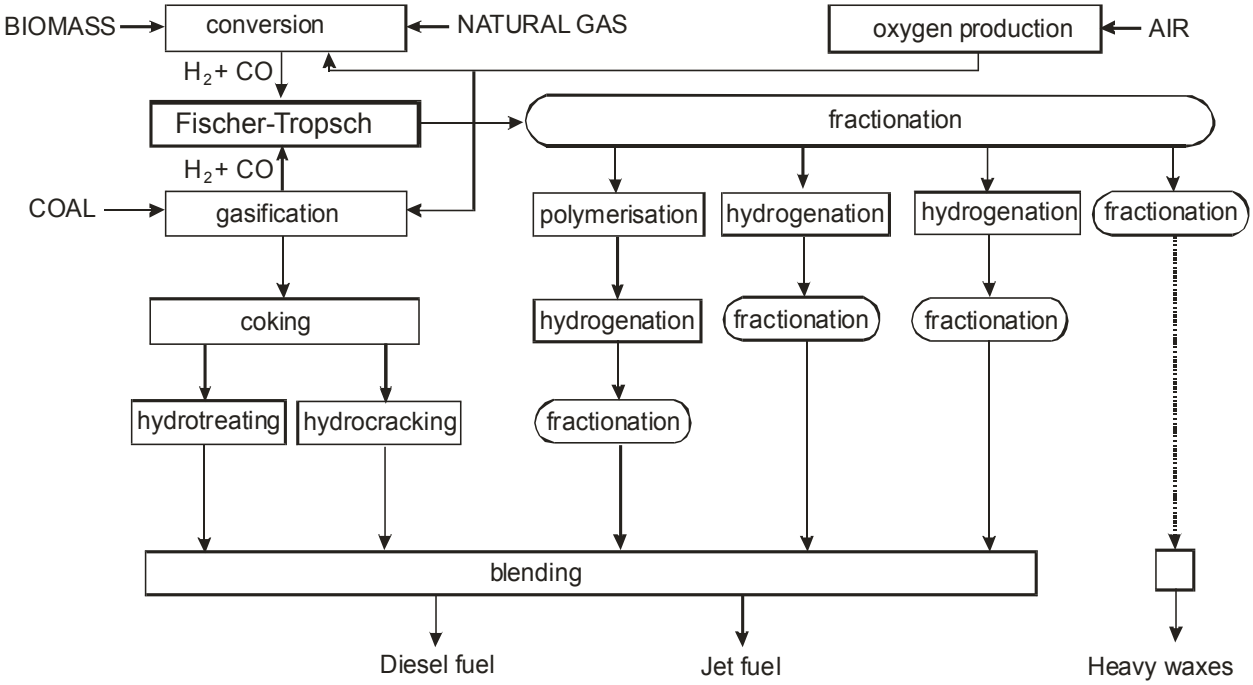

Figure 5: Simplified schematic flow diagram of a Fischer-Tropsch synthesis plant.

Low temperature Fischer-Tropsch (LTFT) and high temperature Fischer-Tropsch (HTFT) synthesis are distinguished. The more common LTFT mainly produces n-alkanes, n-alkenes and some n-alcohols. Depending on process conditions, they range from C_1 up to > C_{120}. If the feedstock is natural gas, products free from e.g. sulphur are obtained. The waxes may be used after fractionation into commercial grades as such or hydrocracked into smaller molecules to deliver diesel or jet fuel.

Comprehensive two-dimensional gas chromatography (GC×GC) analyses of the diesel and jet fuel products indicate that their composition is very similar to products derived from mineral oil sources.

HTFT produces a large range of different compound classes, such as linear and branched alkanes, linear and branched alkenes, branched and unbranched alkylated cycloalkanes, branched and unbranched alkylated cycloalkenes, branched and linear substituted aromatics, alcohols, aldehydes, ketones and some esters. The HTFT product is often used to produce pure solvents such as alcohols, ketones, aldehydes, etc. After hydrogenation the hydrocarbon fraction is used as fuels (Kreutz et al., 2008; van der Westhuizen et al, 2011).

In view of the prospects for oil product demands, availability and pricing, FT processes are of increasing interest. More stringent legislation might limit carbon dioxide emissions, which will boost the use of biomass for conversion into fuels. Large production plants with coal or natural gas as feedstock already exist in South-Africa and Brunei and it is expected that more plants will be put in operation in the near future.

2.5. Physico-chemical characterisation of mineral oil hydrocarbons

Since the oil industry has a long history, quite 'historic' characterisation analyses are still in use. The majority deal with the physical properties of the total product, such as boiling point distribution, density, viscosity, refractive index, pour point, etc. In the majority of product specifications one or more of these properties are defined. These physical properties are useful to understand the behaviour of the products in their applications, but provide little information on the chemical composition.

2.5.1. Boiling range distribution

A method introduced long ago to determine the boiling point distribution (ASTM D86-10a) is still in use for almost all specification. It involves physical distillation of the product in a laboratory unit providing only one theoretical plate. The results are only indicative of the true boiling range. A more accurate boiling point distribution is obtained by so-called SimDist analysis (ASTM D 2887 or D7500-10), which is performed by a GC separation on a non-polar column. This analysis is sometimes referred to as True Boiling Point analysis, since it produces nearly true boiling point information. The two analyses differ: the 5 % point determined by ASTM D86-10a may be 10 or 15 % in reality; this also applies for mineral oil hydrocarbons approved for food use. As an example, Figure 6 shows the distillation curves of a diesel sample according to the ASTM D86-10a distillation and the ASTM D2887 SimDist analysis by GC.

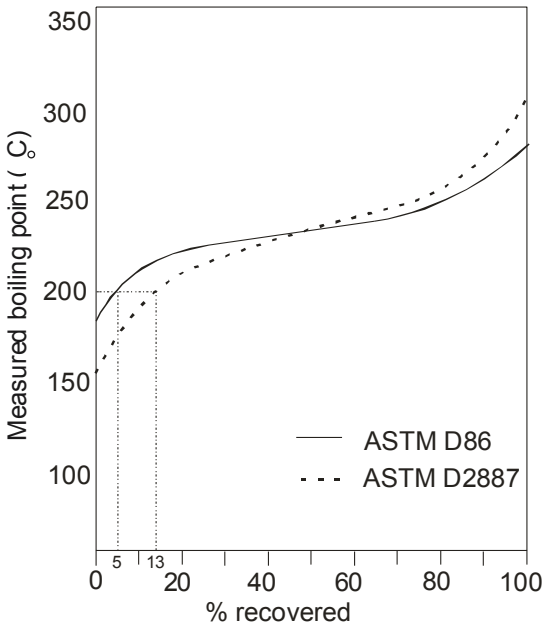

Figure 6: Distillation curves according to ASTM D86 and ASTM D2887.

2.5.2. Viscosity

Especially for lubricants, the kinematic viscosity and the viscosity index are important parameters. However, apart from a rough indication of carbon number distribution, they do not provide compositional information. This is illustrated in Figure 7, where the relationship between carbon number, compound type and viscosity is depicted for classes of hydrocarbons up to C_{11}. The data are from API Project 44 (API, 1961). The principals also apply to higher carbon numbers.

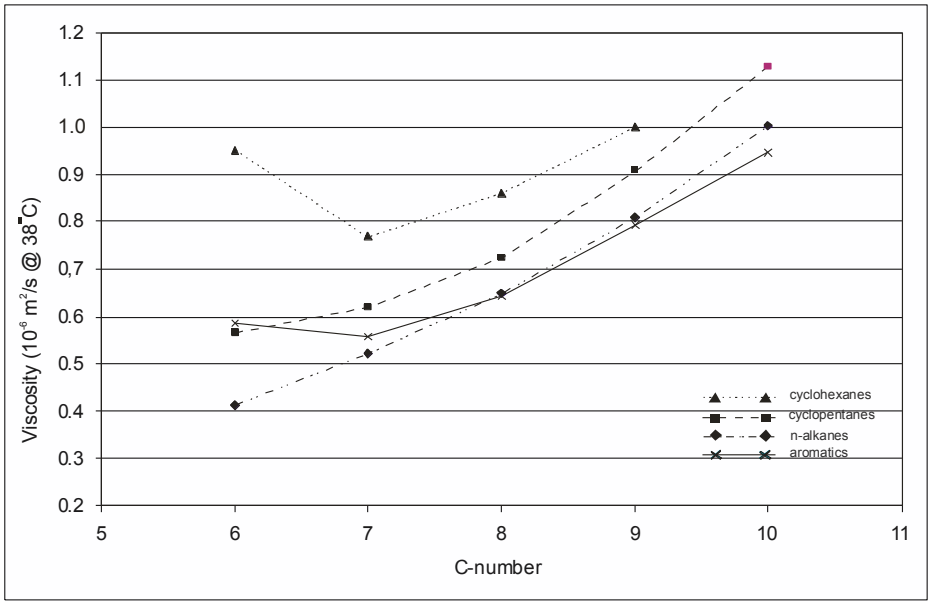

Figure 7: Relationship between carbon number and kinematic viscosity of 4 classes of hydrocarbons.

Carbon numbers and viscosities are related within all four classes of hydrocarbons. There exist differences between the compound classes, but the two classes exhibiting the largest difference in

toxicological behaviour, viz. n-alkanes and aromatics, have more or less the same viscosity. As most mineral oil fractions are composed of various hydrocarbon classes, knowledge of the viscosity does not provide information on composition as long as the carbon number distribution and aromatic content are not known.

2.5.3. Compositional characterisation

Compositional information on MOH is primarily from gas chromatography (GC). GC of MOSH results in a pattern of unresolved peaks of unidentified components (even at highest separation efficiency available) with n-alkanes and some predominant iso- and cycloalkanes on top. GC of MOAH results in a pattern of unresolved peaks with hardly any distinct peak on top.

GC on a non-polar stationary phase provides the boiling point distribution (SimDist as mentioned above). The molecular mass distribution of the MOSH can be derived from the identification of the n-alkanes, keeping in mind that branched species of the same mass may be eluted at a retention time corresponding to an n-alkane of up to about two carbon atoms less. For the MOAH, the conversion of retention time to boiling point and molecular mass is more complex.

GC×GC, a technique introduced in the 1990s, adds a second, independent separation throughout the sample (e.g. Beens et al., 2000; Schoenmakers et al., 2000; Diehl and Di Sanzo, 2005; Edam et al., 2005; Vendeuvre et al., 2005). If the first separation occurs on a non-polar stationary phase, i.e. by boiling point, a second separation on a polar stationary phase enables distinction of, for example, paraffins and naphthenes as well as various types of aromatics by ring number. Separation between MOSH and MOAH is incomplete.

GCxGC enables at least partial separation of cycloalkanes by ring number. Since the cycloalkanes are almost completely alkylated, they form bands stretching through the GCxGC plot. Of a given molecular mass, the species with single long alkyl chains and polyalkylated ones with shorter chains are eluted in a regular fashion and form clusters of partially resolved components, as determined by selected ion mass spectroscopy.

Separation between the benzenes, naphthalenes, benzothiophenes and fluorenes is fairly complete. Dibenzothiophenes are only partially resolved from anthracenes and phenanthrenes, which are no longer separated. Aromatics of higher ring number are separated only partially. The non-alkylated aromatic compounds are almost absent. GCxGC again forms bands of alkylated aromatics with increasing number of carbon atoms in the side chain. Using mass spectrometry, clusters of aromatics with given molecular mass can be monitored, but apart from the least alkylated species, there is no complete resolution. It is difficult to derive the number of alkyl groups and usually impossible to determine the positions of the alkyl groups on the ring system without a reference standard (which are usually not available).

Chemically unmodified mineral oils almost exclusively contain cyclic compounds of which either all rings are saturated (naphthenes) or all are aromatics. Many of the mineral oil products, however, are partially hydrogenated. These contain both saturated and aromatic fused rings and GCxGC forms a three-dimensional pattern of unresolved peaks of unidentified material. The characterisation of these hydrocarbons is virtually impossible.

3. Previous risk assessments

In 1989, the Scientific Committee on Food (SCF) reviewed the toxicity studies in Fischer 344 rats in which abnormalities in various organs had been observed after feeding mixtures of mineral paraffins (SCF, 1989). It was concluded that ‘there was no toxicological justification for the continued use of mineral hydrocarbons as food additives’. A temporary tolerable daily intake (TDI) of 0-0.005 mg/kg body weight (b.w.) was established for oleum-treated mineral hydrocarbons (still containing some aromatics) and of 0-0.05 mg/kg b.w. for hydrogenated products.

In 1995, the SCF evaluated the safety of mineral and synthetic hydrocarbon oils and waxes for use as food additives, in food processing and for use in food packaging materials. The SCF based its assessment on a 90-day study in Fischer 344 rats. In these rats, accumulation of hydrocarbons in liver and lymph nodes was observed, associated with a granulomatous response. No data were available to conclude on possible species differences.

For waxes, largely consisting of n-alkanes, a group acceptable daily intake (ADI) of 0-20 mg/kg b.w. was established for the following specification:

- highly refined products, i.e. virtually free of MOAH;
- average molecular mass of no less than 500 Da (about C_{35});
- a minimum carbon number of 25 at the 5 % boiling point;[5]
- viscosity of no less than 11 mm^2/s at 100 °C.

For white paraffinic oils derived from petroleum-based hydrocarbon feedstocks (high viscosity P100 and medium viscosity P70), the SCF established a Temporary Group ADI of 0-4 mg/kg b.w. based on a no-observed-adverse-effect level (NOAEL) derived from 90-day studies for the hydrogenated paraffinic oil P100(H) and the P70(H) (refer to Table 1 for the classification of mineral oil products), using a safety factor of 500. The ADI was considered temporary, pending submission of a two-year chronic toxicity/carcinogenicity study on the medium viscosity P70(H) mineral oil (SCF, 1995; EFSA, 2009). The specifications are:

- average molecular weight of no less than 480 Da (C_{34} paraffins have a molecular weight of 478 Da);
- a minimum carbon number of 25 at the 5 % boiling point;
- viscosity of no less than 8.5 mm^2/s 100 °C.

The SCF considered the use of paraffinic mineral oils acceptable provided these have a sufficiently high molecular weight, based on the observation that such materials are not absorbed to a relevant extent.

On the other hand, relative to the other oil and wax grades evaluated, the SCF concluded that 'for those hydrocarbons which have been shown to both accumulate and cause toxicity, for which a no adverse effect level is not yet known [...], it is not possible from current information to set a safe level for intake from food. Further research may identify such levels'.

In 2002, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated white mineral oils and waxes from their previous assessments in 1995 (FAO/WHO, 1995) in the light of new studies and established ADIs. The recommendations are summarised as follows (FAO/WHO, 2002).

All mineral oil products except microcrystalline waxes (Table 1[6]) accumulated in tissues in a dose- and time-dependent manner. With the exception of P70(H) and P100(H), the mineral oil accumulation in tissues caused inflammatory effects indicative of a reaction to the presence of a foreign body. Such effects included focal histiocytosis, increased liver weight, lymph nodes, spleen and kidneys, granulomas or microgranulomas in the liver, haematological changes typical of a mild chronic inflammation reaction and biochemical changes associated with mild hepatic damage. For the microcrystalline waxes and the high viscosity P100(H) mineral oils, the ADI of 20 mg/kg b.w. was based on no-observed-effect levels (NOELs) at the highest dose tested in a 90 day study in Fischer

[5] Since the boiling range distribution regarding the '5 % boiling point' is determined by an outdated method, the values now determined by gas chromatography (simulated distillation) may be slightly different. See also Section 2.5.1 for a further explanation.

[6] The CONTAM Panel noted that there are inconsistencies amongst the physico-chemical properties reported in Table 1 (FAO/WHO, 2002) and in the background information received from EC for the various grades of (food grade) white mineral oils. The Panel noted that the same classification reported in the background information is also used in the MOH evaluation by the Committee for Veterinary Medicinal Products (EMEA, 1995) and decided to use the more recent classification reported in Table 1 for the present opinion.

rats. For the class I intermediate and low viscosity P70 mineral oils, the ADI was based on increased incidence of pigmented macrophages in male rats, an effect considered of doubtful biological significance. For classes II and III intermediate and low viscosity mineral oils, a temporary group ADI was established from an increased incidence of histiocytosis in the mesenteric lymph nodes. The temporary nature was due to uncertainty about the long term significance of the observed inflammatory response (FAO/WHO, 2002).

Table 1: Classification and assessment of highly refined mineral hydrocarbons intended for use in food (adapted from FAO/WHO, 2002).

Name	ADI (mg/kg b.w.)	Viscosity at 100 °C (mm^2/s)	Average relative molecular mass	Carbon number at 5 % distillation point
Microcrystalline wax	0-20	≥ 11	≥ 500	≥ 25
High melting point wax				
Low melting point wax	Withdrawn		**No specification**	
Low melting point wax		3.3	380	22
Mineral oil (high viscosity)	0-20	>11	≥ 500	≥ 28
P100		11	520	29
Mineral oil (medium and low viscosity) class I	0-10	8.5 – 11	480-500	≥ 25
P70		9.0	480	27
Medium viscosity liquid petroleum		8.7	480	25
P70(H)		8.6	480	27
Mineral oil (medium and low viscosity) class II	0-0.01[a]	7.0 – 8.5	400-480	≥ 22
N70(H)		7.7	420	23
Mineral oil (medium and low viscosity) class III	0-0.01[a]	3.0 – 7.0	300-400	≥ 17
P15(H)		3.5	350	17
N15(H)		3.5	330	17

P100 oil, crude: paraffinic, viscosity (40 °C): 100 mm^2/s;
P70 oil, crude: paraffinic, viscosity (40 °C): 70 mm^2/s;
P70(H) oil, crude: paraffinic, viscosity (40 °C): 70 mm^2/s, hydrotreated (catalytic hydrogenation);
N70(H) oil, crude: naphtenic, viscosity (40 °C): 70 mm^2/s, hydrotreated (catalytic hydrogenation);
P15(H) oil, crude: naphtenic, viscosity (40 °C): 15 mm^2/s, hydrotreated (catalytic hydrogenation);
N15(H) oil, crude: naphtenic, viscosity (40 °C): 15 mm^2/s, hydrotreated (catalytic hydrogenation).
[a]Temporary group ADI

For waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstocks to be used as lubricant in polymers for contact with food, the EFSA former Panel on additives, flavourings, processing aids and materials in contact with food (AFC Panel) established a specific migration limit of 0.05 mg/kg food. The evaluation was based on the absence of genotoxic potential observed in three *in vitro* mutagenicity tests performed with dimethyl sulfoxide (DMSO) extracts.

Specifications are:

- average molecular weight not less than 350;
- viscosity at 100 °C min 2.5 mm^2/s;
- content of hydrocarbons with carbon number less than 25, not more than 40 % (w/w). (EFSA, 2006).

Three evaluations have been performed and published by the EFSA Pesticide Risk Assessment and Peer Review Unit (PRAPeR):

- CAS 8042-47-5, chain lengths C_{18}-C_{30}, reliable boiling point range not available (EFSA (2008a);

- CAS 8042-47-5, chain lengths C_{17}-C_{31}, boiling point 280-460 °C (EFSA (2008b);
- CAS 64742-46-7 chain lengths C_{11}-C_{25}, CAS 72623-86-0 chain lengths C_{15}-C_{30}, CAS 97862-82-3 chain lengths C_{11}-C_{30} (EFSA (2008c).

In all assessments, data gaps in terms of characterisation and impurities profile have been identified. The toxicological dossiers were based on the claim that paraffin oils are similar to mineral oils used in human medicine. At least regarding the levels of relevant impurities (possible high level of polycyclic aromatic hydrocarbons), this could not be confirmed. No information on potential levels of residues in food or feed items was available. A consumer risk assessment could therefore not be finalised.

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) evaluated the safety of high viscosity white mineral oils (CAS Registry Number 8042-47-5, chain lengths C_{22}-C_{60}, average molecular weight: > 500 g/mol, viscosity at 100 °C ≥ 11 mm^2/s, carbon number > 25 at 5 % distillation point) when used as food additives and established an ADI of 12 mg/kg b.w. based on a NOAEL of 1 200 mg/kg b.w. per day in a chronic (12 months) study in Fischer 344 rats (highest dose tested). The ANS Panel concluded that the same ADI could have been potentially applicable also to medium and low viscosity mineral oil class I (as defined in Table 1), but acknowledged that this class was not covered by the terms of reference of the European Commission (EFSA, 2009).

The German Federal Institute for Risk Assessment (BfR) has evaluated findings on the transfer of MOH from recycled carton-board packagings into food which were attributed to the recycling of newspapers (BfR, 2009). The MOH had chain lengths up to C_{25} and contained 10 – 25 % MOAH. BfR concluded that given:

- the high fraction of MOAH and the possible existence of carcinogenic substances in this fraction,
- the high transfer rate of MOH into food,
- the accumulation of MOH in human tissues,
- the JECFA temporary group ADI of 0.01 mg/kg b.w. for low and intermediate viscosity class II and III mineral oils,

there was an urgent need to minimise the transfer of mineral oils from printing inks into food.

BfR has further evaluated hydrocarbon solvents used for the formulation of additives in the manufacture of paper and board for food contact (liquid paraffinic oils, chain length < C_{17}, not containing MOAH) (BfR, 2011). For this evaluation apart from studies on genotoxicity (Ames-test, chromosome aberration in vitro, micronucleus test in vivo; all negative) also two 90 day-studies (for MOH mixtures with carbon numbers in the range C_{11}-C_{14} and C_{10}-C_{13}) were available from which a NOAEL of 100 mg/kg b.w. per day was obtained. Because the rat strain used in these studies (Sprague Dawley) was not regarded as the most sensitive and data on toxicokinetics were not available, an extra factor of 5 was applied in the derivation of the tolerable daily intake in addition to the usual factor of 100. Assuming the consumption of 1 kg food contaminated with hydrocarbon solvents with carbon number in the range C_{10} - C_{16} by a person weighing 60 kg it was concluded that transfer of these substances into food should not exceed 12 mg/kg. By corresponding purity requirements MOAH are excluded. The evaluation was regarded as provisional. It was indicated the extra factor of 5 could be dispensed, subject to an evaluation on the relevance of the inflammatory effects observed in Fischer 344-rats in humans, which is expected by JECFA.

In a survey performed by the UK Food Standards Agency (FSA) the levels of mineral oils in recycled and non-recycled carton-board packagings were investigated (FSA, 2011). For a preliminary risk assessment it was conservatively assumed that all the mineral oils detected in the packaging could potentially migrate into food and that a portion of these foods were consumed on a daily basis. The FSA concluded that the presence of mineral oils in the packaging at the levels found did not indicate any specific food safety concerns. A toxicological reasoning for this conclusion is not given in the report.

4. Legislation

4.1. Food Contact Materials (FCM)

Regulation 1935/2004[7] lays down the general provisions and principles for food contact materials and articles. There are no specific measures regarding mineral oils, except for the provisions on their use as additives in plastic materials and articles intended to come into food contact laid down by Regulation (EU) 10/2011.[8] The following mineral oils are covered by the positive list of additives:

- FCM substance No 95: White mineral oils, paraffinic, derived from petroleum-based hydrocarbon feedstocks. No specific migration limit (SML) is defined (i.e. its use is restricted only by the overall migration limit of 60 mg/kg food or 10 mg/dm^2 food contact surface). The product must comply with the following specifications:
  - hydrocarbons with carbon number less than 25, not more than 5 % (w/w);
  - viscosity not less than 8.5 mm^2/s at 100 °C;
  - average molecular weight not less than 480 Da.

- FCM substance No 94 - Waxes, refined, derived from petroleum-based or synthetic hydrocarbon feedstocks. No SML is specified (i.e. its use is restricted only by the overall migration limit). The product must comply with the following specifications:
  - hydrocarbons with carbon number less than 25, not more than 5 % (w/w);
  - viscosity not less than 11 mm^2/s at 100 °C;
  - average molecular weight not less than 500 Da.

- FCM substance No 93 - Waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstocks. An SML of 0.05 mg/kg food is specified. In addition, these oils are not to be used for articles in contact with fatty foods. The product must comply with the following specifications:
  - hydrocarbons with carbon number less than 25, not more than 40 % w/w;
  - viscosity at 100 °C min 2.5 mm^2/s;
  - average molecular weight not less than 350 Da.

The Swiss legislation includes a section on printing inks, which lists mineral oils containing MOAH under the non-evaluated substances, the migration of which must be below 0.01 mg/kg (Verordnung 817.023.21, 2005).

4.2. Food additives

According to Directive 95/2/EEC,[9] on food additives other than colours and sweeteners, microcrystalline waxes (E 905) are approved for use in the surface treatment of confectionery excluding chocolate, of chewing gum and of melons, papaya, mango and avocado at *quantum satis*. By Directive 2009/10/EC[10] on purity requirements, the waxes are defined as refined mixtures of solid, saturated hydrocarbons, obtained from petroleum or synthetic feedstocks. The molecular weight must

[7] Regulation (EC) No 1935/2004 of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC.

[8] Commission Regulation (EU) No 10/2011of 14 January 2011on plastic materials and articles intended to come into contact with food

[9] Council Directive No. 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1. The CONTAM Panel noted that the Commission Regulation (EC) No. 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives, entering into force on June 2013, confirmed the approved uses of microcrystalline waxes as food additives at quantum satis for use in the surface treatment of confectionery other than cocoa and chocolate products, entire fruits (melons, papaya, mango and avocado), chewing gum and decorations, coatings and fillings (except fruit-based fillings).

[10] Commission Directive 2009/10/EC of 13 February 2009 amending Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 44, 14.2.2009, p. 62-78.

be not less than 500 Da on average, viscosity must be not less than 11 mm^2/s at 100 °C or not less than 8 mm^2/s at 120 °C if solid at 100 °C. The microcrystalline waxes must not contain more than 5 % of molecules with carbon numbers less than 25. In addition, restrictions regarding the presence of polycyclic aromatic hydrocarbons are specified.

4.3. Pesticides

Paraffin oils with the following CAS numbers are included in Annex to the EC implementing Regulation 540/2011[11] on active substances authorised for use in plant protection products, as foreseen by EC Regulation 1107/2009[12] concerning the placing of plant protection products on the market: 64742-46-7 ($C_{11} - C_{25}$), 72623-86-0 ($C_{15} - C_{30}$) and 97862-82-3 ($C_{11} - C_{30}$). As regards purity requirements it is referred to in the European Pharmacopoeia 6.0.

According to Regulation 889/2008[13] laying down detailed rules for the implementation of Regulation (EC) No 834/2007[14] on organic production and labelling of organic products with regard to organic production, labelling and control the following MOH are allowed to be used as pesticides in the production of organic food:

- paraffin oil (as insecticide and acaricide);
- mineral oils (as insecticide and fungicide; only in fruit trees, vines, olive trees and tropical crops (e.g. bananas)).

The CONTAM Panel noted that specifications and limits for this MOH are not established by the Regulations on organic foods.

5. Sampling and methods of analysis

5.1. Sampling

There are no specific guidelines for sampling of foods to be analysed for their MOH content. Therefore, basic rules for organic contaminants or pesticides should be followed. Respective requirements are, for example, laid down in Commission Regulation (EC) No 1883/2006[15] on methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs. This Regulation contains inter alia a number of provisions concerning methods of sampling depending on the size of the lot, packaging, transport, storage, sealing and labelling. The primary objective is to obtain a representative and homogeneous laboratory sample with no secondary contamination.

5.2. Methods of analysis

5.2.1. Principles

Foods may contain endogenous hydrocarbons, such as odd-numbered n-alkanes from wax layers of leaves and fruits, as well as MOH or oligomers from polyolefins (POSH). Similar methods are applied for the analysis of all of them, but they also have to be designed to distinguish them as best possible.

[11] Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 1-186.

[12] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1-50.

[13] Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. OJ L 250, 18.9.2008, p. 1–84.

[14] Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. OJ L 189, 20.7.2007, p. 1-23.

[15] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

In the past, the sum of the MOH was often determined by infra red (IR) spectroscopy. Extracts in carbon tetrachloride were purified by retention of polar constituents on Florisil or silica gel followed by quantitative IR analysis in the carbon-hydrogen stretching region. Detection limits were reported as 1 mg/kg for feeds and 10 mg/kg for tissue (e.g. Walters et al., 1994). However, since these methods do not distinguish between MOH and natural hydrocarbons, which may be present in foods at levels of several 100 mg/kg, they are not used for the determination of MOH in foods.

Present methods for measuring MOH in foods are commonly based on GC with flame ionisation detection (FID). FID is chosen because of calibration problems encountered with other detection methods, such as mass spectrometry (MS): FID is the only method available for a quantitative determination of mixtures of hydrocarbons which are not available as standards. However, FID is not selective and of modest sensitivity, which are serious drawbacks in view of the broad patterns of unresolved peaks of unidentified components formed by mineral oil products (Biedermann et al., 2009).

GC is the separation technique of choice because it enables the distinction between MOH and the hydrocarbons naturally occurring in foods. It enables characterisation of the mineral oil products by molecular mass range as well as presence or absence of n-alkanes, but it is far removed from achieving resolution into individual substances. It is used to determine the sum of the MOSH or MOAH, potentially with specified molecular mass ranges (Biedermann et al., 2009; Biedermann and Grob, 2010). Commonly short columns with thin films of non-polar stationary phase are used.

Further (but still not complete) resolution can be achieved by comprehensive two-dimensional GC (GCxGC; see above). Often a normal size column with a non-polar stationary phase is combined with 1-2 m x 0.1-0.2 mm i.d. second dimension column coated by a polar stationary phase, such as a phenyl methyl polysiloxane. GCxGC is widely used by the mineral oil industry. It was occasionally used for the characterisation of mineral oils in packaging materials (such as paperboard) or foods after isolation of the MOSH or MOAH fraction, e.g. by HPLC (see Section 6.1.4.7).

GC-MS or GCxGC-MS may be used for extracting and quantifying marker components, such as steranes and hopanes considered as proof of mineral origin (Populin et al., 2004).

The separation of polyolefin oligomeric saturated hydrocarbons (POSH) released by polyethylene (PE) from MOSH is impossible: both form broad patterns of unresolved peaks of unidentified material. POSH from PE usually contain some n-alkanes with an even number of carbons, primarily C_{12}, C_{14} and C_{16}, as well as some characteristic minor peaks which suggest the presence of POSH. When both MOSH and POSH are present, they cannot be distinguished quantitatively. POSH from polypropylene (PP) form characteristic clusters of peaks which are more easily recognized.

Liquid chromatography may separate MOH into paraffins, naphthenes and aromatics, but since there is no suitable detector, it is merely used for pre-separation prior to GC analysis to achieve the following separations:

1. Isolation of the MOH from the sample matrix.
    a. Removal of lipids: as mineral oil is commonly in the fat phase of foods, large amounts of lipids may need to be removed, most pronounced in the analysis of edible oils and fats.
    b. Separation from potentially interfering food components, such as squalene and its isomerisation products, carotenes and wax esters.
    c. Removal of interfering hydrocarbons of plant origin, predominantly n-alkanes of odd-numbered carbon atoms from C_{23} to C_{35}.

2. Separation between MOSH and MOAH, possibly also between the paraffins and the naphthenes.

Some methods start with the saponification of lipids and the removal of the resulting soaps (e.g. Castle et al., 1993b; Guinda et al., 1996; Koprivniak et al., 1997). They are derived from methods for the analysis of minor components in edible fats and oils. However, the saponification procedure is tedious, time- and solvent-consuming, and increases the possibility of accidental contamination during the analysis. This step can be avoided when liquid chromatographic columns have sufficient capacity to retain the lipids, first of all the triglycerides.

5.2.2. Extraction

Extraction needs to be complete for the relevant MOSH and MOAH, be in a solvent adequate for the pre-separation and – in the case of certain packaging materials – discriminate from the high molecular mass hydrocarbons, such as hot melts and polyethylene oligomers which disturb GC analysis (Lorenzini et al., 2010).

Solvent accessibility of MOH in dry foods or water-rich foods may be limited, resulting in incomplete recovery of these lipophilic contaminants.

The extraction yield for solids cannot be tested by standard addition, since the added standards will remain on the outside. Completeness must be checked by re-extraction under accentuated conditions, such as substantially longer times and increased temperature. As every product may behave differently, it is advisable to apply prolonged times (e.g. over 24 hours). Some solids (e.g. some milk powders for baby bottles or noodles) cannot be satisfactorily extracted with hexane, even with long durations at 60 °C. Milk powders can be extracted after acidic hydrolysis, i.e. by the standard methods applied for milk. Noodles must be soaked in hot water and then extracted like wet foods.

For wet samples, the water needs to be removed before extraction with hexane is possible. If they include volatile MOH, this cannot be performed by evaporation. Water can, however, be largely replaced by a solvent which is also miscible with hexane, such as ethanol. The amount of ethanol added should exceed that of the water in the sample by at least 5-fold, such that after equilibration the pores of the sample are filled with >80 % ethanol. Equilibration needs an adequate amount of time (e.g. one hour), before extraction is carried out with hexane. As the ethanol contains some MOH, it needs to be recombined with the hexane and then extracted with water (Biedermann-Brem and Grob, 2011).

5.2.3. On-line coupled HPLC-GC-FID

A large part of the MOH analysis was achieved by on-line coupled HPLC-GC-FID with 25 cm x 2 mm i.d. columns and transfer of complete HPLC fractions (300-500 µl) into GC by specially designed evaporation techniques (Grob, 1991). Up to 2009, this method was exclusively used for MOSH analysis. Some data on the MOAH, including separation by ring number, was published by Moret et al. (1996), but this method was not suitable for routine analysis.

Silica gel used in HPLC provides complete separation between MOSH and MOAH as long as the eluent used, hexane or pentane, is free of polar impurities (Biedermann et al., 2009). Paraffins and naphthenes are separated at least partly, but the method has not been elaborated for this separation. The critical separation is between the naphthenes of several rings and the highly alkylated benzenes.

Reviews on the on-line coupled HPLC-GC-FID are published by Biedermann and Grob (2012a,b). On-line coupled HPLC-GC-FID provides highest sensitivity, is automated and avoids manipulations risking the introduction of contaminants, but corresponding instrumentation is available only in a few laboratories.

Detection limits depend on the MOH distribution and the sample matrix, but are usually around 5 mg/kg in edible oils (20 mg oil being injected) and 0.1-0.5 mg/kg in foods with a low fat content. The measurement uncertainty is largely determined by drawing the baseline underneath the pattern of

unresolved peaks and the contour line on top of the pattern of unresolved peaks separating from food components. Commonly it varies between 10 and 30 %.

Pre-separation by means of off-line HPLC and possibly automated fraction collection is an alternative (Castle et al., 1993a). To achieve comparable sensitivity, a large aliquot of the HPLC fraction must be injected into the GC, which calls for large volume injection.

5.2.4. Manual methods

Various manual methods for pre-separation before GC-FID analysis are in use. They are termed "manual", since they are not automated. They involve a conventional liquid chromatography column of a dimension usually determined by the required capacity to retain lipids. A method involving aluminium oxide pre-separation was ring tested and described by Wagner et al. (2001a). The detection limit was 3-20 mg/kg, depending on the distribution of the mineral paraffins and the interferences by sample components.

Activated silica gel provides higher capacity to retain lipids than aluminium oxide and better separation of MOSH from interfering olefins, particularly squalene and its isomerisation products. A manual method only for MOSH with a detection limit of about 10 mg/kg (Fiselier and Grob, 2008; BfR, 2012) has been successfully used by numerous participants of a ring trial (JRC, 2008). A modified version using a GC-FID method was published by Fiorini et al. (2010), with the limit of detection (LOD) and the limit of quantification (LOQ) of 5 and 15 mg/kg, respectively, for oils and 0.3 and 1 mg/kg for dried fruit samples.

In conventional LC with silica gel or aluminium oxide, separation between MOSH and MOAH is incomplete. It can be improved with silver nitrate, but then the separation between the MOAH and the wax esters (long chain alcohols with saturated fatty acids) becomes critical. Best results were obtained by mixing silica gel with a low amount of silver nitrate with activated silica gel and an eluent containing dichloromethane/toluene for the elution of the MOAH (Grundböck et al., 2010a; BfR, 2011).

Possibilities to reconcentrate the prepared sample by solvent evaporation are limited by losses of volatile components. The poor sensitivity obtained with FID for the broad patterns of unresolved peaks formed by the MOAH calls for large volume injection into GC (some 50 µl), which is possible by the on-column/retention gap technique, by splitless injection with concurrent solvent recondensation or programmed temperature vaporizing techniques.

5.2.5. Auxiliary techniques

The methods outlined above are suitable for the majority of samples, but for some applications additional steps are required.

The analysis of the MOAH may require the removal of interfering olefins, such as squalene and olefins formed during vegetable oil raffination (isomerisation products of squalene, sterenes and derivatives of carotenes). This can be achieved by selective epoxidation, rendering the olefins more polar and increasing their retention beyond that of the MOAH (Biedermann et al., 2009).

The analysis of the MOSH may require the removal of the n-alkanes present in foods, since the latter may severely overload the GC (e.g. wheat germ oil or apples). This can be achieved with strongly activated aluminium oxide: using pentane or hexane as eluent, it retains long chain n-alkanes ($> C_{22}$), whereas iso-alkanes and naphthenes pass unretained. This technique can be used provided the samples do not contain mineral waxes (Fiselier et al., 2009a, b).

To reduce detection limits for MOSH in edible fats and oils to around 0.1 mg/kg, higher capacities for retaining lipids and n-alkanes of food origin are needed. This was achieved by a conventional LC

column packed with a double bed of activated silica gel and activated aluminium oxide (Fiselier and Grob, 2009). Final analysis occurred by on-line HPLC-GC-FID.

5.2.6. Methods of analysis in human samples

On-line coupled HPLC-GC-FID was used to analyse human body fat and milk. Samples were hydrolysed with hydrochloric acid and extracted with pentane (Noti et al., 2003; Concin et al., 2008). HPLC pre-separation involved two columns in series. Detection limits related to the fat were between 3 and 10 mg/kg.

5.2.7. Interlaboratory studies and certified reference materials (CRMs)

The quality assurance for controlling the analytical method relies on the laboratory's internal measures as there are no certified or standard reference materials available for MOH in food. Quality assurance (QA) for the HPLC-GC-FID method was based on verification (Grob, 2007): next to the internal standards, verification standards were added which check for the adequate position of the HPLC windows of MOSH and MOAH and rule out loss of volatile components during sample preparation or HPLC-GC transfer. Similar techniques are used for some of the manual methods. Blanks controls are used frequently to avoid contamination during sample preparation.

In 2001, a collaborative study on a manual method involving aluminium oxide pre-separation and GC-FID (Wagner et al., 2001a) with 8 laboratories (7 of which were inexperienced) showed suitability for the control of the Swiss limit of 30 mg/kg paraffins in fats for animal feeds.

In 2008, the Joint Research Centre (JRC) Geel organised a proficiency test for MOH in sunflower oil to check for a 50 mg/kg limit (JRC, 2008) with 55 participants from 17 EU Member States plus Switzerland and Ukraine (JRC, 2008). Two laboratories used automated on-line HPLC–GC-FID, one off-line HPLC-GC-FID and the others manual methods. Test samples comprising both naturally-contaminated and 'spiked' sunflower oil were dispatched to the laboratories, which then had to measure these blind samples using their in-house methods of analysis. The JRC analysed the results, and determined that between 78 % and 85 % of the laboratories were able to measure satisfactorily, depending on the test material.

None of the current methods of analysis to determine MOH in food has been formally validated. The CONTAM Panel noted that certified reference standards and reference materials for MOH need to be provided to allow method development and (inter-laboratory) validation.

6. Sources, occurrence and exposure assessment

6.1. Sources

MOH enter food from many sources: there is an environmental contribution from the air or through the aquatic ecosystem. Machinery during harvesting and processing adds more MOH in several ways. Mineral oils hydrocarbons are used as processing aids. Also food contact materials (primarily packaging) may release MOH into food.

There is a correspondingly high variation in the composition of the MOH. There are food grade oils (virtually MOAH-free), but the majority contain 10-30 % MOAH. Also the molecular mass varies over a broad range, mainly from fairly volatile diluents up to lubricating oils. Some mineral oils also contain impurities, such as the used motor oils present in the exhaust of diesel engines deposited onto plants. For this reason, not only the concentration of the contaminating MOH, but also their impurities may be relevant for evaluation.

The contaminations vary in their mode of entering the food: those entering through the gas phase are restricted to hydrocarbons of sufficient volatility, whereas contamination by wetting contact is not influenced by volatility. Some contaminations can be more easily avoided than others: for instance, oils used to clean or lubricate cylinders of milling machinery and release agents preventing dough

from sticking to surfaces can more easily be replaced than oils migrating from recycled paperboard or deposited onto plants by dust from the roads.

Only a few laboratories have analysed MOH in a wide range of foods, as on-line HPLC-GC is the method of choice and this technique is used only by a few laboratories. Most of the work was done by the Official Food Control Authority of Zürich (Kantonales Labor), which explains why most data are from Switzerland. However, many samples were imported, and since manufacturing practices are the same throughout Europe, no major differences are expected throughout Europe.

6.1.1. Saturated hydrocarbons naturally occurring in biota

A number of saturated hydrocarbons present in MOH also occur naturally in biota. The relevant information is therefore briefly reviewed below.

6.1.1.1. Marine biota

Most marine organisms contain an n-hydrocarbon series ranging from C_{13} to C_{33} with odd chain predominance. In marine algae, C_{15}, C_{17} and C_{19} are generally the predominant n-alkanes. As shown for the cyanobacteria *Nostoc muscorum*, the n-alkanes are formed by decarboxylation of fatty acids, e.g. stearic acid yielding n-C_{17} (Han and Calvin, 1969). However, in algae n-alkanes are also formed through biosynthesis from acetate and pyruvate, since when these precursors were incubated as radio-labelled material with the blue-green algae *Anabena variabilis*, incorporation of radioactivity into heptadecane was observed (Fehler and Light, 1972, cited by Lester, 1979).

The monounsaturated homologues ($C_{15:1}$, $C_{17:1}$, $C_{19:1}$) as well as monomethyl alkanes are also important in some phytoplankton. In zooplankton and fish, the major n-alkanes are generally C_{27} or C_{29} (Sargent, 1976). Nevertheless, the predominant hydrocarbons of most marine algae are polyunsaturated, particularly the 21:6 hydrocarbon, all-cis-heneicosa-3,6,9,12,15,18-hexaene. This alkene is the major hydrocarbon in photosynthetic diatoms, dinoflagellates, cryptomonads, and other eukaryotic marine phytoplankton. Other polyunsaturated hydrocarbons, such as $C_{19:4}$, $C_{19:5}$, $C_{19:6}$, $C_{21:4}$ and $C_{21:5}$, have also been identified in marine algae. Heneicosahexaene is generally absent from zooplankton, with the exception of copepod species feeding on heneicosahexaene-rich algae.

The terpenoid hydrocarbons derived from isopentenyl pyrophosphate have been reported from a wide variety of marine organisms. Squalene (2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene) and pristane (2,6,10,14-Tetramethylpentadecane) are widely distributed in marine biota. Squalene accounts for a large proportion of the lipids from the liver of certain sharks and the eulachon (*Thaleichthys pacificus*), a fish rich in lipids. Pristane is the major hydrocarbon in copepods and other zooplankton (Whittle et al., 1977). Avigan and Blumer (1968) showed that the source of pristane in copepods of the genus *Calanus* was dietary phytol derived from the phytoplankton.

In spite of the capability of most of the higher aquatic animals to metabolise alkanes and naphthenic hydrocarbons (Cravedi and Tulliez, 1981, 1986), some of these compounds are known to biomagnify in the aquatic food chain and to bioaccumulate in fish (Cravedi and Tulliez, 1982). For example, the bioconcentration factor (BCF = Concentration in fish/Concentration in water) of two dodecane isomers, n-dodecane and 2,2,4,6,6-pentamethylheptane, in fish (*Pimephales promelas*) is 240 and 880, respectively (Tolls and van Dijk, 2002).

6.1.1.2. Terrestrial biota

<u>Bacteria and fungi</u>

If most photosynthetic bacteria contain predominantly C_{14}-C_{20} hydrocarbons, most non-photosynthetic bacteria have higher molecular weight (C_{26}-C_{30}) hydrocarbons (Albro, 1976). These may amount to 20 % of their total lipids and are usually a mixture of saturated and monounsaturated normal and methyl-branched alkanes. Odd chain lengths tend to predominate over even. However, the culture

medium as well as culture age can affect the hydrocarbon composition, both in terms of carbon number distribution and degree of saturation.

Various mechanisms for the biosynthesis of long chain alkanes in bacteria have been reported. The first one involves the condensation of two molecules of fatty acid, one of which is decarboxylated in the process. Subsequent reduction of the ketone to the secondary alcohol, followed by a dehydration step, results in a monounsaturated hydrocarbon. This alkene can then be reduced to an alkane. The second mechanism involves elongation of a fatty acid chain via acetate units, oxidative decarboxylation and reduction.

Generally the alkane content of fungal spores is relatively low, ranging from 40 to 150 mg/kg dry weight (Weete, 1976). The distribution of alkanes in fungal spores and mycelium ranged from C_{14} to C_{37}, with C_{27}, C_{29} and C_{31} being the predominant ones. Alkanes with odd numbers of carbon atoms are generally more abundant than those with even numbers. In some species, methyl-branched alkanes are present in the spores. Although hydrocarbon fractions from the fungal tissue extracts contain substances that are certainly fungal products, the similarity in alkane distribution between fungal and plant host tissue in some studies makes it difficult to determine the origin of the alkanes.

Hydrocarbons have also been reported for several yeasts. *Candida sp.* and *Saccharomyces sp.* reportedly produce normal unsaturated and saturated hydrocarbons with chain lengths ranging from C_{14} to C_{19} and from C_{15} to C_{34}, respectively (Weete, 1976).

Plants

n-Alkanes are probably present in all plant waxes, but the percentage may vary from traces to 90 %, depending on the species (Tulloch, 1976). The major alkanes contain an odd number of carbon atoms, ranging from about C_{21} to C_{37}, C_{29} and C_{31} being generally the most frequent.

The cuticle waxes of fruits, such as apples, contain considerable quantities of n-alkanes, mainly C_{29}, C_{31} and C_{33}. A kilogram of unpeeled apples provides about 10 mg of n-alkanes (Salvayre et al., 1988).

Small percentages of branched alkanes have been reported in the surface lipids of higher plants, such as tobacco. A number of unsaturated hydrocarbons have also been isolated from plant lipid extracts. Alkanes containing a cyclohexyl group were reported as minor components of plant waxes (Kuksis, 1964; Mold et al., 1966).

In plants, as in bacteria, the head-to-head condensation mechanism and elongation-decarboxylation pathway have been proposed (Kaneda, 1968; Khan and Kolattukudy, 1974). Experimental evidence obtained by radiotracer techniques, carried out in vitro and in vivo, strongly supports the latter mechanism (Kolattukudy et al., 1976).

The origins of branched alkanes present in higher plants are probably the *iso* and *anteiso* branched starter pieces derived from the correspondingly branched amino acids, such as valine, leucine and isoleucine. Elongation and subsequent decarboxylation of iso starter pieces result in long fatty acids with an even number of carbon atoms and odd chain alkanes. Similarly, long fatty acids and alkanes derived from *anteiso* precursors contain an odd and even number of carbon atom, respectively (Kolattukudy et al., 1976).

Insects

Hydrocarbons are important components of the cuticular lipids of many insects and it is presumed that their role is in contributing to the control of the animal water balance. They comprise between 60 and 90 % of the cuticular lipids of cockroaches and grasshoppers (Jackson and Blomquist, 1976). The most commonly encountered hydrocarbons are n-alkanes, methyl branched alkanes and alkenes. The predominant n-alkanes have an odd number of carbon atoms, usually from C_{21} to C_{33}.

In beeswax, the alkanes are primarily in the range of C_{23} to C_{31}, with a predominance of C_{25}, C_{27} and C_{29}. The alkenes are primarily cis-configured and have a chain length distribution similar to that of the alkanes.

Mono-, di- and tri-methyl alkanes are also present in many insects. The majority of branched monomethyl alkanes have the methyl branch on an odd-numbered carbon (usually 11, 13 and 15), the chain length ranging from C_{21} to C_{36}, but chain lengths up to C_{50} have been reported.

Higher animals

Hydrocarbons represent about 0.5 % of wool wax. They consist of a large number of normal and branched alkanes, ranging from C_{13} to C_{33} (Motiuk, 1980) and include highly branched alkanes as well as cycloalkanes, the origin of which is unclear.

Although hydrocarbons have been found in goat's milk (Cerbulis et al., 1985, cited by RIKILT, 2008), it is unclear to what extent petroleum hydrocarbons are transferred from feed to milk or meat. Uptake of mineral oil by cows from feed can be concluded from studies by Coppock et al. (2001, 2002, cited by RIKILT 2008), who found temporary accumulation of n-alkanes (C_{10}-C_{19}) in the adipose tissue of cows exposed to crude oil and diesel in the feed. The same was reported by Grob et al. (2001).

6.1.2. Environmental contamination

6.1.2.1. Mineral oil hydrocarbons from the atmosphere

The principal atmospheric contaminants containing MOH are exhaust gases from vehicles, smoke from fuel oils as well as debris from tyres and road tar (shown for wheat in Figure 8). Plants are contaminated with MOH from the atmosphere through absorption from the gas phase (volatile compounds up to approximately C_{24}) and deposition of particulate matter (MOH beyond about C_{16}). The former is an equilibration process, the latter depends on the deposition of dust.

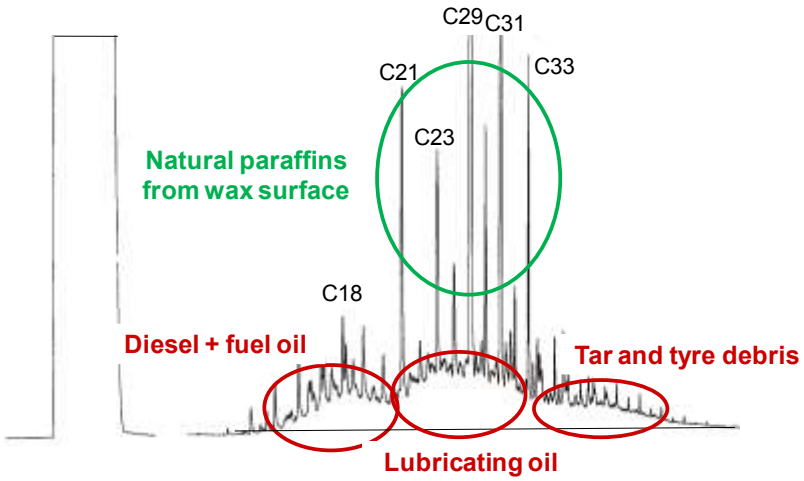

Figure 8: MOSH fraction from wheat contaminated from the atmosphere analysed by on-line HPLC-GC-FID after removal of most long chain n-alkanes by activated aluminium oxide (adapted from Neukom et al., 2002).

Leaves from lettuce and a beach tree in the region of Zürich (Figure 9) contained about 4 mg/kg MOSH per dry mass, approximately ranging from C_{16} to C_{40}, centred on C_{25}-C_{26}. This distribution corresponded to the MOH found in the particulate matter in air and suggested that lubricating oil

emitted from diesel engines was the predominant source of the contamination (Neukom et al., 2002). Particularly cool diesel engines also emit unburned diesel oil (Brandenberger et al., 2005), characterised by MOSH from C_{14} to C_{24}, including n-alkanes, but this was a less important contaminant in foods.

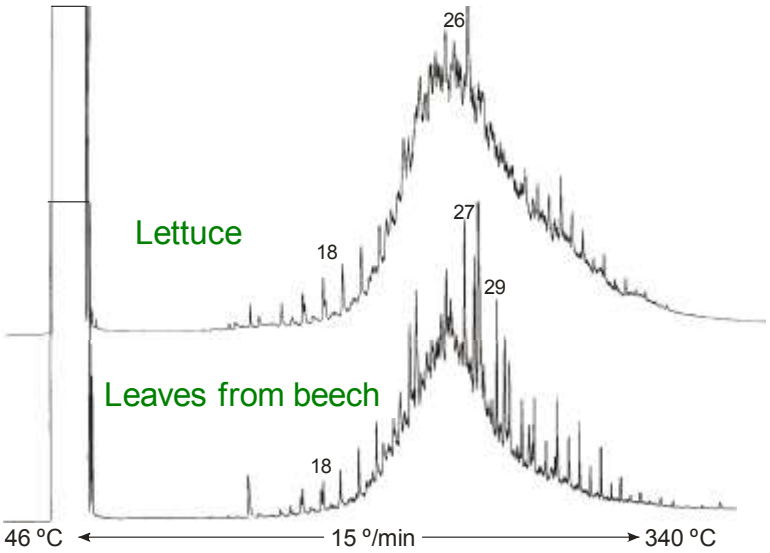

Figure 9: MOSH from lettuce and leaves of the beach tree. Samples pre-separated on activated aluminium oxide to remove the long chain n-alkanes mainly the paraffins from the leaf surface (adapted from Neukom et al., 2002).

The oil extracted from sunflower seeds manually picked from fields in north-eastern Switzerland contained 0.1-2.4 mg/kg MOSH ranging from C_{20} to C_{36} and centred on C_{27}, as typical for lubricating oils. The highest concentrations were found in seeds from fields in the suburbs of Zürich (Grundböck et al., 2010b).

A single apple from a private garden without pesticide treatment contained 55 µg MOSH (corresponding to a concentration of 0.4 mg/kg) ranging from C_{15} to C_{28} (in addition to 500 µg natural n-alkanes C_{27} and C_{29}, corresponding to a concentration of 3.6 mg/kg natural alkanes). The MOSH included n-alkanes, suggesting predominant contamination with diesel or heating oil (Fiselier et al., 2009b).

In the area of airports, landing aircraft could contaminate fruits and vegetables by the release of kerosene. Various types of fruits and vegetables were analysed for MOSH of corresponding composition (C_{13}-C_{16}), but no increased concentrations could be detected at detection limits below 1 mg/kg dry mass (Grob, personal communication).

6.1.2.2. Mineral oil hydrocarbons in marine and fresh water ecosystems

Rather little is known about the contamination of fish and seafood with MOH apart from accidents and other oil spills. The sources could only be speculated about. In freshwater fish from populated regions the sources could include dust washed into the water or debris/extracts from road tar.

Forty samples of fish from sea and fresh water contained 20 - 800 mg/kg MOSH in the fat, with molecular mass distributions centred between C_{17} and C_{28} (3 - 150 mg/kg related to the entire fish; Grob et al., 1997). None of the samples was from an area with a known oil spill. A trout containing 400 mg/kg MOSH in the fat was from a river in a remote area north-east of Zürich without housing and asphalt on roads (unpublished data, Kantonales Labor Zürich).

For a fresh water fish containing 220 mg/kg MOSH in the fat it was shown that it also contained 25 mg/kg alkylated naphthalenes and a smaller amount of fluorenes (Moret et al., 1997).

6.1.3. Food processing

6.1.3.1. Hydrocarbons formed from food components during food processing

Food processing, such as strong heating or treatment with acidic materials, such as bleaching earth (used for refining edible oils and fats), forms hydrocarbons which do not occur in nature. Examples are the sterenes formed by dehydroxylation of sterols with heat and acid during vegetable oil refining, the isomerisation products of squalene or derivatives from carotenoids. Heat treatment also forms polyaromatic hydrocarbons (EFSA, 2008d). These hydrocarbons are not taken into consideration in this text, as they are neither of mineral origin, nor of the same composition.

6.1.3.2. Release agents

Up to some years ago, release agents were probably the predominant source of MOH in food. Paraffin oils, typically centred on about C_{23}, were used in large amounts in the bakery industry to spray surfaces of channels through which the dough should slide, knives to cut the dough into portions or to cut freshly baked bread to slices. Mineral oils were also used for wetting pans for baking bread and biscuits to ease the release of the final product (Grob et al., 1991a). Concentrations in the contaminated products were typically in the range of 500-3 000 mg/kg, with a maximum found in biscuits of 11 000 mg/kg. The same types of oils were used in industry working with sugar, such as candy manufacturers.

Figure 10 shows the MOH from a sample of rusk analysed in 2008 containing 910 mg/kg MOSH. The virtually complete absence of MOAH indicates that the mineral oil was "white".

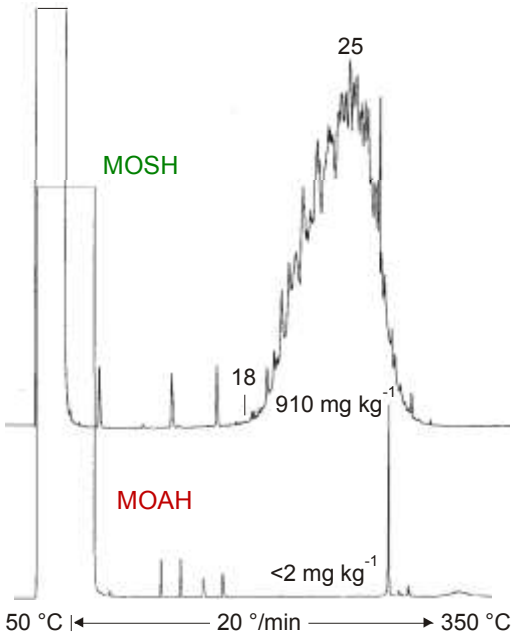

Figure 10: Rusk with mineral oil used as a release agent: MOSH (top) and MOAH (bottom) (adapted from Biedermann et al., 2009).

6.1.3.3. De-dusting agents

Dust released during transport and storage of grain or rice (e.g. pumping into silos) may form explosive mixtures. To bind the dust, the US Federal Drug Administration (US FDA) authorised the use of highly refined mineral oils for grain and rice with limits reaching 800 mg/kg in rice. Such practices were never authorised in Europe.

6.1.3.4. Machine oils

A wide range of machine oils contaminate foods. Harvesters were shown to contaminate sunflower seeds with diesel oil at up to 3 mg/kg and with lubricating oil at up to 5 mg/kg (Grundböck et al., 2010b). These MOH largely end up in the extracted oil (with reconcentration to result in about doubled concentration). It is assumed that other crops are contaminated at similar levels.

Of three collection stations receiving sunflower seeds from farmers, one contaminated seeds with fuel or diesel oil at 12 mg/kg during drying, a second one at a lower concentration. Heating occurred indirectly (Grundböck et al., 2010). The source of the contamination is still unclear.

Oil mills use mineral oils for various purposes, the main applications probably being cleaning and maintenance of machinery. Levels in edible oils produced in large quantities were in the order of 10-30 mg/kg (Fiselier and Grob, 2009), in speciality oils produced in smaller amounts, such as cold pressed oils or oils from special seeds, they were frequently in the range of 100-1 000 mg/kg, maxima reaching about 3000 mg/kg (Grob et al., 1994; Wagner et al., 2001b; Moret et al., 2003). Producers claim to use mineral oils virtually free of MOAH, and in recent years this could be confirmed analytically (Biedermann et al., 2009). The molecular mass distribution was centred at C_{24}-C_{30}.

Chocolate and chocolate products are frequently contaminated with a broad variety of MOH at levels usually below 10 mg/kg, with maxima around 100 mg/kg. A contamination of 80 mg/kg detected in 2010 could be traced back to milling of nuts: mineral oil was used for machinery maintenance (Grob, personal communication).

Syringe-type dosing machinery (e.g. for dosing ice-cream into beakers) uses oils to lubricate the plunger. When the lubricating oil is applied at higher pressure in order to prevent cream getting behind the plunger, there is a constant leakage of lubricating oil into the food (Grob, personal communication).

There is a wide use of lubricating oils, which occasionally leak into foods, in stirring units located above a paste or cream.

6.1.3.5. Coating of foods

Up to about 1990 it was common practice to spray refined rice with mineral oil in order to render it shiny. Concentrations were usually in the range of 1 000 – 3 000 mg/kg. Oils virtually free of MOAH were used with a molecular mass distribution centred on about C_{24}. This practice has never been authorised, either in Switzerland or in the EU, and in Switzerland it was stopped in 1989. However, even in 2009, a rice sample sprayed in this way was found (Grob, personal communication).

It is widely believed that apples as well as other fruits and vegetables are regularly treated with mineral waxes for their preservation and to improve their appearance. According to Directive 95/2/EEC[9] microcrystalline waxes (E 905) are approved for coating of melons, papayas, mangos, and avocados only. The surface of apples is covered by a large amount of natural wax primarily consisting of n-C_{27} and n-C_{29} (Fiselier and Grob, 2009).

6.1.4. Mineral oil migrating from food contact materials

Mineral oil constituents can also be released from food contact materials (FCM), primarily from food packages, into food, either by direct contact between the package and the liquid or semi-solid food or

through the gas phase (evaporation and recondensation) into 'dry' food. Penetration from outer packaging parts through inner pouches or bags represents another possible mechanism of mass transport (permeation) into foods.

6.1.4.1. Jute and sisal bags

Jute and sisal fibres for manufacturing sacks (of around 1-1.5 kg weight for around 50 kg of content) were batched with about 7 % mineral oil to improve their spinning properties (Grob et al., 1991c). The volatile part of this oil (up to about C_{25}) was transferred to the packed foods, primarily hazelnuts, cocoa beans, coffee, rice from Asia (Figure 11) and oil seeds at typically 10-100 mg/kg (Grob et al., 1992a, b; Grob et al., 1993; Moret et al., 1997).

Such a batching oil contained 23 % MOAH of at least two rings, over 99.5 % alkylated, including 8.2 % naphthalenes, 1.8 % fluorenes, 3.2 % dibenzothiophenes, 2.2 % phenanthrenes and anthracenes, 1.0 % fluoranthenes and pyrenes and 0.1 % chrysenes (Grob et al., 1991d).

In 1998, the International Jute Organisation (IJO) adopted 'special criteria for the manufacture of jute bags used in the packaging of selected foods (cocoa beans, coffee beans and shelled nuts)'. The batching oil shall only contain non-toxic ingredients and it shall not contain compounds that produce off-flavours or off-tastes in food. The IJO also specifies limits for the presence of unsaponifiable material in the bags (less than 1 250 mg/kg jute fibre). The EFSA (2004) evaluated these criteria and concluded that:

- if these specifications for unsaponifiable residues in the bags are followed, the use of mineral oils as batching oils, and thus contamination of food, is effectively ruled out and the release of semivolatile mineral hydrocarbons from jute and sisal bags is expected to be significantly reduced;
- if the proposed specifications are followed, human exposure to semivolatile mineral hydrocarbons from jute and sisal bags is estimated to be well below the temporary ADI for mineral hydrocarbons set by the Scientific Committee on Food in 1995;
- adherence to the specifications can be monitored in the producing countries with simple laboratory equipment.

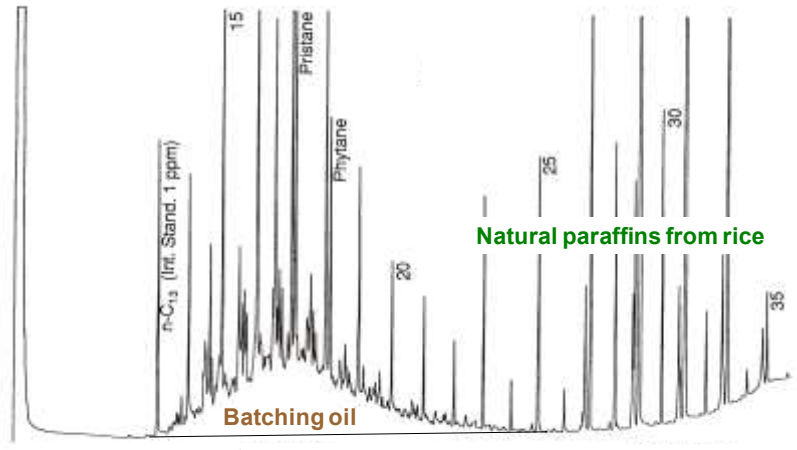

Figure 11: MOSH fraction from rice: contamination by batching oil from a jute bag (Grob et al., 1991c).

Today sacks for the European market are batched with plant oils (and for many applications they have been replaced altogether), but sacks for Asia and Africa are still produced with mineral batching oil, with the effect that rice from these regions (e.g. Basmati) is frequently contaminated with 0.5-10 mg/kg MOH, typically centred on C_{17}-C_{20} and including 20-25 % MOAH (Grob, personal communication).

6.1.4.2. Waxed packaging materials

Waxed paper used for meat products, cheese, bakery ware or candies release waxes into the food (Grob et al., 1991b; Castle et al., 1993a). Concentrations in food strongly depend on the ratio of food contact surface per amount of food and temperature (e.g. high migration when contacting fat is molten). Waxes largely consist of n-alkanes (Figure 12). Waxed paperboard was used, e.g., to pack honey. Large amounts of mineral waxes are still used to render paperboard more water-resistant, including food containers (Biedermann and Grob, 2010).

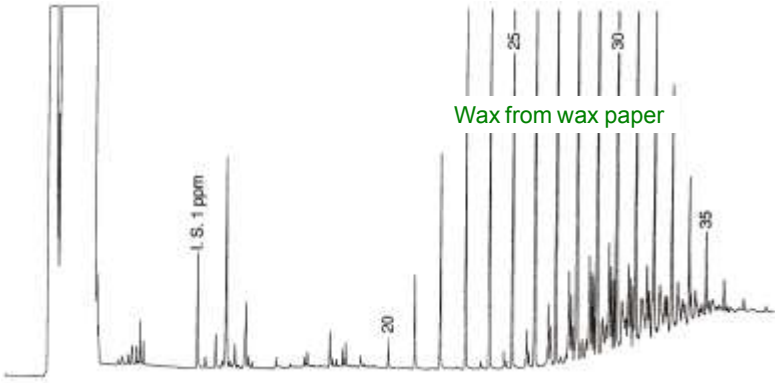

Figure 12: MOSH (90 mg/kg) from salami packed into waxed paper (adapted from Grob et al., 1991b).

6.1.4.3. Wax coatings applied directly on food

Some foods (primarily cheeses) are coated with a wax layer. For cheese it was shown that the migration into the 5 mm surface layer was 15 mg/kg but deeper in the cheese the wax was not detectable (Grob et al., 1991b). Castle et al. (1993b) found 10-150 mg/kg in the outermost 2 mm layer and calculated for 20 samples an average concentration range of < 1 to 27 mg/kg cheese.

6.1.4.4. Plastic materials

Specific grades of mineral oils are approved as additives (e.g. internal lubricants) for use in plastics for food contact according to Regulation (EU) No 10/2011[8] (see Section 4.1). Castle et al. (1991) investigated the migration from polystyrene and acrylonitrile/butadiene/styrene (ABS) containers containing up to 7-8 % mineral oil into aqueous simulants and various beverages. Migration was found to be below 0.5 mg/kg even for hot beverages which can be attributed to the limited solubility of MOH in aqueous foods and the low diffusion out of the ABS polymer. Migrations between 1 and 5 mg/kg were found for fatty dry foods, like powdered formula for babies and cocoa powder (Biedermann-Brem et al., 2012).

Polyolefins contain low molecular weight oligomers, including saturated hydrocarbons (POSH) and olefins. In most polyolefins, POSH largely consist of branched components forming patterns of unresolved peaks, but for some polyethylenes the even numbered n-alkanes predominate. Depending on the type of food (dry, aqueous or fatty), the polymer type and thickness of the polyolefin layer, as well as the nature of contact with the packed food (temperature, time) these oligomers can migrate into the food at levels from the low ppm range (1 – 10 mg/kg; Biedermann et al., 2012) up to the overall migration limit of 60 mg/kg (Alnafouri and Franz, 1999; Kasprick et al., 2010). Although structurally related to the MOSH, polyolefin oligomeric saturated hydrocarbons are not a subject of this evaluation.

6.1.4.5. Lubricating oils for cans

Mineral hydrocarbon oils and waxes may be used in three parts of the can making process; (i) as sheet lubricants; (ii) as wax additives in can coatings (also known as lacquers); and (iii) as components of can sealing compounds.

Mineral oils are used to lubricate sheets of metal as they are cut and shaped into can bodies and ends. For a two-piece drawn and wall ironed (DWI) can any residual lubricant is washed off before the coating (lacquer) is sprayed onto the can interior. All beverage cans and some food cans are washed and coated in this way (Hill, 2007).

For two-piece draw redraw (DRD) cans , for the body of three-piece cans, and for can ends (both classic and easy-open), the sheet of metal is already coated before cutting and forming. Consequently, hydrocarbon waxes or natural waxes such as lanolin or carnauba wax may be used as an additive in the polymeric can coating to protect both the coating itself as well as the tools and dies used during metal forming. These cans are either not washed before filling or are only rinsed with plain water on air (Stoker, 2007) and so any waxes or lubricant oils are not removed and may migrate into the canned food.

Mineral hydrocarbons may also be used as components of the soft sealing compounds used between the can body and the can end, where the metal pieces are curled together to make the hermetic can seal. It is generally considered that there is minimal contact between the sealing compound and the can contents.

A level of up to about 100 mg of oil or wax per square metre of metal is needed to provide adequate lubrication during can making. Assuming that none is transferred to the can-making machinery but is all retained in the can, and considering as a worst case the smallest can with the highest area to volume ratio as being ca. 1 800 cm^2 per kg food (e.g. a small fish can) the maximum theoretical migration may be up to 18 mg/kg food.

Jickells et al. (1994) analysed solvent extracts of 12 types of unused food and beverage cans. The 2- and 3-piece cans tested were obtained from industry and they were closed but were empty of contents. In this way they represented cans in a state immediately prior to filling. Residual levels of hydrocarbons were between 0.05 to 1.1 mg per can. Based on the capacity of the cans and assuming that all mineral hydrocarbons were transferred to the contents, maximum concentrations in foods and beverages were estimated to be between 0.1 to 4.4 mg/kg. They also tested 35 retail samples of canned foods and beverages purchased in the UK. Press lubricants were considered to be present in 50 % of the products at levels ranging from 0.05 - 1.0 mg per can, equivalent to 0.1 - 3.6 mg/kg of food. Additionally, mineral oil of unknown origin was detected in a further 10 of the retail sample at levels of 0.1 - 4.7 mg/kg.

In a more recent UK survey (FSA, 2003) 21 samples of canned food and 5 samples of canned beverages were analysed. No hydrocarbons were detected in 25 of the samples (detection limit 2 mg/kg for mineral oil, 0.3 to 0.5 mg/kg for individual wax hydrocarbons). The one sample that did contain detectable mineral oil, at 8 mg/kg, was a can of hot dog sausages. It is possible, but it was not established, that the mineral oil detected in that sample was as a residue from the manufacture of skinless sausages using temporary sausage casings that were commonly lubricated with mineral oil at that time (Castle et al., 1993b).

In contrast, very high levels of mineral oils have been reported in canned oily foods, such as canned fish in oil. Concentrations in the oil were usually around 100 mg/kg and reached 820 mg/kg (Grob et al., 1997). It seems highly unlikely that such very high levels originated from the cans but probably originated from the oil or the fish before canning.

6.1.4.6. Printing inks

Cold-set printing of paper and board with conventional offset printing inks containing 20-30 % mineral oil (the most common technique for printing paperboard boxes for food packaging in the past) involves drying by setting, which means that the solvent is absorbed into the fibres rather than being removed by evaporation (heat-set printing). However, these MOH (typically centred on C_{17}-C_{19}, see Figure 13) are so volatile that they slowly evaporate either outwards into atmosphere or inwards into

the packed food. Since the freshly packed foods are usually packed into larger transport boxes and stacked onto pallets, most of the oil migrates inwards into the foods if not blocked by a barrier layer between the food and the print layer. Examples of such functional barrier structures are packs with an internal bag consisting of aluminium, polyethylene terephthalate (PET), metallised or coated (acrylic, polyvinylidene chloride, silicium oxide (SiOx), aluminium oxide (AlOx)) polymer films or multi-layer films containing a polyamide or ethylene vinyl alcohol layer and other. Oils for offset printing inks contain 1-50 % MOAH, the MOAH enhancing the solubility of binders and pigments.

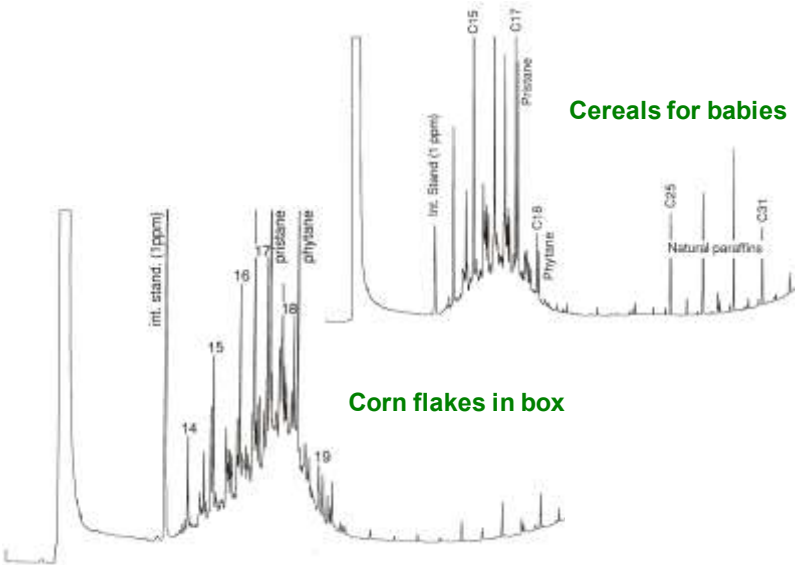

Figure 13: MOSH from mineral oils used as diluents for printing inks migrated into dry foods (see also Droz and Grob, 1997).

Contamination of dry foods in paperboard boxes at concentrations sometimes exceeding 100 mg/kg was shown by Grob et al. (1992a) as well as Droz and Grob (1997). It was also shown that internal paper or polyethylene bags had no barrier effect. In 2010, still a substantial proportion of the offset printing inks applied to food packaging contained mineral oil, often including 15-20 % MOAH (Vollmer et al., 2011). A maximum of 210 mg/kg MOH was found in cocoa powder packed into a paper bag and printed virgin fibre paperboard.

6.1.4.7. Recycled board

Recycled paper and board contains MOH from materials such as newspaper and other heavily printed paper fed into the recycling process, including oils from printing inks, adhesives and solvents used as carriers for binders and additives as well as waxes to improve water resistance (Biedermann et al., 2011; top chromatograms in Figure 14). Since recycled board is not used for liquid food contact, migration is limited to the components of sufficient volatility enabling transfer through the gas phase, which is up to about C_{24} (Lorenzini et al., 2010). This means that the offset printing inks are of primary concern particularly those used for newspaper (Biedermann and Grob, 2010).

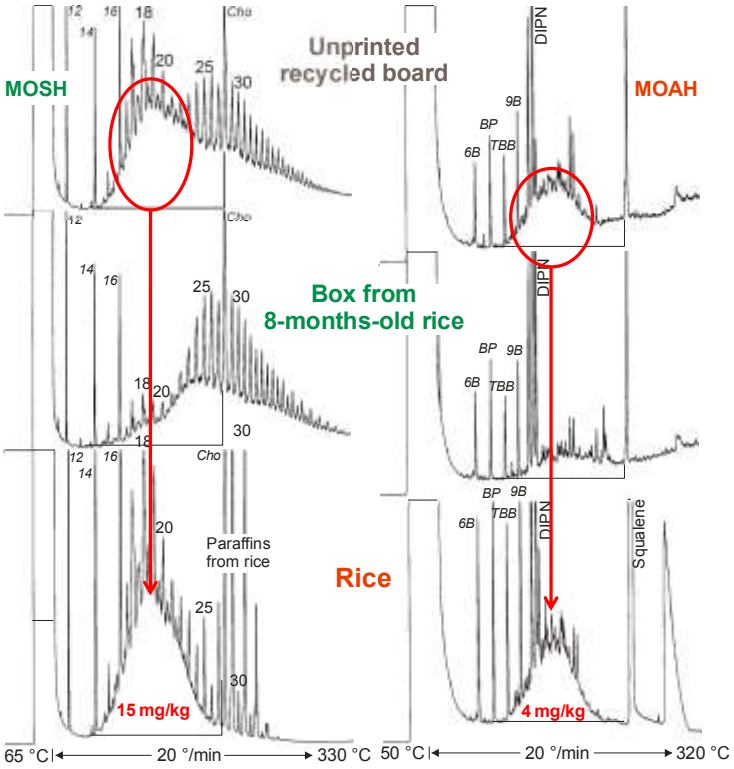

Figure 14: Migration of mineral oil from recycled board into rice (adapted from Biedermann and Grob, 2010).

Within the scope of the German Food Ministry of food, Agriculture and Consumer Protection (BMELV)-Project 'Degree of migration of undesirable substances from packaging materials made of recycled paper into food samples', 120 samples of dry foods intended for long term storage at ambient temperature from the German market were analysed for MOSH in the packaging materials (paperboard and internal bag) as well as the food (Vollmer et al., 2011). The initial concentration of MOSH < C_{24} in the paperboard, i.e. at the moment of packing the food, was estimated by calculating the amounts of MOSH transferred into the internal bag and the food backwards to the board. The mean and maximum concentrations calculated for boxes largely consisting of recycled fibres (n = 107) were 433 mg/kg paper and 1 820 mg/kg paper respectively. In boxes made of virgin fibres (n = 13) the corresponding concentrations were 175 mg/kg paper and 402 mg/kg paper.

The mean MOSH concentration in 60 products packed in recycled board without an internal bag acting as a functional barrier (aluminium and suitable plastic based bags keeping the migration below the detection limit of 0.2-0.5 mg/kg food) was 10.9 mg/kg food, that in the 13 products packed in fresh fibre boxes was 6.2 mg/kg food. The maximum (80 mg/kg food) was found in a 200 g pack of semolina in recycled paperboard with an internal paper bag.

For a selection of samples, also MOAH were analysed in the foods. The maximum determined concentration was 6.1 mg/kg food. The mineral oils transferred into food commonly contained 10-20 % MOAH.

The above data were obtained in April 2010 immediately after collection of the samples. Most of them were 2-3 months old and had a shelf life of 1.5-3 years. Only in a few samples the migration approached the maximum value, assumed to correspond to 70 % mass transfer of the MOSH and

MOAH present in the packaging material eluting from GC before n-C_{24}. Based on this assumption, the average migration was expected to increase to 31 mg/kg and in four samples the migration was anticipated to exceed 100 mg/kg food. In August 2010, the same samples were re-analysed. In fact, the migration proceeded towards the extrapolated value and some of the internal bags still providing a barrier in April no longer acted as an efficient barrier. These data are further explored in Section 6.2.2.4.

Within the scope of the UK Food Standard Agency (FSA) Survey on migration of selected ink components from printed packaging materials and screening of printed packaging for the presence of mineral oils (FSA, 2011), food products collected from the UK market, packed in virgin and recycled carton board, were screened to ascertain whether MOH were present in the packaging material. MOSH was detected in all 51 samples tested and concentrations of MOAH exceeded the limit of detection in 17 of the 51 samples tested (10 mg/kg paper for MOAH with carbon number < C_{24} and 15 mg/kg paper for MOAH with < C_{35}). In the carton board samples, MOSH < C_{24} was determined between 8 and 2149 mg/kg (mean 160 mg/kg). For MOSH < C_{35} concentrations between 11 and 3 028 mg/kg were found (mean 310 mg/kg. Setting the negative samples at the detection limit, concentrations of MOAH < C_{24} were 10 – 154 mg/kg (mean 31 mg/kg) and MOAH < C_{35} ranged from 15 to 229 mg/kg (mean 46 mg/kg). There is no information on the initial concentrations in the packaging or on the time of contact between the packaging and the food.

Another survey on MOH in foods packed in paper and board was conducted for the Austrian market (results provided to EFSA in March 2012 by Austrian Agency for Health and Food Safety (AGES), see also Section 6.2.2.4). 38 samples were collected and analysed for both the concentration in the foods and in the packaging material. Results of the concentration in foods are presented in Section 6.2.2.4. Table 2 presents the results found for the concentration in the packaging material.

Table 2: Concentration of MOSH and MOAH in carton board packaging in samples collected in the Austrian survey.

	MOSH C_{10}-C_{16}	MOSH C_{16}-C_{24}	MOSH C_{24}-C_{35}	MOAH < C_{24}	MOAH C_{24}-C_{35}
	Concentration in packaging, mg/kg				
Mean	12	140	135	48	32
Median	9	99	147	35	25
Minimum	0,1	3	8	2	0,0
Maximum	51	345	344	166	146

MOSH: mineral oil saturated hydrocarbons; MOAH: mineral oil aromatic hydrocarbons.

Some of the foods packaged in paper and board packaging materials screened in the above mentioned surveys are consumed without further processing. It is the case of breakfast cereals and bakery wares. Other foods, like rice for example, are cooked before consumption. However, cooking in boiling water removes only a minor part of the migrated MOH, probably because the MOH are located in the food matrix pores and water entering these pores is an almost perfect barrier to prevent MOH to be transferred into the boiling water (Biedermann-Brem and Grob, 2011).

6.1.4.8. Adhesives

Some glues and adhesives contain mineral oil components to render them sticky. Migration from these particular types of adhesives from bags, boxes and labels has not been systematically investigated so far.

Hot-melt adhesives were investigated in a UK survey since they had been suggested as one potential source of migration of mineral hydrocarbons into foods (FSA, 2003). No mineral hydrocarbons were detected in the adhesives and so, for the samples investigated, the hot-melts were not a potential source of mineral hydrocarbons in food. Several alternative materials to mineral hydrocarbons, such

as polyterpene resins, tall oil rosin esters, and sterol-like natural products, were detected in the adhesives (Bradley and Castle, 2007).

6.1.5. Food additives

According to the European legislation microcrystalline waxes are approved as food additives. EFSA has evaluated the use of high viscosity mineral oils (P100) as food additives for several applications, such as glazing agents in confectionery and frozen meat, protective coatings in fruit and vegetables or antifoaming agents in the wash water for sliced potatoes. The maximum use levels reported for the various applications range from 80 mg/kg in the wash water for sliced potatoes to 6 000 mg/kg for the use as firming or glazing agents in capsules and tablets (EFSA, 2009). This evaluation is not yet taken into account within the legislation on food additives (Directive 95/2/EC[9]).

6.1.6. Pesticides

In certain pesticide formulations, MOSH are used as the active component. They are applied in winter treatment on plants without leaves and suffocate pest organisms by forming a thin gas-impermeable layer.

Mineral oils are also used as formulation aid in products with other active compounds.

Data on residues in food and feed are not known. Such data were also not available in the evaluation process of the paraffin oils authorised as active substances for use in plant protection products (EFSA, 2008c). The relatively high mineral oil content in grape seed oil (50-250 mg/kg) was assumed to be from pesticide formulations, but the final proof was not provided (Fiorini et al., 2008).

6.1.7. MOH entering food chain through feed

6.1.7.1. MOH from edible oil refining

The (predominantly applied) physical refining of edible oils (removal of free fatty acids in the deodorisation step) yields a condensate containing the free fatty acids, some valuable components like tocopherols, but also most of the MOH below about $<C_{27}$ contaminating crude edible oils (see above). This condensate is commonly used for the production of mixed animal feed (possibly after extraction of some valuable components). 200-3 000 mg/kg MOSH were measured in such condensates (Wagner et al., 2001b).

6.1.7.2. Binders for additives

MOH were used as a binder to facilitate admixture of minor components to feeds, like minerals and vitamins in powder form (Grob et al., 2001). Concentrations in feeds typically ranged from 500-1 000 mg/kg. In about 50 samples of eggs, the fat phase of the egg yolk contained on average about 30 mg/kg MOSH centred on C_{21}-C_{24}, with a maximum of 80 mg/kg; nearly all eggs were contaminated (Grob et al., 2001; surveillance data from the Official Food Control Zürich). The same source caused pork meat to be contaminated, particularly that of the dams.

6.1.7.3. Motor oils and other wastes entering feed

In 1999, it was detected that spent frying oils were contaminated with mineral oil wastes, such as used motor oils (Grob et al., 2001) The wastes contaminated the spent edible oil along the collection process in restaurants and public recycling stations: the collection did not always separate edible and technical oils well enough. These oils also contained components other than MOH, such as additives, paint (in diluents), metals (e.g. used crankcase oil) and possibly pesticides. The oils collected were used in animal feed.

In the used edible oils collected, mineral oils were frequently present at the level of 1 %, sometimes above 10 %. As a consequence, half of 36 samples of body fat from pigs or cattle analysed contained

more than 10 mg/kg MOSH (average, 25 mg/kg; maximum, 100 mg/kg). This contamination disappeared after the use of spent edible oils for feeds was banned.

In Europe, feeding of farmed animals other than fur animals with catering waste or feed material containing or derived from catering waste is prohibited under the EC Regulation 1774/2002.[16]

6.1.8. Other sources

6.1.8.1. Unidentified sources in food

Analysing foods for MOH often reveals contamination which cannot be related to the sources listed above. Tracing these tends to be tedious and finally to reveal particular cases. Often the quality manager of a producer feels sure that no MOH are used in their processes, but finally detects that, for instance, one of the workers struggling with a problem quietly solved it using an oil obtained from a colleague in a mechanics shop or a pharmacy.

Dry butter-fat for cooking contained around 100 mg/kg MOSH. It was produced from butter returned from the sale as it had exceeded the shelf life for fresh butter. Instead of laborious unpacking, it was molten out of the packaging material and the warm butter fat extracted the packaging material (Grob, personal communication).

Mixed pralines came in fresh fibre packing with printing ink free of mineral oil. Nevertheless they contained 30-80 mg/kg MOH including some 20 % MOH as typically encountered in recycled paperboard. It turned out that the pralines were produced in batches and stored on large sheets to recycled paperboard (of course with a clean white surface layer) before being combined to the collection.

One out of about twenty samples of fat from pork (from totally about 250 samples mainly collected in the slaughter house) contained around 200 mg/kg MOH ranging from C_{10} to C_{14} (unpublished; Kantonales Labor Zürich). It turned out that a product essentially consisting of MOH with some herbs was fed to mother pigs in order to stop diarrhoea of piglets. The product was sold as 'manure conditioner'. Figure 15 shows the MOSH from the body fat of a mother pig with a relatively low concentration of 'manure conditioner' (40 mg/kg), but also a MOH used as binder for adding minor components to feeds (60 mg/kg)

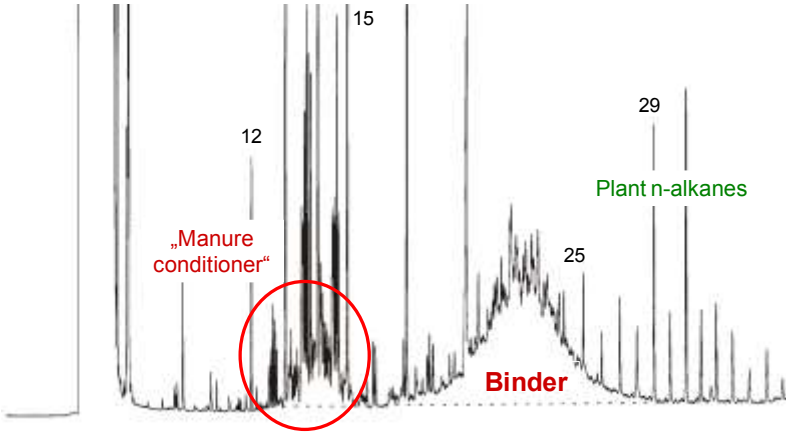

Figure 15: MOSH from the body fat of a mother pig with hydrocarbons corresponding to 'manure conditioner' (C_{12} to C_{15}, 150 mg/kg fat) and a MOH centred on n-C_{23} used as binder in feeds (unpublished; Kantonales Labor Zürich).

[16] Regulation (EC) No 1774/2002 of 3 October2002 laying down health rules concerning animal by-products not intended for human consumption. OJ L 273, 10.10.2002, p. 1-95.

These examples are particular cases, mostly contributing little to the overall exposure to MOH. However, in the routine analysis of MOH in food many such cases are observed, suggesting that there are many more so far unidentified cases of food contamination with MOH which sum up to a relevant contribution to the total exposure. It may be argued that most of these uses are illegal, but, firstly, they are reality and, secondly, usually there is no clear regulation in place.

6.1.8.2. Breast feeding

Breast milk (33 samples from Switzerland) was found to contain C_{15} to C_{45} MOSH at a mean concentration of 95 mg/kg fat and a maximum of 1 300 mg/kg (Noti et al., 2003). For the primiparous women and on the first days of breast feeding, typical concentrations of mineral hydrocarbons in milk were around 50 mg/kg fat. Continuing breast feeding decreased the concentrations several fold, but application of breast salves increased it (ointments and salves were responsible for the maximum value found).

Figure 16 shows an example of the MOSH in breast milk from day 4 after birth of the child. There are prominent peaks for diterpenic hydrocarbons eluted around n-C_{18} and predominantly odd-numbered n-alkanes eluted late (mainly C_{25} to C_{31}, presumably originating from plants), but virtually no mineral n-alkanes. The MOSH form the broad pattern of unresolved peaks ranging from the retention times of n-C_{16} to that of about n-C_{32}, with a maximum at n-C_{23}. This composition corresponds to that of the MOSH in the body fat (Section 7.1).

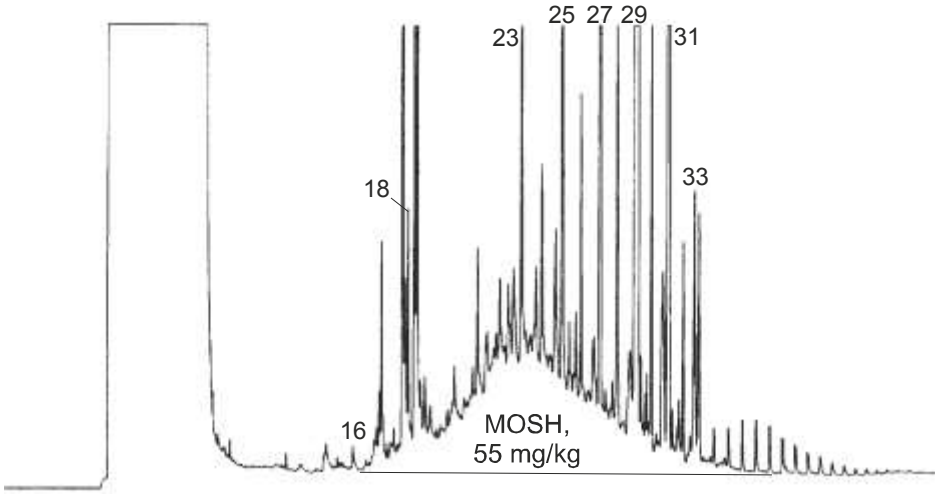

Figure 16: MOSH (55 mg/kg fat) from breast milk (adapted from Concin et al., 2008).

Babies may also be exposed to mineral paraffins by directly licking off salves (often consisting of petroleum jelly) from the breast. Their worst case daily intake from breast care products was estimated to reach 40 mg/kg b.w. (Noti et al., 2003).

MOSH in breast milk from 144 volunteers were analysed on days 4 and 20 after birth (Concin et al., 2008). The samples from day 4 contained virtually the same mixture as the abdominal tissue fat with concentrations ranging between 10 and 355 mg/kg (average, 44.6 mg/kg; median, 30 mg/kg). The fats from the day 20 milks contained <5-285 mg/kg mineral oil paraffins (average, 21.7; median, 10 mg/kg), whereby almost all elevated concentrations were linked with a modified composition, suggesting a new source, such as use of breast salves. The contamination of the milk with MOH seemed to decrease more rapidly than known for other organic contaminants.

6.1.8.3. Fat substitute

MOSH ('paraffin oils') have been recommended for years in weight-reducing diets and are used widely and freely in several countries in salad dressing in replacement of vegetable oil. Although no data were identified on the occurrence of this practice in EU member states, the recent encouragement in best seller books to use MOSH in weight-loss programs (e.g. Dukan, 2010) could result in an increased dietary exposure to MOSH for consumers following these recommendations.

6.1.8.4. Cosmetics, pharmaceuticals and medicinal use

White mineral oils are ingredients of pharmaceutical products or are used as a medicine. People have been exposed to oral doses of up to 100 ml/day (approximately 1 500 mg/kg b.w. per day) over several days or even longer without evidence of major adverse effects (EMEA, 1995).

White mineral oils have also been used in cosmetic preparations, and are a common ingredient in nearly all types of personal care products, from emulsions to anhydrous cosmetics (Morrison et al., 1996; Nash et al. 1996). They are excellent moisturizers and emollients as well as a lipophilic base in which to deliver active ingredients. Naphthenic medicinal white oils have shown good emulsion properties (Shell, 2009). Formulas for baby oils, breast salves, creams and lotions, bath oils, lipsticks and lip gloss, sunscreens, hair products and make-up bases and removers contain MOH (Heimbach et al., 2002; Noti et al. 2003).

Common skin cream formulations (oil-in-water emulsions) contain up to 15 % medicinal white oil. Lotions contain approximately 15 % oil, sunbathing oils up to 50 % and baby oils may contain in excess of 95 %. Many other cosmetics also contain medicinal white oils, e.g. shaving creams (up to 8 %), make-ups (up to 5 %), face creams (approximately 2 %), lip sticks (approx 2 %) and mascara sticks (approx. 4 %) and some soaps with re-fatting properties (up to 10 %). Typically lower viscosity paraffinic medicinal white oils are used.

In animal pharmacy, MOH of non-medicinal quality as well as medicinal white oils (Shell, 2009) are often used as adjuvants in vaccines, for ointments, anti-dusting agents in medicated feeds, vehicles in antimastitic intramammary infusions, emollients in teat dips, ingredients in parasiticides, and vehicles in some subcutaneously-applied depot products (EMEA, 1995). For the hydrocarbons with higher chain length (> C30) it is known that, like in humans, they are not absorbed in ruminants and have a laxative effect. Crude oil has been seen as having this effect in cattle (RIKILT, 2008).

6.1.9. Influence of multiple sources of MOH on occurrence data

As outlined in the sections above, in the analysis of the occurrence data it should be noticed that for many foods MOH can be present from several sources with different overlapping distributions. Rice is an extreme case. Figure 17 schematically shows the known sources with an estimate of the proportion of rice samples containing MOH from the given source. The estimated concentration range is reported on a logarithmic scale in the horizontal axis:

- nearly all rice is contaminated at a low concentration from the environment (usually below 1 mg/kg);
- harvesting adds lubricating or fuel oil to many samples at a slightly higher concentration;
- jute bags primarily contaminate Asian rice, such as Basmati, in a typical range of 2-15 mg/kg;
- most rice packed in paperboard boxes is contaminated from printing inks or recycled board;
- no data are available about real concentrations and frequency of occurrence from antidusting by mineral oil;
- occasionally rice is still sprayed with mineral oil, resulting in concentrations around 1 000 mg/kg. If 1 % of the samples were treated in this way (no data available), this would still be a major source for average exposure and an extremely high one for band-loyal consumers.

Also the quality of the mineral oils, e.g. in terms of molecular mass range and MOAH content, differs for the sources.

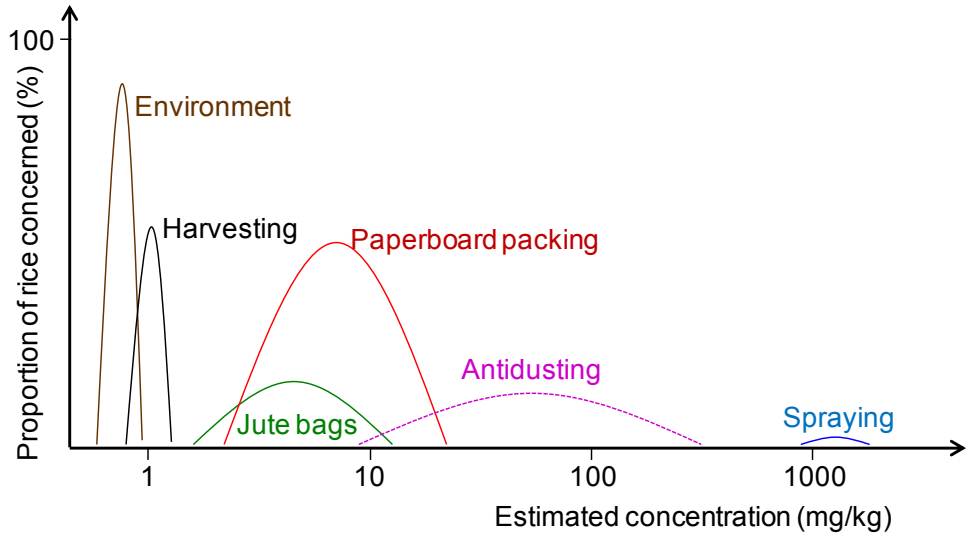

Figure 17: The various sources of MOH potentially contaminating rice: proportion of samples being affected (%) against the concentration expected. Estimates in the figure are intended for illustration of the principles (Grob, personal communication).

6.2. Occurrence

6.2.1. Published occurrence data

Many foods are contaminated with MOH (Section 6.1). Available published results are reported in Appendix A.

Concentrations of MOH in European food were reviewed by Tennant (2004), who used the scientific literature and information provided by industry, but made no measurements. The levels ranged from less than 1 mg/kg in drinks held in polystyrene containers to approximately 250 mg/kg in bread and sugar confectionery. Because of their hydrophobic nature, mineral waxes are not expected to migrate into non-fatty foods. Levels ranged from less than 1 mg/kg in sliced meats and milk/cream to 200 mg/kg in tropical fruit peels and confectionery. Tennant (2004) noted that the occurrence data were generally drawn from old analytical studies with limited numbers of samples. Such samples may have been selected as the analyst expected elevated concentrations and thus some bias could have been introduced.

In addition to the natural hydrocarbons, high concentrations of MOH were found in vegetable oils (Moret et al., 1997; Wagner et al., 2001b; Moret et al., 2003; Fiorini et al., 2008; Biedermann et al., 2009; Biedermann and Grob, 2009; Fiselier and Grob, 2009). MOSH concentrations in the about 220 samples summarised by Wagner et al. (2001b) ranged from < 3 in soybean to 260 mg/kg in rice bran oil.

MOSH concentrations in food samples contaminated by mineral batching oil from jute and sisal sacks, such as hazelnuts, cocoa beans, coffee, rice and oil seeds, ranged from < LOD to 500 mg/kg (Grob et al., 1991b; MOAH concentrations were not measured). Use of MOH as batching oil in sacks sent to Europe was largely stopped in the late 1990s, but is still standard for all others.

MOSH concentrations in meat and eggs reflected the mineral oil content of the feeds, as outlined in Section 6.1.7.6.

In sea-water and fresh-water fish, MOSH concentrations ranged from < 10 to 1 200 mg/kg fat, with most concentrations being around 200 mg/kg fat (Moret et al., 1997). The concentrations in canned fish were mostly around 100 mg/kg in the oil phase (maximum 820 mg/kg) and 10-15 mg/kg in the sea foods (maximum 370 mg/kg) (Grob et al., 1997).

In rice, the concentrations ranged from 1.8 to 160 mg/kg (Grob et al.,. 1991b; Moret et al., 1997; Biedermann et al. 2009; Biedermann and Grob, 2010).

In chocolate and cocoa, the concentrations varied between 5 and 1,300 mg/kg (Grob et al. 1991a,b; Moret et al., 1997; Biedermann et al., 2009).

In powdered baby formula, the MOH reached up to 33 mg/kg when packed in paper bags and were not detected in formula from aluminium-laminated bag (Droz and Grob, 1997).

As described in Section 6.1.8.1., babies are exposed to MOSH through breast milk. Similar results were published by Concin et al. (2008) as part of a study also including human body fat (see Section 7.4.1).

The contamination of food with MOH migrating from recycled paperboard is specifically addressed in Section 6.2.2.4, based on data from a survey of the German market (Vollmer et al., 2011) and from a survey of the Austrian market. The samples from the German market survey were analysed twice. The data from the first measurement were included in the occurrence data presented in the food categories. Those from the second measurement, after additional 4 months of storage, were used in Section 6.2.2.4 on occurrence from food packaging materials.

Outside Europe, two studies have estimated the concentrations of 'white mineral oils, paraffin wax and microcrystalline wax' in various categories of foods, based on the amounts added to foods and migration studies from coatings and packaging materials (Heimbach et al., 2002 and WHO/IPCS, 2003). The results of this analysis are summarised in Appendix C.

6.2.2. EFSA call for data

6.2.2.1. Source of data

In August 2010, EFSA launched a call for data on MOHs in food, addressing National Authorities, Universities, Food business operators and other stakeholders. A few organisations answered by providing data.

- Food business operators/associations and academia provided analytical results on MOH in oils and references to published literature.
- The majority of data in different food groups was actually provided by the Official Food Control Authority of the Canton of Zürich (Kantonales Labor Zürich, KLZH) reporting analyses performed from 1997 to 2010. Apart from a few results on bread and rice, older data were not included since they may no longer be pertinent for the present situation. In many cases, the data provided by KLZH were from the publications cited in Section 6.2.1. KLZH provided also 141 analytical results for feed samples produced and consumed in Switzerland. These data, not used in the current opinion, are reported in Appendix B.

6.2.2.2. General remarks

The entire dataset consists of 1 455 data points. Many different factors can affect the suitability of these data to represent the situation in Europe.

- On the one hand, the food market in Europe may be considered relatively homogeneous in terms of technologies and food sources. On the other hand, the different regulatory framework

in Switzerland might have differently influenced the levels of MOH contamination with respect to other countries.

- Since the KLZH is an enforcement laboratory, the selection of many samples was targeted and this may lead to an overestimation of occurrence and exposure estimates. However, high concentrations found in early control activities prompted measures for reduction which may have improved the situation there more than in other countries. This would lead, as said, to an underestimation of the MOH exposure in Europe. However, some of the products analysed by KLZH were produced by other European countries, so they are not solely representative of the situation in Switzerland.
- It is unknown whether the analysed food groups cover all relevant products in terms of MOH contamination.

In the absence of more comprehensive data, the Panel decided to use the available dataset for occurrence analysis and subsequent exposure assessment while highlighting the identified uncertainties in Section 9.

The introduction of a method in 2009 allowing a separate quantification of MOAH confirmed previous expectations that most of the MOH present in food included a substantial proportion of MOAH (15-30 % of total MOH; Grob, personal communication). However, the food grade oils normally found at the highest concentrations, e.g. those from release agents, are usually virtually free of MOAH.

In the absence of data on MOAH, the occurrence evaluation is limited to MOSH. The proportion of MOAH estimated by the KLZH is reported in Table 3.

6.2.2.3. Occurrence data collected on food

Out of the 1 455 single analytical data on MOSH in food collected by EFSA, 338 data originated from Germany, France and Italy (all regarding vegetable oils), the remaining 1 117 were submitted by KLZH. The data from KLZH (many of which have also been published) covered the food products discussed in Sections 6.1 and 6.2.1. As shown in Figure 18, most of the data was from the years 1997-2000 and 2008-2010. Some data on bread and rice from the years 1989-1994 are also included. Though these analyses are old, the measured levels of MOH refer to production practices that are encountered occasionally even today (like use of food grade MOH during dough handling and as release agents or for spraying of rice). Therefore they were kept in the dataset for occurrence analysis, but only included in a worst case scenario.

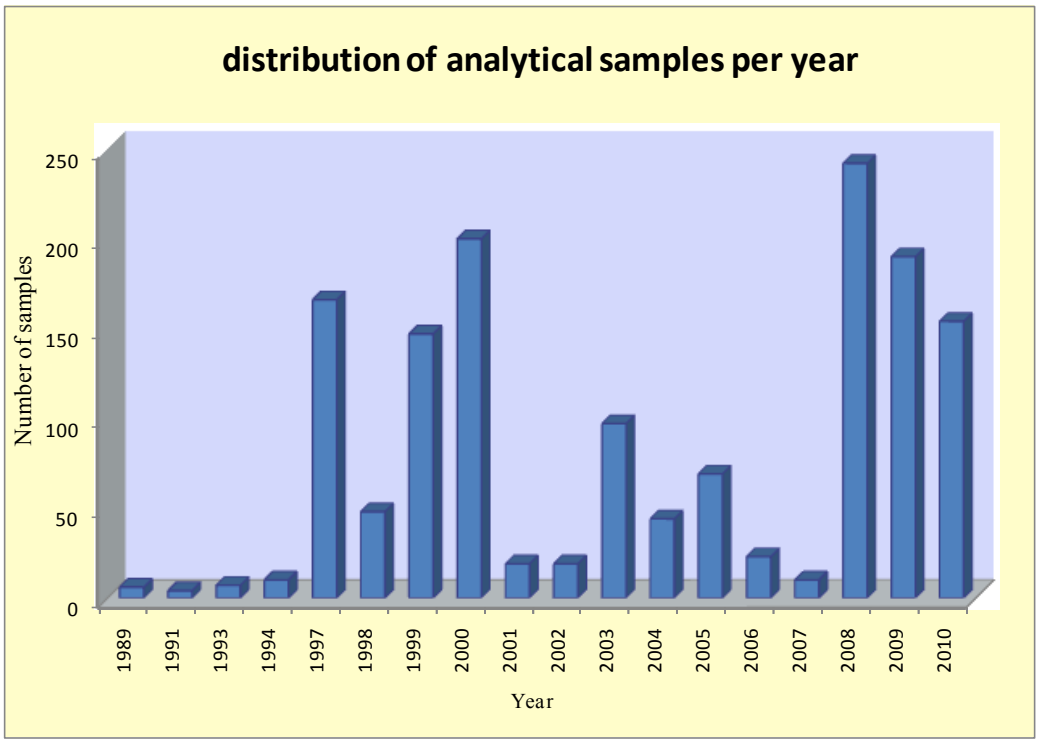

Figure 18: Number of MOH measurements reported by year of analysis

The food samples were classified according to the FoodEx classification system adopted by EFSA for coding the Comprehensive Food Consumption Database (EFSA, 2011a). FoodEx is a flexible food classification system developed by the EFSA Dietary & Chemical Monitoring Unit (DCM Unit) in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food groups (first level), which are further divided into subgroups having 140 items at the second level, 1 261 items at the third level and reaching about 1 800 end-points (food names or generic food names) at the fourth level.

Data relative to food items rarely consumed as such, or consumed in minor amounts, were converted to food groups commonly reported in the food consumption database by using empirical conversion factors. In particular:

- data on egg yolk were recalculated to whole edible egg, dividing concentrations by 3 (Sinclair, 2005);
- data on cocoa butter were extrapolated to chocolate, dividing concentrations by 3 (Sinclair, 2005; Danish Food Composition Databank, 2011; Fineli ®, 2011);
- data on dried bread and bread products were recalculated to fresh bread as widely consumed, dividing concentrations by 1.5 (Danish Food Composition Databank, 2011; Fineli ®, 2011);
- in the absence of data on meat as such, data on animal fat were also converted to livestock meat dividing by the average value of 11.9, calculated from the fat values of meat recorded in the Comprehensive Food Consumption Database;
- data on fish fat were converted to fish meat dividing by the average value of 8.3, calculated from the fat values of fish recorded in the Comprehensive Food Consumption Database;
- data on the oil from canned fish (FoodEx group 'Fish products') were divided by 4 (Grob et al., 1997), under the assumption that the oil in the can is consumed together with the fish.

Overall, 26 FoodEx groups were represented in the dataset; almost all of them were at level 2, apart from, 'breast milk' and 'potato flakes' (level 3 group).

Figure 19 presents the distribution of reported analytical values among food groups

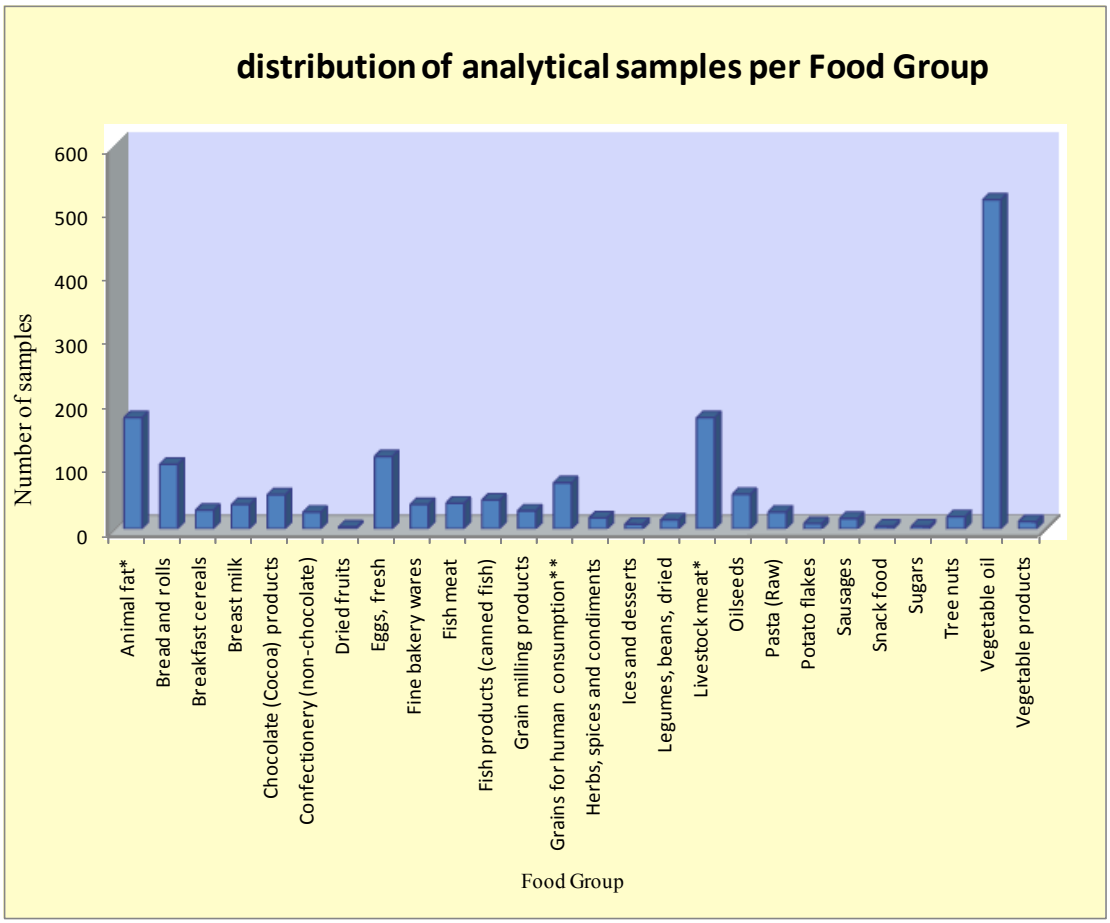

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Figure 19: Number of MOH measurements reported by food group. (* 'Animal fat' and 'Livestock meat' share the same original data, with a conversion factor; ** 'Grains for human consumption' is predominantly represented by rice).

The group 'Vegetable oil' (515 results, corresponding to 35 % of all the data) was the most represented in the data set, followed by 'Livestock meat' ('Animal fat') (174 results = 12 %), 'Eggs, fresh' (113 results = 8 %), 'Bread' (101 results = 7 %) and 'Grains for human consumption' (predominantly represented by rice) (72 results = 5 %).

Almost one third of the data, 431 results (30 %), were reported as not detected (< LOD) or not quantified (< LOQ). These data are usually referred to as 'left-censored' (LC). To attribute a value to LC results, the substitution method was used, as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009) and the EFSA scientific report 'Management of LC data in dietary exposure assessment of chemical substances' (EFSA, 2010). The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). This approach, based on the consideration that the true value for a LC result is a value between 0 and the limit, compares the two extreme scenarios. The LB approach assumes that the substance is absent, thus to LC results reported as < LOD or < LOQ a value of 0 is assigned. The UB approach assumes that the substance is present at the level of the limit thus assigning the value of the LOD or LOQ to results reported as < LOD or < LOQ, respectively.

The range (minimum-maximum) of LODs reported in the different food groups is summarised in Figure 20.

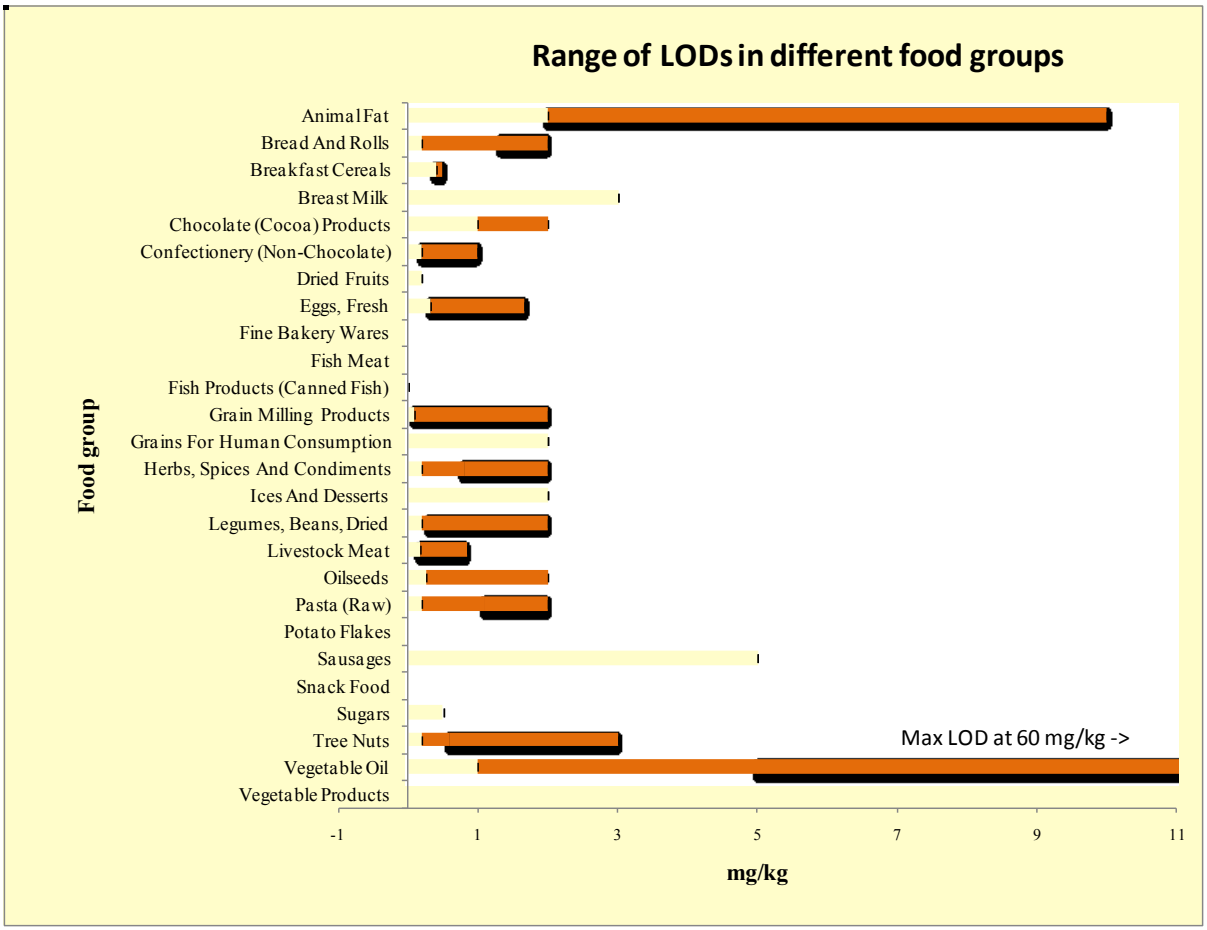


Figure 20: Range (minimum-maximum) of LOD reported by food group.

Apart from a few data points in the 'Vegetable oil' and 'Animal fat' groups, most of the reported LOD are around or below 3 mg/kg.

A statistical analysis of the data per food group was performed calculating mean, median (P50), 75th percentile (P75), 95th percentile (P95) and maximum of occurrence. The analysis is reported in Table 2 both for the lower bound and upper bound approach. The number of samples and percentages of LC data in each food group are also included.

The statistical robustness of the percentiles is dependent on the number of samples. The Guidance on the use of the Comprehensive food consumption database warns that the 95th percentile estimates obtained with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should be considered with caution. Applying the same rationale to evaluate the other percentiles, the minimum number of observations for the 75th percentile is 11 and for the median (50th percentile) is 6.

Additionally, the range of carbon number distribution and the carbon number the mixture is centred on, as well as an estimated percentage of MOAH as determined from the analytical experience of KLZH are reported as reference.

Some food groups show a few high values. In particular, the high values in the groups 'Bread and rolls' and 'Grains for human consumption' (mainly represented by rice) can be related to specific production practices. In these cases, the distribution of values showed two different maxima, therefore it was modelled using a maximum likelihood log-normal fitting (Du, 2002) in order to identify a mean value for both the 'background' occurrence and the high levels of occurrence (Appendix D). The mean values calculated from the model are reported at the end of Table 3. The 'background' occurrence may actually include MOSH from different sources, as highlighted in Section 6.1.9 and therefore be constituted by many different contributions. However, the fittings of the lower distributions for both bread and grains were considered to well represent the total background occurrence levels for these food groups.

Table 3: Summary of MOSH occurrence data (lower and upper bound) presented by food group: number of samples, percentage of LC data, mean, median, 75th percentile (P75), 95th percentile (P95) and maximum of occurrence; typical molecular mass distribution (carbon numbers) of MOSH and estimated proportion of MOAH in the total MOH (***), based on values communicated by KLZH.

FOODEX 1 Category	N	LC %	Occurrence (mg/kg)						Carbon number		% MOAH in MOH
			LB / UB	mean	median	P75	P95	max	range	centre	
Animal fat*	174	67 %	LB UB	22 24	0 5.0	10.0 10.0	200 200	379	12-35	22-27	20
Bread and rolls	101	31 %	LB UB	261 261	4.2 4.2	210 210	1 740 1 740	2 800	17-30	24	<1
Breakfast cereals	29	10 %	LB UB	6.0 6.0	4.9 4.9	8.4 8.4	13 13	25	12-25	18	15
Breast milk	38	8 %	LB UB	1.8 2.0	0.4 0.4	1.3 1.7	11 11	21	16-28	24	-
Chocolate (Cocoa) products	53	9 %	LB UB	11 11	5.0 5.0	12 12	40 40	80	20-35	27	20
Confectionery (non-chocolate)	26	42 %	LB UB	46 46	1.0 1.0	30 30	193 193	516	16-35	18-28	25
Dried fruits	3	33 %	LB UB	1.1 1.2	0.6 0.6	2.8 2.8	2.8 2.8	2.8	12-25	18	15
Eggs, fresh	113	9 %	LB UB	3.4 3.4	2.3 2.3	5.0 5.0	10.0 10.0	12	19-40	29-34	-
Fine bakery wares	38	24 %	LB UB	4.5 4.7	2.4 2.4	4.7 4.7	30 30	38	16-28	23	20
Fish meat	40	0 %	LB UB	21 21	8.2 8.2	34 34	75 75	96	12-24	18	17
Fish products (canned fish)	45	0 %	LB UB	40 40	31 31	44 44	106 106	206	12-25	18	20
Grain milling products	28	36 %	LB UB	9.1 9.4	5.2 5.2	10 10	34 34	80	12-35	18	15
Grains for human consumption**	72	24 %	LB UB	131 132	5.0 5.0	15 15	1 560 1 560	2 050	12-35	17-28	15-30
Herbs, spices and condiments	17	29 %	LB UB	4.4 4.7	1.0 2.0	6.4 6.4	25 25	25	12-26	18	30

Table 3: Continued.

FOODEX 1 Category	N	LC %	Occurrence (mg/kg)						Carbon number		% MOAH in MOH
			LB / UB	mean	median	P75	P95	max	range	centre	
Ices and desserts	7	14 %	LB	14	8.4	28	49	49	12-25	18	15
			UB	14	8.4	28	49				
Legumes, beans, dried	14	86 %	LB	0.8	0	0	10	10	12-35	18-28	15
			UB	1.2	0.4	0.5	10				
Livestock meat	174	67 %	LB	1.8	0	0.8	17	32	12-35	22-27	20
			UB	2.0	0.4	0.8	17				
Oilseeds	54	37 %	LB	38	2.0	6.9	61	950	11-35	16-28	18-35
			UB	38	2.1	6.9	61				
Pasta (Raw)	26	15 %	LB	11	4.0	13	40	83	12-25	18	15
			UB	11	4.0	13	40				
Potato flakes	8	0 %	LB	12	7.2	13	39	39	-[a]	-	-
			UB	12	7.2	13	39				
Sausages	16	38 %	LB	7.2	2.1	15	20	20	-	-	-
			UB	9.0	5.0	15	20				
Snack food	4	0 %	LB	1.6	1.1	3.1	4.1	4.1	-	-	-
			UB	1.6	1.1	3.1	4.1				
Sugars	4	25 %	LB	3.5	2.9	5.8	8.4	8.4	-	-	-
			UB	3.7	2.9	5.8	8.4				
Tree nuts	19	32 %	LB	20	1.7	18	204	204	12-30	18-25	15
			UB	21	3.0	18	204				
Vegetable oil	515	31 %	LB	41	14	50	178	618	18-35	27	< 1-30
			UB	45	15	60	178				
Vegetable products	11	0 %	LB	9.6	9.9	11	21	21	18-35	27	25
			UB	9.6	9.9	11	21				
Bread and rolls - modelled background				1.8							
Bread and rolls - modelled worst case				532							
Grains for human consumption - modelled background				4.1							
Grains for human consumption - modelled worst case				977							

* The same samples are also used to estimate the contamination of 'livestock meat'.
** This group is predominantly represented by rice samples.
*** Values only reported for reference, but not used in the exposure assessment.
a: No data available for the estimate.
MOSH: mineral oil saturated hydrocarbons; MOAH: mineral oil aromatic hydrocarbons; MOH: mineral oil hydrocarbons; KLZH: The Official Food Control Authority of the Canton of Zürich - Kantonales Labor Zürich; N = Number of samples; LC: Left censored data; LB: Lower bound; UB: Upper bound; P75: 75th percentile; P95: 95th percentile; Max: Maximum reported value.

The percentage of left-censored data is highly variable across groups, ranging from 0 to 86 %. Overall, the mean of MOSH occurrence in the LB and UB approaches shows little differences in most categories.

Excluding the high values for 'Bread and rolls' and 'Grains for human consumption', the highest mean occurrence values (LB-UB) are in 'Confectionery (non-chocolate)' (46 mg MOSH/kg), 'Vegetable oil' (41-45 mg MOSH/kg), 'Fish products' (canned fish) (40 mg MOSH/kg) and 'Oilseeds' (38 mg MOSH/kg), followed by 'Animal fat' (22-24 mg MOSH/kg), 'Fish meat' (21 mg MOSH/kg), 'Tree nuts' (20-21 mg MOSH/kg) and 'Ices and desserts' (14 mg MOSH/kg).

The mean 'background' occurrence estimates for 'Bread and rolls' is 1.8 mg MOSH/kg whereas for 'Grains for human consumption' it is 4.1 mg MOSH/kg. The mean values calculated for the same two groups assuming the presence of MOSH from release agents and spraying agents are 532 mg MOSH/kg and 977 mg MOSH/kg, respectively. However, it should be noted that while the background levels are of unknown chemical composition and most probably contain also MOAH, the high levels in 'Bread and rolls' or 'Grains for human consumption' refer only to food grade MOSH.

6.2.2.4. Occurrence in dry food packaged in recycled paperboard

Two recent surveys performed in two EU Member States have focused on MOH occurrence in dry foods packaged in paperboard largely consisting of recycled fibres. Often an inner bag made of different materials was part of the packaging system. Data from these two surveys are further explored in this section.

Data from the German Survey BMELV-Project "Degree of migration of undesirable substances from packaging materials made of recycled paper into food samples" with samples collected in April 2010 from the Stuttgart region were analysed in detail (this study was already referred to in Section 6.1.4.6, Vollmer et al., 2011).

The 120 surveyed samples of dry foods intended for long term storage at ambient temperature from the German market were analysed shortly after collection as well as 4 months later, but only the values from the second analysis are used here as they are expected to represent a more conservative case of migration of MOH into food.

The number of samples analysed per food category and packaging assembly is presented in Table 4. Most of the packaging samples largely consisted of recycled fibres (107) and only 13 were made of virgin fibres.

Table 4: Number of samples per food category and packaging assembly analysed in the German survey.

Class	Fibres					Inner bag					
	Total	Fresh	Recycled	Alu[1]	CP[2]	None	Paper	PE[3]	PET[4]	PP[5]	PPacr[6]
Bakery wares	21	2	19	6		1			1	9	4
Baking mix	6		6				4	1	1		
Breadcrumbs	12	1	11			7	2	2		1	
Breakfast cereals	24	3	21	1			1	17		5	
Creme/Pudding powder	6	1	5	1	1		4				
Drink powder	1		1	1							
Dumpling mix	14		14	6			4	4			
Noodles	4		4			3				1	
Rice	11	3	8			5		4	2		
Salt	5		5			5					
Semolina	8	2	6			5	2			1	
Starch/sugar	8	1	7			1	4	1	1		1
Total	**120**	**13**	**107**	**15**	**1**	**27**	**21**	**29**	**5**	**17**	**5**

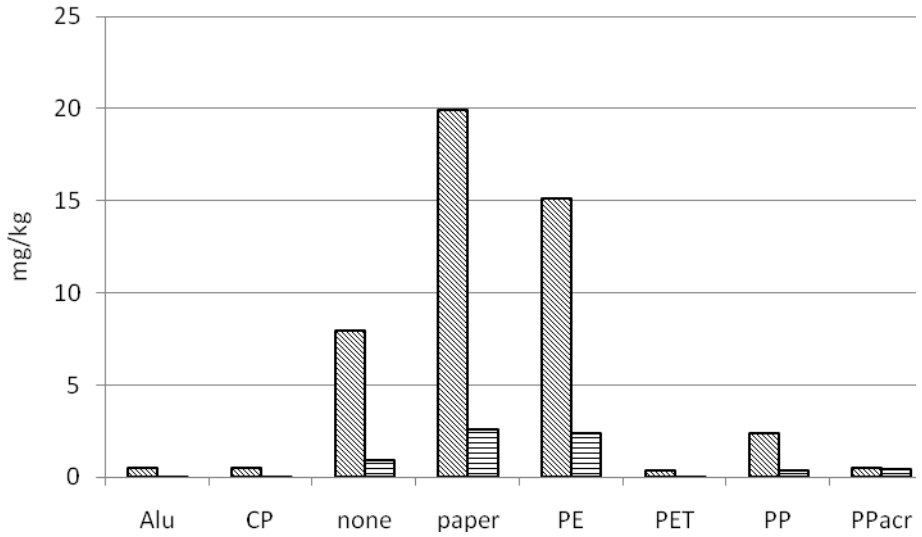
[1]aluminium foil; [2]paper coated with cellulose propionate; [3]polyethylene; [4]polyethylene terephthalate; [5]polypropylene; [6]polypropylene, acrylate coated.

Analysis focused on what was assumed to represent migrated MOH, judged by the molecular mass distribution and the peak pattern: mass range from C_{13} to C_{25}, typically centred on C_{17} to C_{19}.

The source of the MOSH in food was traced back to paper by comparison of the molecular mass distribution, the presence of MOAH and diisopropyl naphthalenes as well as by the peak shape between the food and the recycled paperboard packaging.

The samples in which the food was packaged in direct contact with the board (without an inner bag) or with an inner bag made of non-barrier materials were analysed for MOSH migrating from the packaging. A value equal to LOQ (= 0.5 mg/kg) was attributed to the samples packed with an inner bag functioning as a barrier (26 samples, not analysed). Samples were also analysed for MOAH with detectable MOAH found in 60 samples. The samples analysed for both migrated MOSH and MOAH were used to draw a relationship between these two groups of substances. Data showed that when migrated MOSH increases, MOAH also increases showing a linear trend (R^2 = 0.865). The concentration of migrated MOAH was found to be around 14 % of that of migrated MOSH. The samples not analysed for MOAH were therefore attributed an estimated MOAH concentration equal to 14 % of the MOSH concentration found. Experimental data and estimated data for MOAH were subsequently treated together in the following analysis.

Figure 21 shows the mean (all food categories) of MOSH and MOAH according to the type of packaging system. In most samples (93) the paper and board was not in direct contact with the food and the packaging system included an inner bag. The highest concentrations of MOSH were found in foods packaged in recycled paper with no inner bag or with a paper, polyethylene (PE) or polypropylene (PP) bag. Bags made of aluminium, cellulose propionate (CP), polyethylene terephthalate (PET) or acrylate coated polypropylene (PPacr) provided a barrier against transfer of MOH from paper and board for the duration of storage tested. Consequently, the exposure scenarios drawn in Section 6.3.8 considered the recycled paper packages without a barrier bag as the worst case (no inner bag or bag made from paper, PE or PP).



ALU: aluminium foil; CP: paper coated with cellulose propionate; PE: polyethylene; PET: polyethylene terephthalate; PP: polypropylene; PPacr: polypropylene, acrylate coated.

Figure 21: Average concentration of MOSH (▨) and MOAH (▭) in foods packed in recycled paperboard with different inner bags over all food categories. (German survey, BMELV-Project).

Table 5 presents the mean, median, minimum and maximum concentrations of MOSH and MOAH found in each food category when only the packaging systems without an inner barrier bag are considered. Semolina, creme/pudding powder, rice and baking mix were the categories with the highest concentrations (≥ 15 mg/kg). In breakfast cereals, breadcrumbs, dumpling mix and starch/sugar concentrations were between 5 and 10 mg/kg.

Table 5: Concentration of MOSH and MOAH in food packed in recycled paperboard without inner barrier bag. (German survey, BMELV-Project).

	MOSH, mg/kg				[1]MOAH, mg/kg			
Class	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**
Bakery wares	4.1	4.4	0.5	9.6	0.5	0.5	0.1	1.3
Baking mix	20.0	15.8	7.8	38.6	2.8	3.1	1.1	4.8
Breadcrumbs	9.0	8.9	0.5	13.8	1.3	1.2	0.1	2.6
Breakfast cereals	9.8	9.4	0.3	56.0	1.7	1.3	0.1	7.8
Creme/Pudding	32.4	29.3	9.2	61.8	3.7	2.6	1.3	8.5
Dumpling mix	12.8	9.0	3.4	48.3	1.7	1.3	0.5	6.3
Noodles	1.4	1.4	0.2	2.7	0.1	0.1	0.0	0.2
Rice	18.1	18.3	1.0	46.0	2.2	2.1	0.1	4.5
Salt	0.4	0.3	0.1	0.7	0.1	0.0	0.0	0.1
Semolina	23.9	14.5	0.2	100.0	3.1	1.4	0.0	15.8
Starch/sugar	7.6	5.8	1.9	17.6	0.8	0.5	0.1	1.7
Value for all foods considered together	**11.8**	**8.7**	**0.1**	**100.0**	**1.6**	**1.1**	**0.0**	**15.8**

[1]These data are based on experimental data and, when not available, on estimated data based on the linear relationship found between MOAH and MOSH.

Comparison of the mean concentration values presented in Table 3 with those in Table 5 shows that occurrence values for MOH in some dry foods are significantly higher when only foods packaged in recycled paper without a barrier are considered as compared to the same foods independently of their type of packaging. For example: breakfast cereals have a mean value of 6.0 mg MOSH/kg when all

data are considered, and a mean value of 9.8 mg MOSH/kg when only packages of recycled paper without barrier are considered. For grains for human consumption (including predominantly rice) the mean concentration of MOSH from all sources, excluding spraying with MOH, is 4.1 mg/kg while if only rice packaged in recycled paper without a barrier is considered the mean concentration of MOSH is 18.1 mg/kg.

Data from an Austrian survey were also available (results provided to EFSA in March 2012 by AGES). This survey included analysis of 38 food samples, mostly from packages with recycled fibres (n=34). Analysis was performed after 6 month storage of the samples wrapped in aluminium foil in order to simulate the situation closer to the end of the shelf-life. The composition of the packaging systems regarding the presence and type of inner bag is presented in Table 6.

Table 6: Number of samples per food category and packaging assembly analysed in the Austrian survey

	Total	Fibres			Inner bag			
		Fresh	Recycled	Alu[1]	None	PE[2]	PP[3]	Unknown
Bakery wares	10	1	9	3	1		5	1
Breadcrumbs	2		2		2			
Breakfast cereals	6		6	3		2	1	
Confectionary	2	2	0		2			
Flour	1		1		1			
Noodles	4		4		4			
Pulses	1		1		1			
Rice	4	1	3		3			1
Semolina	8		8	3	3	2		
Total	**38**	**4**	**34**	**9**	**17**	**4**	**6**	**2**

[1]aluminium foil; [2]polyethylene; [3]polypropylene.

All samples were analysed for MOAH (> C_{10}) and for MOSH (C_{10}-C_{35}). Table 7 presents the values for the mean, median, minimum and maximum concentration found in each food class when only the packaging systems without a barrier are considered. The number of samples of some food classes analysed for MOSH was low, therefore the data for MOSH are only indicative.

The mean concentration for all foods in packages made of recycled fibres and without barrier to MOH migration was 7.0 mg/kg for MOSH and 2 mg/kg for MOAH. MOSH concentration in foods ranged from non-detected (detection limit of 0.2 mg/kg) up to 40 mg/kg. The highest values for MOSH were found in noodles (40 mg/kg), bakery wares (21 mg/kg) and rice (24 mg/kg). MOAH concentration in foods was up to 17 mg/kg. The highest values for MOAH were found in bakery wares (6 mg/kg), rice (5 mg/kg), breakfast cereals (4 mg/kg) and noodles (17 mg/kg).

Table 7: Concentration of MOH in food packed in recycled paperboard without barrier. Austrian survey.

	MOSH (C_{10}-C_{35}), mg/kg				MOAH (>C_{10}), mg/kg			
Class	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**
Bakery wares	7.5	7.0	0.2	20.8	1.6	0.6	0.2	5.8
Breadcrumbs	6.8	6.8	5.9	7.6	1.2	1.2	0.9	1.5
Breakfast cereals	5.0	3.6	1.5	10.0	2.2	1.6	0.9	4.0
Noodles	12.1	3.5	1.4	40.0	4.5	0.4	0.3	16.9
Rice	12.7	13.2	0.2	24.6	2.5	2.1	0.3	4.9
Semolina	3.3	0.2	0.2	10.0	1.1	0.6	0.4	2.1
Value for all foods considered together	**7.0**	**5.0**	**0.2**	**40.0**	**2.0**	**0.9**	**0.2**	**16.9**

The results from the Austrian survey are in the same range as those from the German survey.

Occurrence data for MOH found in recycled paper and board and in the packaged foods indicate that when food is packaged in recycled paper and board without a barrier, significant transfer of MOH into food can occur.

6.2.3. Food consumption

6.2.3.1. EFSA's Comprehensive European Food Consumption Database

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built from existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data from the most recent national dietary survey in their country at the level of consumption by the individual consumer. This included food consumption data concerning infants (2 surveys from 2 countries), toddlers (8 surveys from 8 countries), children (17 surveys from 14 countries), adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries), elderly (9 surveys from 9 countries) and very elderly (8 surveys from 8 countries) for a total of 32 different dietary surveys carried out in 22 different countries. Surveys on children were mainly obtained through the Article 36 project 'Individual food consumption data and exposure assessment studies for children' (acronym EXPOCHI) (Huybrechts et al., 2011).

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, consumption data were collected by using different methodologies and thus they are not suitable for direct country-to-country comparison.

The CONTAM Panel considered that only chronic exposure to MOSH has to be assessed (see Section 7.2). Therefore, as suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b), dietary surveys with only one day per subject were not considered for the calculation of exposure, as they are not adequate to assess chronic exposure. Similarly, subjects who participated only one day in the dietary studies although the protocol prescribed more reporting days per individual were also excluded. Thus, for the present assessment, food consumption data were available from 28 different dietary surveys carried out in 17 different European countries as follows:

1. Infants: 2 countries; 2 dietary surveys
2. Toddlers: 7 countries; 9 dietary surveys
3. Other children: 13 countries; 17 dietary surveys
4. Adolescents: 10 countries; 12 dietary surveys
5. Adults: 14 countries; 15 dietary surveys
6. Elderly: 7 countries; 7 dietary surveys
7. Very elderly: 6 countries; 6 dietary surveys

Within the dietary studies subjects were classified in different age classes as defined below in Table 8.

Table 8: Age classes available in the Comprehensive Food Consumption Database.

Infants	< 1 year old
Toddlers (Young children)	≥ 1 year to < 3 year old
Other Children	≥ 3 year old to < 10 years old
Adolescents	≥ 10 years to < 18 years old
Adults	≥ 18 years to < 65 years old
Elderly	≥ 65 years to < 75 years old
Very elderly	≥ 75 years old

For a specific scenario, an additional sub-class is considered to cover the period when infants are presumably only breast fed (or formula-fed):

Breast-fed infants	reference age: 3 months old

6.2.3.2. Food consumption data for specific age and consumers group

Infants and young children are often more exposed to chemicals than adults when considering the food intake in relation to their body weight. The Comprehensive European Food Consumption Database includes detailed food consumption data for children. Results from consumption surveys for children from 13 Member States were gathered by means of the EFSA Article 36 project '"Individual food consumption data and exposure assessment studies for children', described in the EFSA Guidance on Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011b). All food consumption data was collected from infants to children of 18 years and grouped according to the ranges given in Table 5. Consumption records were codified according to the FoodEx classification system developed by the DCM Unit in 2009 (EFSA, 2011a). The EXPOCHI data have been integrated in the Comprehensive Food Consumption Database to allow calculating dietary intake of children according to the different age classes.

Particular attention was put in determining breast milk consumption in infants. Estimating MOH exposure for infants from breast milk or infant formula requires information about the quantity of liquid consumed per day and the duration over which such consumption occurs. According to the Institute of Medicine of the U.S. National Academies of Sciences (IOM), average breast milk consumption is about 750-800 g per day (range, 450-1 200 g per day) for the first 4-5 months of life (IOM, 1991). Infant birth weight and nursing frequency have been shown to influence consumption (IOM, 1991). The WHO related breast milk consumption to body weight rather than age with an estimated 125 mL/kg or 763 mL for a 3 month old child weighing 6.1 kg (Onyango et al., 2002). According to the German DONALD study, the mean consumption of infant formula for a three months old child weighing on average 6.1 kg was 780 mL per day with a 95th percentile consumption of 1 060 mL per day (Kersting et al., 1998). A rounded mean consumption value of 800 g per day, with a high of 1 200 g per day for breast milk and infant formula (Kent et al., 1999) for a 3 month old child will be used here as in other recent EFSA opinions on contaminants to calculate exposure in breast-fed infants. In line with the Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data (EFSA, 2012), a body weight of 5 kg will be assumed for breast-fed infants.

The dietary surveys considered for the chronic dietary exposure assessment are presented in Appendix E. For each survey, the number of subjects in the different age classes is provided. Further details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011b).

6.3. Exposure assessment

An exposure assessment has been carried out considering the overall consumption of foods with occurrence of MOH from various sources. The results of this assessment and their analysis are reported in Sections 6.3.3 and 6.3.4 respectively.

A specific scenario of exposure to MOH from consumption of selected dry foods packaged in recycled paper is described separately in Section 6.3.5.

6.3.1. MOSH dietary exposure scenarios in Europe

Both the average and high (P95) chronic exposure scenario were calculated across different European dietary surveys using the mean occurrence data for different food groups as reported in Table 3 (background exposure scenario). For bread and grains for human consumption, the modelled mean of concentrations excluding values from the use of release agents and spraying was used. It was considered that specific consumers might be exposed during long periods to bread or cereal grains

(rice) with high levels of MOSH, due to restricted choice in the food supply or to brand loyalty. Two additional chronic exposure scenarios were thus calculated to address these sub-groups, one for bread with high levels of MOSH, and the other for grains with high levels of MOSH. An additional exposure scenario was calculated to estimate the exposure of exclusively breast-fed infants.

Acute scenarios were excluded on the ground of toxicological assessment, i.e. none of the MOH preparations was acutely toxic.

6.3.2. Chronic exposure to MOSH in different age classes

For calculating the chronic dietary exposure to MOSH in the general population, food consumption and body weight data at the individual level from the Comprehensive Database were combined with mean occurrence levels. For each country, exposure estimates were calculated per dietary survey and age class. Distributions of individual exposure estimates were therefore calculated for 28 different dietary surveys carried out in 17 different European countries, in each of the available age classes. Not all countries provided consumption information for all age classes; some countries provided more than one consumption survey for the same age class.

For each exposure distribution (defined by dietary survey and age class) mean and high percentile (95^{th} percentile) exposure estimates were calculated. Exposure estimates were calculated for both LB and UB occurrence scenarios.

Depending on the dietary habits, each national dietary survey shows different exposure statistics. Therefore, in the summary table each value is presented as median and range (minimum and maximum) across countries, thus showing the consumption-based exposure variability across Europe.

In accordance with the specifications of the EFSA Guidance on the use of the Comprehensive database (EFSA, 2011b), 95^{th} percentile estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust and therefore they were excluded from the calculation.

The summary statistics of the chronic dietary exposure scenarios for MOSH across European dietary surveys are reported in Table 9.

Table 9: Summary statistics of the chronic dietary exposure scenarios for MOSH (mg/kg b.w. per day) in the general population across European dietary surveys**.** Where a difference is observed between UB and LB, the range is provided.

Mean chronic exposure (mg/kg b.w. per day) across national dietary surveys			
	min (LB-UB)	**median (LB-UB)**	**max (LB-UB)**
Infants [(a)]	0.038 - 0.041	0.10 - 0.11	0.16 - 0.18
Toddlers	0.083 - 0.087	0.11	0.19
Other children	0.066 - 0.068	0.11	0.16 - 0.17
Adolescents	0.028	0.064 - 0.066	0.091 - 0.096
Adults	0.031 - 0.032	0.038 - 0.039	0.064 - 0.068
Elderly	0.031 - 0.032	0.040 - 0.042	0.056 - 0.059
Very elderly	0.032 - 0.033	0.037 - 0.039	0.051 - 0.054

P95 chronic exposure (mg/kg b.w. per day) across national dietary surveys[(b)]			
	min (LB-UB)	**median (LB-UB)**	**max (LB-UB)**
Infants [(a)]		0.12 - 0.13	
Toddlers	0.18	0.22	0.25 - 0.26
Other children	0.14	0.21 - 0.22	0.31 - 0.32
Adolescents	0.063 - 0.065	0.12 - 0.13	0.19 - 0.20
Adults	0.059 - 0.061	0.082 - 0.085	0.11 - 0.12
Elderly	0.058 - 0.060	0.074 - 0.078	0.093 - 0.096
Very elderly	0.069 - 0.070	0.076 - 0.079	0.081 - 0.084

b.w.: body weight; LB: lower-bound; UB: upper-bound;
(a): estimates available only from two dietary surveys for the mean and only one for the 95th percentile;
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation. Those estimates were not included in this table.

6.3.2.1. Infants (< 1 year old)

Only two dietary surveys reported consumption data for children younger than 1 year, therefore the dietary exposure estimate cannot be considered as representative of the European infant population. One of the surveys did not qualify for the calculation of the 95th percentile exposure (number of subjects < 60). Taking into account these limitations, the mean exposure of infants to MOSH ranged from 0.038 to 0.18 mg/kg b.w. per day (minimum LB and maximum UB across national dietary surveys). In the case of high consumers (P95) the UB exposure in the only suitable available survey was estimated to be 0.13 mg/kg b.w. per day (LB = 0.12).

For infants < 3 months old, assuming only breast feeding, a separate estimate is presented in Section 6.3.5 using standard body weight and consumption levels.

6.3.2.2. Toddlers (Young children) (≥ 1 year to < 3 year old)

In the age class 'Toddlers', the exposure to MOSH (minimum LB and maximum UB across national dietary surveys) ranged from 0.083 to 0.19 mg/kg b.w. per day and from 0.18 to 0.26 mg/kg b.w. per day for mean and high consumption (P95), respectively. These values are the highest among the different age classes, due to the ratio between body weight and amount of food consumed.

6.3.2.3. Other Children (≥ 3 year old to < 10 years old)

In the age class 'Other Children', the exposure to MOSH (minimum LB and maximum UB across national dietary surveys) ranged from 0.066 to 0.17 mg/kg b.w. per day and from 0.14 to 0.32 mg/kg b.w. per day for mean and high consumption (P95), respectively. These values are comparable to those for toddlers, with a slight decrease in minimum and median values and further increase in

maximum. In this case exposure is affected by both the dietary habits and the issue relating to body weight previously described for toddlers.

6.3.2.4. Adolescents (≥ 10 years to < 18 years old)

In the age class 'Adolescents', the exposure to MOSH (minimum LB and maximum UB across national dietary surveys) ranged from 0.028 to 0.096 mg/kg b.w. per day and from 0.063 to 0.20 mg/kg b.w. per day for mean and high consumption (P95), respectively. These values are lower with respect to the younger age classes, although the high consumers show an exposure comparable to younger children.

6.3.2.5. Adults (≥ 18 years to < 65 years old)

In the age class 'Adults', the exposure to MOSH (minimum LB and maximum UB across national dietary surveys) ranged from 0.031 to 0.068 mg/kg b.w. per day and from 0.059 to 0.12 mg/kg b.w. per day for mean and high consumption (P95), respectively.

6.3.2.6. Elderly (≥ 65 years to < 75 years old)

In the age class 'Elderly', the exposure to MOSH (minimum LB and maximum UB across national dietary surveys) ranged from 0.031 to 0.059 (median = 0.042) mg/kg b.w. per day and from 0.058 to 0.096 mg/kg b.w. per day for mean and high consumption (P95), respectively.

6.3.2.7. Very elderly (≥ 75 years old)

In the age class 'Very elderly', the exposure to MOSH (minimum LB and maximum UB across national dietary surveys) ranged from 0.032 to 0.054 mg/kg b.w. per day and from 0.069 to 0.084 mg/kg b.w. per day for mean and high consumption (P95), respectively. The values for both 'Elderly' and 'Very elderly' are similar to those of adults, with a minimal decrease.

6.3.2.8. Conclusions on intake in different age classes

According to the present analysis, the dietary exposure to MOSH ranges in the general population between approximately 0.03 and 0.3 mg/kg b.w. per day and is higher in younger consumers than in adults and older age classes. Particularly, the highest exposures were estimated for toddlers and other children, for both average and high consumers, with the maximum found in high consumers among 'other children'. With increasing age, the exposure per kg b.w. considerably decreases. This is, at least partly, explained by the lower intake of food per kg b.w. per day in higher age classes. The similarity in exposure in 'Toddlers' and 'Other children', despite the considerable increase in body weight in the period from one to ten years, depends most probably on age-related differences in dietary habits. In addition, within each age class a relatively high variation is observed between the exposure estimates in different dietary surveys.

6.3.3. Percentage contribution of different food groups

The percentage contribution of the different food groups to the exposure distributions was calculated for each available survey / age class combination. The contribution of a food group to the exposure in any age class varies across dietary surveys therefore the results are reported as median and range (minimum-maximum).

Tables 10 and 11 summarise the contribution of each food group as percent of the total exposure for LB and UB occurrence values, respectively.

Table 10: Contribution of different food sources to chronic dietary exposure to MOSH across European dietary surveys (Lower bound).

Contribution (%) of food groups to mean chronic exposure median (min-max) across national dietary surveys													
Lower bound													
	Infants		**Toddlers**		**Other children**		**Adolescents**		**Adults**		**Elderly**		**Very elderly**	
Animal Fat	10	(<0.1 - 20)	1.5	(<0.1 - 11)	1.4	(<0.1 - 13)	2.8	(<0.1 - 13)	4.4	(1.4 - 13)	10	(1.7 - 18)	11	(2 - 19)
Bread and Rolls	4.1	(<0.1 - 8.1)	8.1	(2.2 - 12)	6.3	(2.8 - 9.6)	6	(4.3 - 11)	7.9	(3.7 - 11)	8.8	(5.6 - 13)	8.6	(7 - 12)
Breakfast Cereals		<0.1	2.1	(<0.1 - 2.9)	2.5	(<0.1 - 11)	2.5	(<0.1 - 7.1)	1.6	(<0.1 - 11)	1.3	(<0.1 - 3.4)	<0.1	(<0.1 - 3.5)
Breast Milk	37	(2.4 - 71)	1.7	(<0.1 - 5)		-		-		-		-		-
Chocolate (Cocoa) Products	0.4	(0.1 - 0.6)	2.1	(<0.1 - 14)	3.8	(2 - 9.8)	3.7	(1.7 - 9)	3.3	(<0.1 - 5.8)	<0.1	(<0.1 - 3.4)	1.6	(<0.1 - 2.9)
Confectionery (Non-Chocolate)		0.2	4.2	(<0.1 - 18)	8.4	(<0.1 - 25)	6	(<0.1 - 30)	2.6	(<0.1 - 21)	1.8	(<0.1 - 8.2)	1.7	(<0.1 - 3.6)
Dried Fruits		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1
Eggs, Fresh	1.8	(<0.1 - 3.4)	1.2	(<0.1 - 2.6)	1.6	(<0.1 - 2.1)	1.2	(<0.1 - 2.2)	1.7	(<0.1 - 2.4)	1.7	(<0.1 - 2.9)	2	(<0.1 - 2.2)
Fine Bakery Wares		9.9	5.1	(3.7 - 11)	8.1	(1.2 - 17)	8	(1.4 - 12)	8.3	(1.4 - 11)	4.9	(2 - 12)	4.8	(2.5 - 13)
Fish Meat	2.8	(2.8 - 2.9)	7.1	(1.6 - 25)	7.4	(2.3 - 20)	7.9	(3.6 - 20)	14	(3.7 - 28)	20	(3.4 - 24)	18	(3.1 - 24)
Fish Products (Canned Fish)		<0.1	4.5	(<0.1 - 9.7)	6.4	(<0.1 - 10)	2.7	(<0.1 - 11)	2.8	(<0.1 - 9)	<0.1	(<0.1 - 3.4)	1.7	(1.2 - 3.2)
Grain Milling Products	5.9	(1.4 - 11)	3	(<0.1 - 6)	2.5	(<0.1 - 9.6)	2.6	(<0.1 - 11)	3.3	(<0.1 - 9.7)	3.1	(<0.1 - 11)	2.8	(<0.1 - 11)
Grains For Human Consumption	2.1	(1 - 3.3)	1.9	(<0.1 - 3)	1.4	(<0.1 - 3.5)	1.7	(<0.1 - 4.7)	1.7	(1 - 4.5)	1.5	(<0.1 - 2.2)	1.3	(<0.1 - 2.2)
Herbs, Spices And Condiments	0.6	(0.1 - 1)	1.2	(<0.1 - 8.8)	2.2	(<0.1 - 5.3)	2.6	(<0.1 - 6.9)	2.9	(<0.1 - 7)	2.1	(<0.1 - 5.4)	2.4	(1.1 - 4.7)
Ices And Desserts		0.4	18	(2.2 - 31)	16	(5.4 - 26)	8.5	(5.2 - 16)	6.4	(<0.1 - 19)	3.9	(<0.1 - 11)	7.2	(2.2 - 13)
Legumes, Beans, Dried		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1
Livestock Meat	1	(0.4 - 1.5)	1	(<0.1 - 4.3)	1.9	(<0.1 - 3.4)	2.8	(1.3 - 4.5)	2.8	(2 - 5.4)	2.9	(2 - 6)	3.2	(1.8 - 5.2)
Oilseeds		<0.1		<0.1	<0.1	(<0.1 - 1.5)	<0.1	(<0.1 - 4.3)	<0.1	(<0.1 - 1.6)	<0.1	(<0.1 - 1.3)		<0.1
Pasta (Raw)	5.3	(3.3 - 7.3)	7.4	(2.6 - 19)	7	(1.9 - 16)	7.3	(1.3 - 16)	4	(<0.1 - 17)	5.9	(1.2 - 15)	4.9	(1.1 - 17)
Potato Flakes		<0.1	<0.1	(<0.1 - 1.2)		<0.1		<0.1		<0.1		<0.1		<0.1
Sausages		1.4	4.8	(<0.1 - 11)	5.5	(<0.1 - 11)	5.2	(1.3 - 13)	4.7	(1.8 - 12)	4.1	(1.2 - 9.2)	3.9	(<0.1 - 9)
Snack Food		0.3		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1
Sugars	5.3	(0.6 - 10)	<0.1	(<0.1 - 3.2)	<0.1	(<0.1 - 1.8)	<0.1	(<0.1 - 1.6)	1.5	(<0.1 - 2.6)	1.4	(<0.1 - 3.1)	1.4	(<0.1 - 3.9)
Tree Nuts		0.1	<0.1	(<0.1 - 1.2)	<0.1	(<0.1 - 1)	<0.1	(<0.1 - 3.7)	<0.1	(<0.1 - 2.3)	<0.1	(<0.1 - 1.8)	<0.1	(<0.1 - 1)
Vegetable Oil	17	(14 - 20)	9.1	(<0.1 - 29)	4.8	(<0.1 - 30)	11	(<0.1 - 33)	8.1	(<0.1 - 36)	7	(<0.1 - 38)	13	(<0.1 - 37)
Vegetable Products		0.4	1.2	(<0.1 - 4.3)	<0.1	(<0.1 - 3.3)	<0.1	(<0.1 - 4.4)	1.3	(<0.1 - 11)	1.3	(<0.1 - 12)	1.1	(<0.1 - 11)

Table 11: Contribution of different food sources to chronic dietary exposure to MOSH across European dietary surveys (Upper bound).

Contribution (%) of food groups to mean chronic exposure median (min-max) across national dietary surveys														
Upper bound														
	Infants		**Toddlers**		**Other children**		**Adolescents**		**Adults**		**Elderly**		**Very elderly**	
Animal Fat	11	(<0.1 - 21)	1.6	(<0.1 - 12)	1.5	(<0.1 - 13)	2.9	(<0.1 - 14)	4.7	(1.5 - 14)	11	(1.8 - 20)	12	(2.1 - 20)
Bread and Rolls	3.9	(<0.1 - 7.7)	7.7	(2.2 - 11)	6.1	(2.7 - 9.3)	5.7	(4.1 - 11)	7.5	(3.5 - 11)	8.4	(5.3 - 12)	8.2	(6.7 - 11)
Breakfast Cereals		<0.1	2.1	(<0.1 - 2.8)	2.4	(<0.1 - 11)	2.4	(<0.1 - 6.9)	1.5	(<0.1 - 11)	1.2	(<0.1 - 3.3)	<0.1	(<0.1 - 3.4)
Breast Milk	38	(2.6 - 73)	1.8	(<0.1 - 5.4)		-		-		-		-		-
Chocolate (Cocoa) Products		<0.1	2	(<0.1 - 13)	3.6	(1.9 - 9.6)	3.6	(1.6 - 8.8)	3.2	(<0.1 - 5.6)	<0.1	(<0.1 - 3.3)	1.6	(<0.1 - 2.8)
Confectionery (Non-Chocolate)		<0.1	4	(<0.1 - 17)	8.1	(<0.1 - 24)	5.7	(<0.1 - 29)	2.5	(<0.1 - 20)	1.8	(<0.1 - 7.8)	1.6	(<0.1 - 3.5)
Dried Fruits		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1
Eggs, Fresh	1.7	(<0.1 - 3.3)	1.2	(<0.1 - 2.5)	1.5	(<0.1 - 2)	1.2	(<0.1 - 2.1)	1.6	(<0.1 - 2.3)	1.7	(<0.1 - 2.8)	1.9	(<0.1 - 2.2)
Fine Bakery Wares		9.7	5.1	(3.7 - 11)	8.2	(1.2 - 17)	8	(1.4 - 12)	8.3	(1.4 - 11)	4.9	(2 - 12)	4.8	(2.5 - 13)
Fish Meat	2.6	(2.5 - 2.7)	6.7	(1.6 - 24)	7	(2.3 - 20)	7.6	(3.4 - 19)	14	(3.6 - 27)	19	(3.2 - 23)	17	(3 - 23)
Fish Products (Canned Fish)		<0.1	4.3	(<0.1 - 9.2)	6.1	(<0.1 - 9.8)	2.6	(<0.1 - 11)	2.7	(<0.1 - 8.6)	<0.1	(<0.1 - 3.2)	1.7	(1.2 - 3)
Grain Milling Products	5.7	(1.3 - 10)	2.9	(<0.1 - 5.9)	2.5	(<0.1 - 9.5)	2.6	(<0.1 - 10)	3.3	(<0.1 - 9.4)	3.1	(<0.1 - 11)	2.8	(<0.1 - 11)
Grains For Human Consumption	2	(<0.1 - 3.1)	1.8	(<0.1 - 2.9)	1.3	(<0.1 - 3.4)	1.6	(<0.1 - 4.6)	1.6	(<0.1 - 4.4)	1.4	(<0.1 - 2.1)	1.2	(<0.1 - 2.1)
Herbs, Spices And Condiments		<0.1	1.2	(<0.1 - 9)	2.3	(<0.1 - 5.5)	2.6	(<0.1 - 7.1)	2.9	(<0.1 - 7.2)	2.2	(<0.1 - 5.6)	2.4	(1.1 - 4.9)
Ices And Desserts		<0.1	18	(2.1 - 30)	16	(5.3 - 26)	8.4	(5.1 - 15)	6.3	(<0.1 - 19)	3.8	(<0.1 - 10)	7	(2.1 - 13)
Legumes, Beans, Dried		<0.1		<0.1	<0.1	(<0.1 - 1)	<0.1	(<0.1 - 1.2)		<0.1		<0.1		<0.1
Livestock Meat	<0.1	(<0.1 - 1.6)	1.1	(<0.1 - 4.6)	2	(<0.1 - 3.6)	2.9	(1.4 - 4.8)	3	(2.1 - 5.8)	3	(2.1 - 6.3)	3.4	(1.9 - 5.5)
Oilseeds		<0.1		<0.1	<0.1	(<0.1 - 1.5)	<0.1	(<0.1 - 4.2)	<0.1	(<0.1 - 1.6)	<0.1	(<0.1 - 1.3)		<0.1
Pasta (Raw)	4.9	(3.2 - 6.7)	7.2	(2.5 - 19)	6.8	(1.9 - 16)	7.1	(1.2 - 16)	3.9	(<0.1 - 16)	5.6	(1.1 - 14)	4.7	(1.1 - 16)
Potato Flakes		<0.1	<0.1	(<0.1 - 1.2)		<0.1		<0.1		<0.1		<0.1		<0.1
Sausages		1.6	5.7	(<0.1 - 13)	6.7	(1.1 - 13)	6.2	(1.6 - 15)	5.7	(2.2 - 14)	5	(1.4 - 11)	4.7	(1.2 - 11)
Snack Food		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1		<0.1
Sugars	5.2	(<0.1 - 9.7)	<0.1	(<0.1 - 3.2)	<0.1	(<0.1 - 1.8)	<0.1	(<0.1 - 1.5)	1.4	(<0.1 - 2.5)	1.4	(<0.1 - 3)	1.4	(<0.1 - 3.8)
Tree Nuts		<0.1	<0.1	(<0.1 - 1.2)	<0.1	(<0.1 - 1)	<0.1	(<0.1 - 3.6)	<0.1	(<0.1 - 2.2)	<0.1	(<0.1 - 1.7)		<0.1
Vegetable Oil	17	(14 - 21)	9.4	(<0.1 - 30)	4.9	(<0.1 - 32)	11	(<0.1 - 34)	8.4	(<0.1 - 37)	7.2	(<0.1 - 39)	13	(<0.1 - 38)
Vegetable Products		<0.1	1.1	(<0.1 - 4.1)	<0.1	(<0.1 - 3.1)	<0.1	(<0.1 - 4.2)	1.3	(<0.1 - 11)	1.2	(<0.1 - 11)	1	(<0.1 - 10)

In 'Infants', the major contributor (median LB-UB) is 'breast milk' (37-38 %), followed by 'vegetable fat' (17 %) and 'animal fat' (10-11 %). In 'Toddlers', 'Ices and desserts' (18 %) are the most important, followed by 'Vegetable oil' (9.1-9.4 %) and 'Bread and rolls' (8.1-7.7 %). In 'Other children' again 'Ices and desserts' are in first place (16 %), then 'Confectionery (Non-Chocolate)' (8.4-8.1 %) and 'Fine bakery wares' (8.1-8.2 %). For 'Adolescents', first is 'Vegetable oil' (11 %), followed by 'Ices and desserts' (8.5-8.4 %) and 'Fish meat' (7.9-7.6 %). For 'Adults' the rank is 'Fish meat' (14 %), 'Fine bakery wares' (8.3 %), 'Vegetable oil' (8.1-8.4 %) and 'Bread and rolls' (7.9-7.7 %); for 'Elderly', 'Fish meat' (20-19 %), 'Animal fat' (10-11 %), 'Bread and rolls' (8.8-8.4 %) and 'Vegetable oil' (7-7.2 %); finally, for 'Very elderly' 'Fish meat' (18-17 %), 'Vegetable oil' (13 %), 'Animal fat' (11-12 %) and 'Bread and rolls' (8.6-8.2 %).

6.3.4. Additional exposure to MOSH in specific consumers of bread and grains with high levels of MOSH

As highlighted in the occurrence chapter, 'Bread and rolls' and 'Grains for human consumption' may occasionally show high levels of MOSH from the use of white oils as release or spraying agents. On top of the background chronic exposure to MOSH, regular consumers of these products are then subject to additional exposure to the MOSH compositions present in those white oils. The mean occurrence values used to calculate exposure were obtained through bi-modal log-normal fitting of the available data, therefore no distinction is made between UB and LB. Tables 12 and 13 present the additional exposure for consumers of, respectively, 'Bread and rolls' and 'Grains for human consumption' with high values of selected MOSH (from food grade mineral oils).

The consumption data for bread and grains in infants come from only two surveys and a limited number of subjects; moreover the consumption by infants of these food groups progressively starts only after weaning. The interpretation of exposure results in infants is for these reasons particularly difficult. Excluding infants, the additional exposure to MOSH in the 'Bread and rolls' scenario ranges across national dietary surveys between 0.7 and 3.1 mg/kg b.w. per day for average consumers and between 1.4 and 6.4 mg/kg b.w. per day in high consumers. In the 'Grains for human consumption' scenario, the additional exposure to MOSH ranges between 0.02 and 1.3 mg/kg b.w. per day for average consumers and between 0.22 and 3.8 mg/kg b.w. per day in high consumers.

Table 12: Summary statistics of the additional chronic dietary exposure to MOSH (mg/kg b.w. per day) across European dietary surveys, in the case of continued consumption of 'Bread and rolls' with high MOSH levels (from food grade mineral oils) over a long period.

Mean of exposure			
	min	**median**	**max**
Infants	0.036	0.48[(a)]	0.92
Toddlers	1.1	2.3	3.1
Other children	1.1	1.9	2.9
Adolescents	0.83	1.1	1.5
Adults	0.69	0.93	1.2
Elderly	0.89	0.99	1.2
Very elderly	0.89	1	1.1

P95 of exposure			
	min	**median**	**max**
Infants		3.8[(a)]	
Toddlers	3.7	4.7	5.8
Other children	2.5	3.6	6.4
Adolescents	1.9	2.6	3.4
Adults	1.4	1.9	2.5
Elderly	1.8	2	2.1
Very elderly	1.8	2	2.5

b.w.: body weight;
(a): estimates available only from two dietary surveys for the mean and only one for the 95th percentile;

Table 13: Summary statistics of the additional chronic dietary exposure to MOSH (mg/kg b.w. per day) across European dietary surveys, in the case of continued consumption of 'Grains for human consumption' with high MOSH levels (from food grade mineral oils) over a long period.

Mean of exposure			
	min	**median**	**max**
Infants	0.3	0.34[(a)]	0.38
Toddlers	0.24	0.44	1.3
Other children	0.024	0.39	0.88
Adolescents	0.11	0.28	0.65
Adults	0.08	0.16	0.35
Elderly	0.054	0.11	0.29
Very elderly	0.048	0.12	0.26

P95 of exposure			
	min	**median**	**max**
Infants		1.5[(a)]	
Toddlers	1.9	2.1	3.1
Other children	0.68	1.8	3.8
Adolescents	0.45	1.2	2.5
Adults	0.36	0.9	1.3
Elderly	0.22	0.68	1.1
Very elderly	0.53	0.71	1.2

b.w.: body weight;
(a): estimates available only from two dietary surveys for the mean and only one for the 95th percentile;

6.3.5. Exposure to MOSH in breast-fed infants (0-6 months)

In the previous chapters, exposure estimates were presented in the different scenarios also for the age class 'Infants'. Actually, this group includes individuals before and after weaning and cannot therefore be considered homogeneous in terms of diet. Since an accumulation of MOSH has been found in the body fat of mothers and it is also found in milk, especially in early lactation, a specific scenario has been calculated for breast-fed infants. As occurrence values, the mean levels detected in breast milk from Table 2 have been used: LB = 1.8 mg/kg, UB = 2 mg/kg. As consumption, a mean value of 800 g milk per day, with a high of 1 200 g per day have been chosen (Kent et al., 1999). Taking into account that the MOSH content of milk is expected to decrease during lactation (Concin et al., 2008) and – on the other hand - that it is often recommended to continue breast feeding until at least 6 months, it was decided to refer the calculation to a 3 month old child weighing 5 kg (EFSA, 2012).

The resulting estimated exposure (LB-UB) for breast-fed infants ranges from 0.29 – 0.32 mg/kg b.w. per day for average consumers and 0.43 - 0.48 mg/kg b.w. per day for high consumers. These values are higher by a factor of about 3 than those calculated for the whole period up to 1 year for the background scenario for the 'Infants' age class. However, this result must be considered with caution because –as highlighted previously - consumption data for infants are limited and the MOSH content of breast milk is expected to decrease during the nursing period.

6.3.6. Summary conclusions of the exposure assessment to MOSH

The results of the exposure assessment allow some general conclusions. The dietary exposure to MOSH ranges in the general population between approximately 0.03 and 0.3 mg/kg b.w. per day. 'Toddlers' and 'Other children' are often the most exposed age classes, mainly due to a higher amount of food consumed per kg b.w. The exposure is considerably lower for 'Adolescents' and is roughly stable in older age classes. For all age classes, the difference between average consumers and high consumers is modest. A factor of about 2-3 is generally observed between them.

Special cases where identified, when 'Bread and rolls' or 'Grains for human consumption' with high levels of MOSH, due to the use of food grade MOSH as release agents or spraying agents, are consumed on a regular basis. In these cases, the additional exposure to MOSH was up to 6.4 mg/kg b.w. per day for bread and up to 3.8 mg/kg b.w. per day for grains. In contrast to the background exposure, the additional exposure is related to virtually MOAH-free MOH. With the data presently available it is difficult to draw conclusions on how many consumers are affected by this additional exposure.

The analysis of the percentage contribution to exposure was performed for the background scenario and suggests that some food groups need more attention and possibly a deeper investigation. These include e.g. 'bread and rolls', 'Confectionery (non chocolate)', 'fine bakery wares', 'fish meat', 'fish products (canned fish)', 'ices and desserts', 'pasta', 'sausages', 'vegetable oil'.

For infants, the main contributors to MOSH exposure are 'Breast milk' and fats (both vegetable and animal). In particular, for the subgroup of exclusively breast-fed infants an exposure of roughly 0.3-0.5 mg/kg b.w. per day was calculated. This exposure should be further investigated, since the MOSH in breast milk are those the metabolism of the mother was unable to eliminate, so they might have a high chance to accumulate also in infants.

The exposure scenarios and all the conclusions suggested by whose analysis are based on assumptions on the coverage of all relevant food groups, the representativeness of the true levels of occurrence and the representativeness of the variability of occurrence across Europe. The available data came mostly from investigations carried out over many years in one laboratory. It would be advisable to confirm them with a more comprehensive survey with more complete coverage of food groups, representative sampling and analysis of different MOH classes. Sampling should be designed in order to enable distinction amongst sources of contamination. The present analysis of the available data may represent a good starting point for designing a comprehensive survey.

6.3.6.1. Exposure considerations for MOAH

Making assumptions on the nature of the mineral oil product found in food, the MOAH concentrations can be estimated on the basis of typical composition. This has been tentatively done in Table 3 but these data were not used for calculating exposure. For instance, technical MOH, such as lubricating oils or diesel oils, typically contain 20-30 % MOAH, whereas those migrating from paperboard boxes contain around 15 % MOAH (as resulting from the recent surveys mentioned in Section 6.2.2.4). MOH used as release agents, e.g., in the bakery industry, or for spraying grains, are normally white mineral oils virtually free of MOAH.

It would be important to generate MOAH data in future MOH occurrence surveys. Beyond the total concentration in food, it would also be important to characterise the MOAH, since the toxicological relevance might well vary depending on composition.

6.3.7. Exposure estimates for food packaged with recycled paper and board

The data available to the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) on concentration of MOH in food migrating from recycled paperboard packages are from a limited number of food samples and thus not considered sufficient for a full exposure assessment to MOH from all food groups packed in recycled paper. However, these data could allow estimating the potential exposure from this source at the level of single selected food categories. The following categories typically packed in such material and for which a significant level of consumption is expected were selected to this aim: breakfast cereals, semolina, bakery wares and rice.

Consumption data from the EFSA Comprehensive Database (see Section 6.3.1) for these food categories were combined with the occurrence data derived from the BMELV Project (Vollmer et al., 2011, see also Sections 6.1.4.7 and 6.2.2.4) to estimate the exposure to MOSH and MOAH from specific foods packed in direct contact with recycled paper and board packages or with an inner bag offering no barrier to transfer of MOH from the paper and board.

Data on consumption from total population and data from consumers only were considered. These consumption data were combined with occurrence data on MOH from recycled paper as given in Table 6. In each case two scenarios were considered:

- Average chronic exposure scenario based on mean consumption and mean value of occurrence (Scenario I);
- High consumer chronic exposure scenario based on P95 consumption and mean value of occurrence (Scenario II);

For each age class and food category under consideration, the highest value across national surveys of exposure to MOSH for the two different scenarios is reported in Table 14.

Table 14: Exposure to MOSH (mg/kg b.w. per day) from selected foods packaged in recycled paper with no inner barrier to migration, in the country with the highest estimated exposure, consumers only.

	Bakery wares		Breakfast cereals		Rice		Semolina	
Scenario	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
Infants	0.011[1]	0.030[1]	-[3]		0.018[1]	0.051[1]	0.015[1]	0.034[1]
Toddlers	0.015	0.036	0.017[2]	0.031	0.061	-[3]	0.021[2]	0.056
Other children	0.017	0.038	0.024	0.072	0.051	0.110	0.052[2]	-[3]
Adolescents	0.008	0.021	0.008	0.020	0.033	0.067	0.020[2]	-[3]
Adults	0.005	0.014	0.009	0.033	0.021	0.051	0.015[2]	-[3]
Elderly	0.005	0.012	0.009	0.010	0.020	0.033	0.006[2]	-[3]
Very elderly	0.005	0.016	0.012	0.032	0.016	0.034	0.023[2]	-[3]

1: Based on one country
2: Based on 2nd highest entry value from the consumption database due to the low number of consumers in the country with the highest exposure
3: Not possible to estimate, due to the low number of consumers

Exposure from breakfast cereals ranged from 0.008 to 0.024 mg/kg b.w. per day in the exposure scenario I and from 0.01 to 0.072 mg/kg b.w. per day in scenario II. 'Other children' appeared to be the age class potentially more exposed. The adolescents' class showed an unexpected low maximum average value of 0.008 mg/kg b.w. per day (Scenario I).

Exposure from bakery wares consumption ranged from 0.005 to 0.017 mg/kg b.w. per day in the average exposure scenario (I) and from 0.012 to 0.038 mg/kg b.w. per day in scenario II. Toddlers and other children classes appeared as potentially more exposed.

Exposure from rice consumption ranged from 0.016 to 0.061 mg/kg b.w. per day in the average exposure scenario (I) and from 0.030 to 0.111 mg/kg b.w. per day in scenario II. Also for this product toddlers and other children appeared as potentially more exposed.

Exposure from semolina ranged from 0.006 to 0.052 mg/kg b.w. per day in scenario I; the number of consumers was not sufficient to estimate scenario II.

Data for scenario I are also presented in Figure 22.

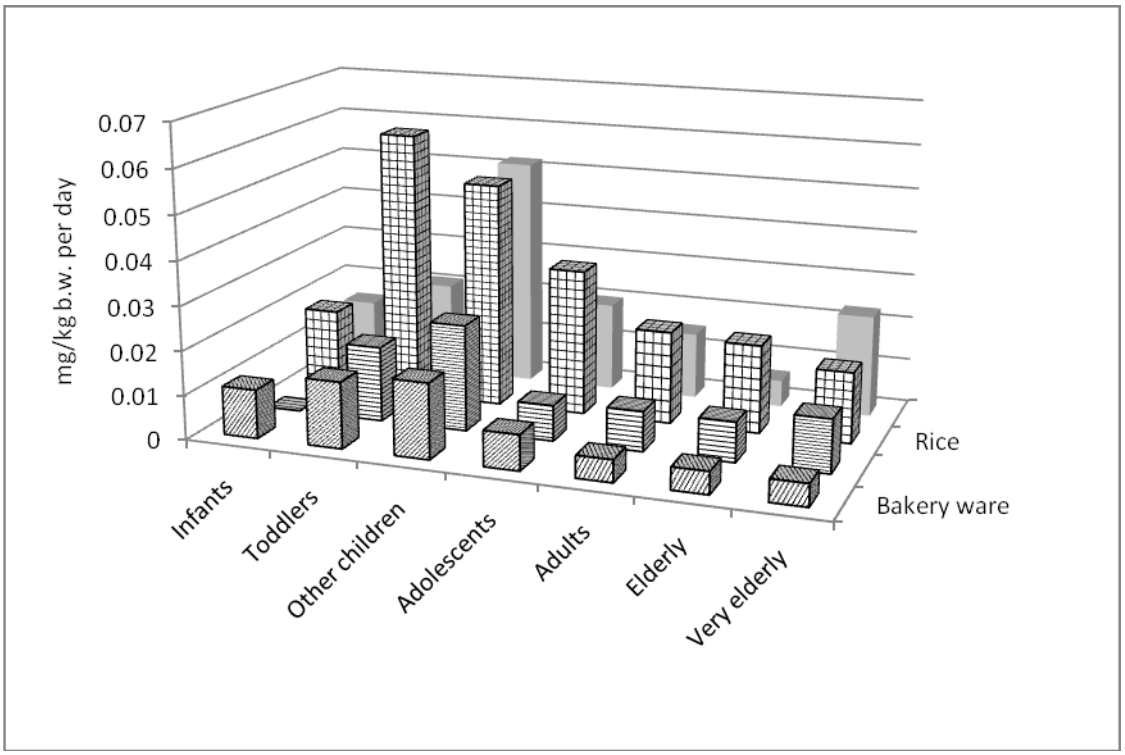


Figure 22: Exposure of consumers only to MOSH from specific foods packaged in recycled paper without an inner barrier to migration. Scenario I based on mean consumption and mean contamination.

Exposure estimates for the total population (not shown) for MOSH from recycled paper via breakfast cereals ranged from the same level to 9 times lower than those for consumers only. On the other hand, estimates for bakery wares show no relevant differences when the total population is considered, except for 'infants' that result in a 3 times lower exposure compared to consumers only. However, this estimate is based on a single survey from one country. Estimates for exposure from rice for consumers only are 2-3 times higher than those for the total population. Finally, exposure to MOSH from recycled paper via semolina for consumers only shows values more than 10 times higher than those for the total population.

Since the MOAH concentration originating from recycled paper and board was estimated as approximately 15 % of the MOSH, this percentage value is reflected in the exposure estimates.

These exposure estimates are indicative only, because of the data available and assumptions underlined. Estimates are based on occurrence data collected in one country only (Germany). Food categories more relevant for the use of paper and board packaging containing recycled fibre were considered, but these foods are only a fraction of the total diet. The scenarios considered that they are always packaged in recycled paper and board without a barrier to migration of MOH. This conservative case does reflect possible consumer brand loyalty. 'Toddlers' and 'other children' appear to be the age classes potentially more exposed. The results show that exposure to MOH from migration into foods packaged in recycled paper and board without an inner barrier may provide a significant contribution to total exposure to MOH in consumers regularly buying these products in recycled paperboard packages consumers. Combined exposure by daily consumption of more than one food of the food groups examined is possible.

7. Hazard identification and characterisation

The toxicokinetic and toxicological data retrieved are reported considering the main chemical classes present in petroleum-derived products. Saturated hydrocarbons (MOSH) were divided into linear alkanes, branched alkanes and cyclic alkanes. Aromatic hydrocarbons (MOAH) were divided into

(poly)aromatic hydrocarbons, alkylated aromatic hydrocarbons and partially hydrogenated aromatic hydrocarbons. Sulphur containing-aromatic compounds were also included among the MOAH, as they can be present in significant concentrations in some petroleum-derived products. If relevant, data on mixtures of MOSH and MOAH were also evaluated for hazard identification and hazard characterisation.

For all chemical classes, information relative to MOSH and MOAH included in the carbon number range C_{10}-C_{50} was considered, since hydrocarbons with < C_{10} are likely not relevant for food contamination in view of the high volatility, whereas hydrocarbons with > C_{50} are unlikely to be systemically absorbed following ingestion.

Oral studies were preferentially selected, but studies carried out by other routes of exposure were also considered when oral studies were not retrieved or in case of information relevant to the hazard assessment (e.g. skin-painting studies were considered to assess the carcinogenic potential).

In some cases information relating to petroleum products unlikely to be relevant for food contamination were taken into account. This approach is justified by considering that the individual hydrocarbon constituents of those products are also likely to be present in petroleum products that may be relevant for food contamination.

7.1. Toxicokinetics

Data on the fate of MOH in mammals are scarce and they essentially come from investigations performed in experimental animals. Human data are limited to the post-mortem analysis of MOH in tissues or organs such as liver or adipose tissues. The majority of the studies were carried out on individual saturated compounds, mainly n-alkanes, whereas only few data were published on alkylated polyaromatic hydrocarbons. However, for the latter class of compounds, it may be assumed that they behave similarly to the well documented PAHs.

7.1.1. Absorption

7.1.1.1. MOSH

It is generally anticipated that absorption and distribution of MOSH occur by passive processes and therefore studies on both odd and even numbered alkanes would be equally informative.

Mixtures

Kolattukudy and Hankin (1966) prepared a radiolabelled unsaponifiable fraction from broccoli plants and mixed this fraction into a standard dry diet given to rats. The fraction contained nearly 90 % n-C_{29}. About 75 % of the administered radioactivity was recovered unchanged in the excreta, suggesting that absorption of this n-alkane was approximately 25 %.

Albro and Fishbein (1970) estimated the gastrointestinal absorption of an aliphatic hydrocarbon mixture (containing both saturated and unsaturated compounds) in rats on the basis of the difference between administered dose (up to 500 mg/kg b.w.) and the amounts recovered in faeces. They found that after a single dose of the hydrocarbon mixture administered to the animals by gavage, the absorption of the aliphatic hydrocarbons was inversely proportional to the number of carbon atoms and ranged from 60 % for C_{14} to 5 % for C_{28} compounds. The absorption was independent of the hydrocarbon type (alkanes, alkenes or alkynes) and was also independent of dose rate, except at very high rates. Popovic et al. (1973) obtained similar results with a mixture of n-alkanes, showing that the absorption of compounds in the C_{18}-C_{28} range varied as a function of carbon number.

Tulliez (1986) estimated the absorption rates of alkanes in rats and pigs by measuring the total amounts recovered in faeces after ingestion of diets containing 0.1 % of two types of mineral oils, denoted oil A and B. The composition of oil A was as follows: 15.7 % of n-alkanes + branched

alkanes, and 84.3 % of cycloalkanes, with an average carbon number of 20. Oil B consisted of 10.9 % of n-alkanes + branched alkanes and 89.1 % of cycloalkanes, with an average carbon number of 28. Faecal hydrocarbon analysis showed that in rat apparent absorption of oil A and B was 52 ± 2 and 35 ± 2 % of ingested dose, respectively, whereas in pigs, the percentage was 38 ± 3 and 20 ± 2 %, respectively. Lower absorption rates were observed in both species when mineral oils were incorporated into the diet at a 1 % level. This study confirms the fact that absorption of alkanes varies as a function of carbon number and of dose.

In the same article Tulliez (1986) investigated the absorption in pig of a mineral oil incorporated into the diet at a 0.65% level and consisting of n-alkanes (60 %), cyclo-alkanes (33 %), and branched-alkanes (7 %), with an average carbon number of 16. The absorption rate of this mixture administered to the animals for 10 days was 88 % of the ingested dose. The author explained the high absorption rate of the mixture by the low carbon number as compared with oil A and oil B mentioned above.

More recently, a study was carried out in female Sprague Dawley and Fischer 344 rats administered by gavage a single dose (0, 20, 200 or 1 500 mg/kg b.w.) of P15(H) white oil. The hydrocarbon concentration in blood and liver was analysed by GC/GC-MS and quantified based on the C_{19}-C_{24} range of alkanes present in P15(H) white oil (Cnubben and van Stee, 2010). Results showed that at similar doses, blood concentrations of Pl5(H) white oil in Fischer 344 rats were approximately 3-fold higher and the areas under the curve (AUC) were about 4-fold higher than P15(H) concentrations in Sprague Dawley rats. For instance, in Fischer 344 rats, single exposure to external doses of 200 mg/kg b.w. (a dose comparable to the 90-day hepatic NOAEL as observed by Smith et al., 1996) and 1 500 mg/kg b.w. resulted in similar calculated maximum blood concentrations of approximately 14 and 15 μg/ml, respectively. On the other hand, maximum blood concentrations of approximately 6 μg/ml for Fischer 344 rats exposed to 20 mg/kg b.w., and 6 and 5 μg/ml for and for Sprague Dawley rats administered 200 or 1 500 mg/kg b.w., respectively, were calculated. Using the same analytical methodology, the absorption of P15(H) white oil was also investigated in human volunteers after a single oral administration (1 mg/kg b.w.) (Bakker, 2011). Blood samples were taken at 2, 4, 8, 24, 48, 72, 96 and 68 h after dosing but the concentration of P15(H) was below the LOD (0.16 μg/ml) in all samples.

Individual compounds

Rats received a single dietary dose (100 mg/kg b.w.) of n-C_{17}, n-C_{20}, n-C_{21}, n-C_{24}, n-C_{32}, dodecylcyclohexane, heptadecylcyclohexane, 2,2,4,4,6,8,8-heptamethylnonane or pristane (Tulliez and Bories, 1975a). Based on the difference between the doses and the fractions eliminated in the faeces apparent absorption levels were 90, 95, 95, 75, 70, 94, 93, 98 and 83 %, respectively.

Studies performed by the same authors in order to determine the fate of radiolabelled heptadecane and dodecylcyclohexane incorporated in rat diet (Tulliez and Bories, 1978, 1979) confirmed that absorption of these alkanes was > 90 % for doses ranging from 1 to 20 mg/kg b.w. Apparent absorption of radiolabelled pristane orally administered to rats (0.5 mg/kg b.w.) was found to be about 45 %, based on unchanged hydrocarbon found in faeces (Le Bon et al., 1988).

In a study performed in rabbits treated *per os* with radiolabelled decalin (decahydronaphthalene), (Elliott et al., 1966), total radioactivity eliminated in urine was 67 and 53 % of the dose for the cis- and trans-isomers, respectively, suggesting an apparent absorption at least equivalent to these values for these conformational isomers.

While there are some discrepancies between studies regarding the degree to which alkanes are absorbed, there is almost complete concordance with the fact that these hydrocarbons are primarily absorbed from the gut into the lymphatic system (Baxter et al., 1967; Albro and Fishbein, 1970; Albro and Thomas, 1974; Tulliez and Bories, 1979). Using radiolabelled hexadecane, Savary and Constantin (1967) specifically addressed the uptake into the lymphatic system of this n-alkane given by gavage to

rats. Lymph was collected over 40 h periods from a thoracic canula. The fraction of the total administered dose to pass to the lymph rose to a peak around 8 h post dosing. The uptake was strongly enhanced by the co-administration of lipids such as oleic acid and triolein. n-Hexadecane and low molecular mass hydrocarbons may also be absorbed into the portal blood (Albro and Fishbein, 1970).

These data indicate that n-alkanes and cycloalkanes are well absorbed when ingested at low levels. The available information on branched alkanes suggests that uptake of this category of compounds occurred to a lower extent as compared with n- or cyclo-alkanes of similar molecular weight (Tulliez and Bories, 1975a).

In order to study the rat strain differences in the toxicokinetics of mineral hydrocarbons, Halladay et al. (2002) exposed by gavage female Fischer 344 rats and Sprague Dawley rats of both sexes to a single oral dose of 0.18 or 1.8 g/kg b.w. mineral oil containing [^{14}C]-1-eicosanylcyclohexane ([^{14}C]-EICO, C_{26}) as tracer. Blood samples were systematically collected up to 96 hours after the administration. At both doses female Fischer 344 rats showed higher [^{14}C]-EICO peak concentrations than Sprague Dawley rats. In particular, a triphasic absorption profile with two peak concentrations in blood (the first after 6 hours and the second after 16 hours after the administration) was observed in Fischer 344, but not in Sprague Dawley rats at the lower tested dose. The two concentration peaks observed in Fischer 344 females were attributed to two independent routes of absorption, the fastest one occurring through the hepatic portal system and the second through the lymphatic system. Based on radioactivity eliminated in urine, absorption of approximately 20 - 25 % of administered EICO for the low dose can be estimated.

7.1.1.2. MOAH

Although several studies have examined the gastrointestinal absorption of PAHs, there is little information on the absorption of aromatic compounds having one or several branched or unbranched alkyl groups. Barrowman et al. (1989), in reviewing early experiments on alkyl-naphthalene, reported that absorption of 2-methylnaphthalene (MNAP) administered orally to guinea pigs (10 mg/kg) may be close to 100 % and that more than 50 % of an unspecified dose of 2,6-dimethylnapthalene administered to rats was found as urinary or biliary excretory products within 24 hr. The same authors mentioned that as much as 85 % of a single oral dose of 2,6-di-isopropylnaphthalene (100 mg/kg b.w.) was absorbed in 48 hr in rats.

A series of experiments on dimethylbenzanthracene given intraduodenally to rats demonstrates that this PAH is readily absorbed through the intestine, since more than 30 % of a dose is recovered in bile in 24 hr and a substantial amount is detected in lymph (Laher et al., 1983; Rahman et al., 1986; Laher and Barrowman, 1987, 1988). Comparable results were obtained under similar experimental conditions, with benzo(*a*)pyrene (BaP) (Laher et al., 1984; Rahman et al., 1986), suggesting that the presence of methyl groups on PAH compounds is of minor effect on their intestinal absorption rate.

No other information has been located that documented the gastrointestinal absorption of alkyl-substituted PAHs in animals or humans.

<u>Sulphur-containing MOAH</u>

There is evidence suggesting that sulphur compounds are absorbed in rodents, however no quantitative data were identified. When dibenzothiophene was incorporated into the diet (concentration not mentioned) and given to rats, metabolites of this thiarene were detected in urine (Ambaye et al., 1961). More than 70 % of thiophene administered to rats by gavage (200-300 mg/kg b.w.) was excreted in expired air and in urine within 24 h (Bray et al., 1971), suggesting that the major part of this compound was absorbed through the gastrointestinal tract.

7.1.2. Distribution, deposition and retention

The tissue distribution of MOH was mainly investigated in rodents after oral administration of radiolabelled or unlabelled individual hydrocarbons, but also in laboratory animals given repeated doses of food-grade mineral oils or waxes. Regarding these mixtures, several studies have examined primarily mesenteric lymph nodes which were considered as the main target organs of MOH.

7.1.2.1. MOSH

Animal experiments with mixtures

Baldwin et al. (1992) compared the fate of two types of food grade mineral oils in Fischer 344 rats. Males and females were given free access for 90 days to diets containing 20 000 mg/kg of either oleum-treated white oil (OTWO) or hydro-treated white oil (HTWO). Total hydrocarbon residues were analysed at the end of the experiment in liver and mesenteric lymph node. The mean hepatic concentrations in males were 2.36 and 1.76 mg/g for OTWO and HTWO groups, respectively, whereas in females, the mean levels were 11.5 and 9.20 mg/g, respectively. The residues found in mesenteric lymph node of treated rats were approximately half of those found in liver. For hepatic residues, higher total hydrocarbons concentrations were observed in females than in males, and in OTWO-fed rats than in those receiving HTWO.

In a 90-day study carried out in female rats (Fischer 344 and CRL-CD Sprague Dawley derived strain) fed a food grade white oil (P15(H), viscosity = 15.0 mm^2/s at 40 °C, carbon number range = C_{18}-C_{30}, mean molecular weight = 350) incorporated into the diet (2 000 or 20 000 mg/kg diet), Firriolo et al. (1995) observed a dose- and strain-related increase in the levels of hydrocarbons in the liver and mesenteric lymph nodes from both rat strains. At the end of the experiment, the average residue levels found in the liver of animals treated with the lowest dose were 5.6 and 1.7 mg/g tissue for Fischer 344 and Sprague Dawley rats, respectively, whereas the values reported for the highest dose were 8.2 and 4.1 mg/g tissue, respectively. As compared with the liver, the hydrocarbon concentration in mesenteric lymph nodes was approximately 2-3 and 5 fold lower for Sprague Dawley and Fischer 344 strains, respectively.

Smith et al. (1996) performed a 90-day feeding study in male and female Fischer 344 rats with seven different white oils (Figure 23). In the liver, saturated hydrocarbon contents ranged from 0.6 to 4.3 mg/g tissue in female rats fed a diet containing 20 g /kg mineral oils, and were 4-5 times greater than in males (exact data in males not reported by the authors). In mesenteric lymph nodes, saturated hydrocarbon contents ranged up to 3.3 mg/g tissue and were higher in females than in males (exact data in males not reported by the authors). In samples of perirenal fat, the hydrocarbon content was similar in males and females. Levels in the spleen and kidney were much lower and did not exceed 0.1 mg/g tissue.

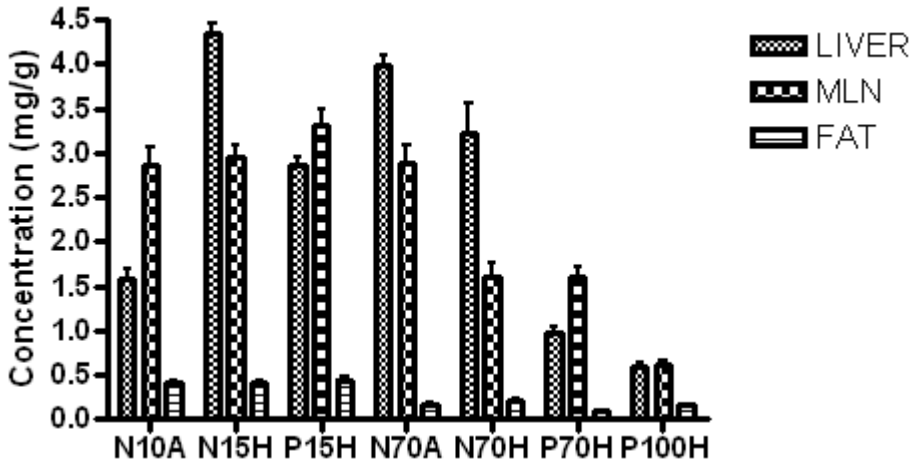


Figure 23: Residues of hydrocarbons in tissues of female Fischer 344 rats fed during 90 days on a diet containing 20 g mineral oils /kg feed. From Smith et al. (1996).

N indicates naphthenic white oils; P indicates paraffinic white oils; A and H indicate the refining methods (acid treatment and hydrogenation, respectively). The viscosity at 40 °C, expressed as mm^2/s, and the average carbon number distribution (indicated in parenthesis) are as follows : N10A = 13.3 (15-30), N15H = 16.6 (17-30), P15H = 15.0 (18-30), N70A = 76.4 (21-35), N70H = 68.0 (22-37), P70H = 69.5 (27-43), P100H = 99.8 (28-45). MLN = mesenteric lymph nodes; FAT = perirenal adipose tissue.

A low melting point wax (LMPW), a synthetic wax (C80W) and three white oils (N15(H), N70(H) and P70(H)) were administered orally to female Fischer 344 rats for 90 days at a dose level of 2% in the diet (Scotter et al., 2003). Tissues (liver, intestine, heart, kidney, cervical lymph nodes, mesenteric lymph nodes) were analysed at the end of the experiment. MOSH was detected in all samples of small intestine, heart and kidney for all groups. For LMPW, the levels found ranged from 0.4 (proximal mesenteric lymph node) to 15.2 mg/g by weight (proximal part of small intestine), whereas for the C80W, the range was from 0.1 (proximal mesenteric lymph node) to 7.5 mg/g % (cervical lymph nodes). In animals exposed to N15(H), N70(H) and P70(H), the higher MOSH concentrations were found in distal small intestine and kidney (liver not analysed). Preferential accumulation occurred in the alkane range approximately C_{20}–C_{35}.

Trimmer et al. (2004) investigated the MOSH deposition in liver, kidneys, mesenteric lymph nodes and spleen of female Fischer 344 rats dietary exposed to P70(H) and P100(H) white oils incorporated into the diet at concentrations to produce dose levels of 60, 120, 240 and 1 200 mg/kg b.w. per day, for up to two years. Following exposure to either oil, MOSH were detected only in the liver (LOQ not specified). At the highest tested dose, the concentration found in the liver at the first sampling time (after 3 months) was about 900 and 1 600 mg/kg for P100(H) and P70(H), respectively, followed by a slow increase to reach approximately 1 400 and 2 300 mg/kg over the following 21 months. For a subgroup, exposure to MOH was stopped after 12 months. During the following year concentrations in the liver strongly fell, but did not reach the level of the control groups. Using the data from Trimmer et al., the proportion of the mineral hydrocarbons persisting after a 12 month depuration period is in the range of 10 - 15 %.

More recently, Griffis et al. (2010), compared the distribution of MOSH in a 90-day dietary study in Fischer 344 and Sprague Dawley rats dietary exposed to mineral oils incorporated into the diet. Two concentrations (2 and 20 g/kg diet) of a low melting point paraffin wax (carbon number of n-alkanes = C_{19} - C_{42}) were tested in female rats and the hydrocarbon residues were measured at 30,

60 and 90 days. Hepatic residues were observed only in Fischer 344 rats (LOQ = 0.5mg/g) and the mean concentrations at the end of the experiments were 19.8 and 13.3 mg/g for the high and low dose groups respectively. There was evidence of accumulation as the mean residue levels increased from 1.86 mg/g at 30 days to 19.8 mg/g at 90 days in the livers and from below the LOQ (< 2.5 mg/g) at 30 and 60 days to 6.2 mg/g at 90 days in mesenteric lymph nodes. In the Sprague Dawley rats, a mean residue levels above the LOQ was observed in mesenteric lymph nodes only in the 20 g/kg group at 90 days (2.64 mg/g of tissue). In both strains, the steady-state level of hydrocarbon accumulation does not appear to have been reached by 3 months. A GC-MS analysis of the liver extract from the Fischer 344 rats indicated that the major part of the residues was linear and branched alkanes with carbon numbers comprised between C_{22} and C_{31}

In another study carried out in female Sprague Dawley and Fischer 344 rats, P15(H) white oil was administered by gavage at a single dose (200 or 1 500 mg/kg b.w.) (Cnubben and van Stee, 2010). Animals were euthanised at 24, 48 and 96 hr post-dose, the livers were sampled and hydrocarbons were extracted by carbon tetrachloride and analysed by GC-GC/MS. The maximum liver concentration occurred at 24 h post-dose and was higher in the Fischer 344 than in the Sprague Dawley rats (0.056 and 0.023 mg/g at the 200 mg/kg dose level, and 0.089 and 0.032 mg/g at the 1 500 mg/kg dose level, respectively).

Data from humans

In humans, the presence of mineral oil derived from medicinal use and food packaging sources in the liver, spleen and lymph nodes has been documented by several authors. Boitnott and Margolis (1970) analysed MOSH content in 60 livers and 34 spleens obtained from 64 patients (4 children and 59 adults) at the time of autopsy. Five of the patients were selected because oil droplets were found in their tissues; the remainders were chosen at random. The concentrations found in the liver and spleen varied from 0.1 to 4.1 mg/g tissue and from 0.1 to 4.5 mg/g tissue, respectively. In children, the concentrations in spleen and liver did not exceed 0.4 mg/g. Alkanes present in tissues were mainly naphthenic compounds and were found to be of similar composition to mineral oils.

Some reports described the presence of mineral oil deposits in the liver of patients suffering various liver disorders, including chronic hepatitis, reactive hepatitis, metastatic carcinomas, portal fibrosis (Blewitt et al., 1977; Dincsoy et al., 1982), or having ingested large amounts of paraffin oil over many years for laxative purposes (Nochomovitz et al., 1975), but the MOSH concentration was not investigated in these studies. Aliquots from six spleens obtained at autopsies from adult subjects from different countries were analysed for mineral oil content by Cruickshank (1984a). In three spleens exhibiting severe follicular lipidosis, the MOSH concentration ranged from 3.4 to 4.2 mg/g tissue, whereas in three spleens considered negative histologically, the alkane concentration was below 0.3 mg/g.

Lipid analysis of tissues from an adult male after sudden death (affected with diffuse visceral granuloma containing lipophilic crystallised material) showed the presence of long-chain n-alkanes with 29, 31 and 33 carbon atoms suspected to be of plant origin (Salvayre et al., 1988). A study of the n-alkane distribution in patient tissues showed a major accumulation in lumbo-aortic lymph nodes, adrenal glands, lung (the highest levels were found in lung granulomas) and liver; significantly lower amounts were detected in myocardium and kidney, whereas no detectable level was found in brain.

Saturated hydrocarbons were analysed in abdominal tissue fat collected during Caesarian section of 144 volunteers living in Austria, as well as in milk fat from days 4 and 20 after birth in the same subjects (Concin et al., 2008). Figure 24 shows a typical example. The chromatogram corresponds to that shown in Figure 16 for breast milk: there are signals for diterpenic hydrocarbons eluted around n-C_{18} and n-alkanes from C_{21} to C_{33}, dominated by the odd-numbered species, as it is typical for paraffins of plant origin. The peak area of the even numbered n-alkanes relative to that of the odd-numbered n-alkanes was typical of plant paraffins, i.e. there is no indication of the presence of mineral

n-alkanes. The branched and/or cyclic MOSH form the pattern of unresolved peaks, possibly with some small peaks on top which are, however, of little relevance for the MOSH concentration. Since humans are exposed to substantial amounts of n-alkanes, e.g. from fuel and diesel oil as well as printing ink diluents, this suggests that the metabolism efficiently removes the n-alkanes.

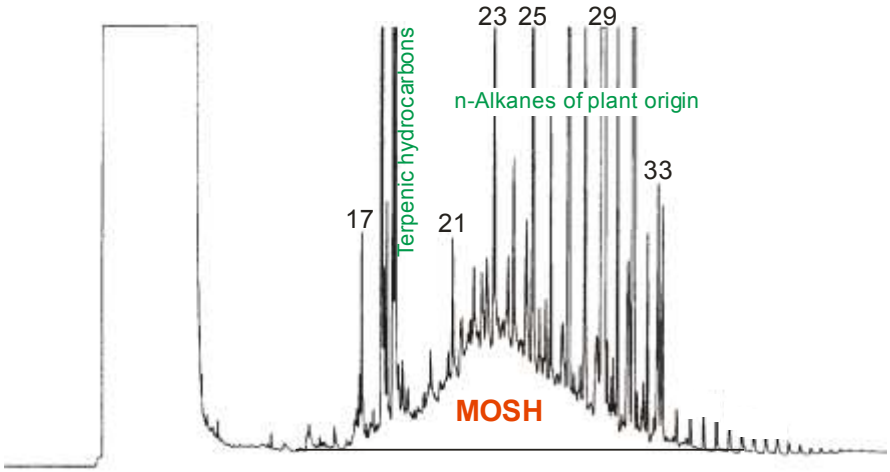


Figure 24: MOSH from human abdominal body fat (concentration, 85 mg/kg). The MOSH are primarily represented by the unresolved pattern of unresolved peaks. Adapted from Concin et al. (2008).

The molecular mass distribution of the mineral paraffins was almost identical for all the 144 samples analysed. Figure 25 shows the extremes, in terms of molecular mass distribution (upper chromatograms) as well as concentrations (lower chromatograms). The MOSH were always centred on n-C_{23} or n-C_{24} and always ranged from about n-C_{16} to n-C_{35}. This distribution does not correspond to the MOSH humans are exposed to (these range from below C_{10} to above C_{45}, and the maxima are rather either below, e.g. for printing inks, or above n-C_{23}, e.g. for lubricating oils). The distribution might be determined by loss (exhalation) of volatiles, limited absorption of high molecular mass hydrocarbons and efficient elimination of certain hydrocarbons such as the n-alkanes.

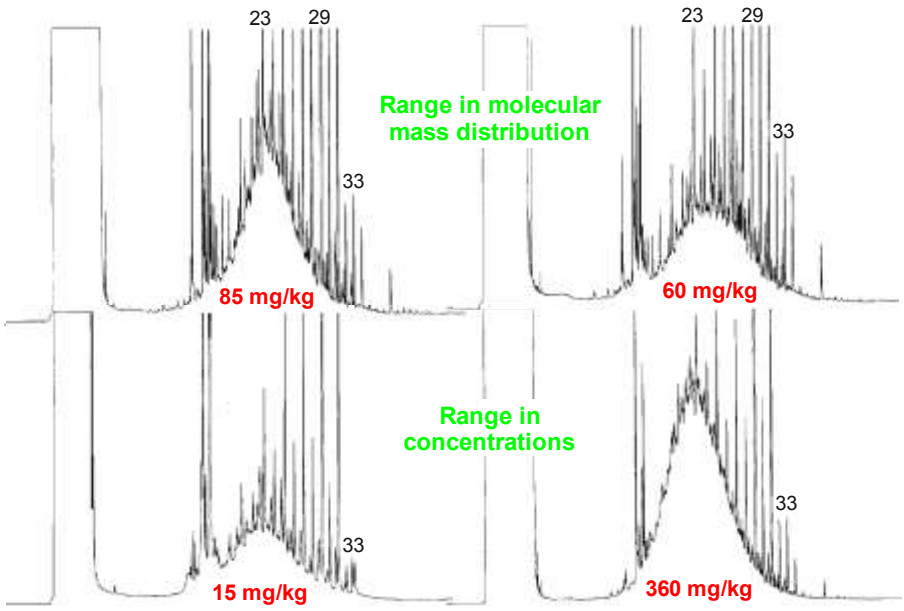


Figure 25: MOSH chromatograms obtained from human body fat representing the range in terms of molecular mass distribution (upper chromatograms) and concentrations (lower chromatograms). Adapted from Concin et al. (2008).

The MOSH concentrations in fat were within a rather narrow range between 15 and 360 mg/kg, with an average of 60.7 mg/kg and median of 52.5 mg/kg. 80 % of the concentrations (10^{th} – 90^{th} percentile) were in the narrow range between 30 and 100 mg/kg.

The subjects of the Concin et al. (2008) study filled in a questionnaire on personal data, nutrition habits, and use of cosmetics. The MOSH concentrations in tissue fats were correlated with data from the questionnaire (Concin et al., 2011). Concentrations increased with age ($P < 0.001$) and decreased with the body mass index ($P = 0.001$). The country of main residence ($P = 0.03$), number of previous childbirths ($P = 0.029$), usage of sun creams in the present pregnancy ($P = 0.002$) and of hand creams and lipsticks in daily life ($P = 0.011$ and $P = 0.007$, respectively) were significant independent determinants. Austrian women and women with more children had a significantly higher MOSH concentration in tissue fat even in multivariate analysis. No association was found with nutritional habits. A strong correlation existed between the MOSH concentration in tissue fat (median 52.5 mg/kg), and the corresponding milk fat sample on day 4 (median 30mg/kg, sample taken at end of breast feeding) ($P < 0.001$.) and day 20 (median 10mg/kg) ($P = 0.028$).

Comment on MOSH accumulation in humans

Assuming that all body fat of a person contains MOSH at approximately the same concentration (not verified) and the MOSH concentration is fairly independent of the body weight (Concin et al., 2011), the total amount of MOSH in the human body fat can be estimated. Concin et al. (2008) estimated that a slim person with 60 mg/kg MOSH in 5 kg body fat contains 0.3 g MOSH. For a person with 30 kg fat this results in almost 2 g. If the 30 kg body fat were contaminated at the maximum concentration found (360 mg/kg), the total content of MOSH would exceed 10 g. Moreover, it should be taken into account that MOSH accumulate also in other human tissues (e.g. liver and spleen).

Under the assumption that 10 g MOSH can be accumulated at an average daily exposure to approximately 0.04 mg/kg b.w. per day (2.8 mg/person per day for a 70 kg person), as estimated in Section 6.3.2 (Table 9), and that 100 % retention occurs (which is unlikely), it could take approximately 10 years to reach the estimated body burden. On the one hand, exposure was substantially higher 10-20 years ago (because of more frequent use in food production, e.g. as release agents for bakery wares) (Grob, personal communication) and some of these MOSH might still have persisted. Furthermore, the possible contribution of non dietary sources of exposure (e.g. dermal uptake) should also be taken into account. On the other hand, some of the MOSH present in food are below or above the range of molecular mass which is known to be accumulated. If it is assumed that 85-90 % of the MOSH absorbed by humans are eliminated within a year (Trimmer et al., 2004, see above), this suggests that part of the MOSH ingested might be accumulated virtually over a lifetime. This possibility of strong accumulation must be taken into consideration when extrapolating data from relatively short term animal experiments (mostly 90 days) to humans of maybe 90 year in age.

MOSH in human breast-milk

In the Concin et al. (2008) study, the fat in the milk samples at day 4 contained virtually the same mixture of mineral paraffins as the tissue fat at concentrations between 10 and 355 mg/kg (average, 44.6 mg/kg; median, 30 mg/kg), similar to the concentration range measured in the body fat of the subjects, particularly when considering that the samples were collected at the end of breast feeding and that the MOH content dropped during breast feeding (Noti et al., 2003). The fats in the day 20 milk samples contained from < 5 to 285 mg/kg mineral paraffins (average, 21.7 mg/kg; median, 10 mg/kg). Fifty-five of 82 fat samples of day 20 milk contained 15 mg/kg mineral paraffins or less: However, as almost all elevated concentrations were linked to a different composition compared to that observed earlier, the authors suggested the presence of a new source, such as the use of breast salves. It was concluded that in the absence of a new source, the MOH concentrations dropped several fold from day 4 to day 20 and that the MOH concentrations decrease more rapidly than those of other organic

contaminants (LaKind et al., 2001). The transfer of mineral paraffins to the baby was estimated to be roughly 1 % of that in the body of the mother.

Individual compounds

Administration of a radiolabelled n-alkane such as ^{14}C-heptadecane or ^{14}C-octadecane to rats at a single oral dose resulted in a rapid distribution of the radioactivity in the organism (Tulliez and Bories, 1978; Pokrovskiĭ et al., 1969). Initially the largest part of radioactivity was found in the liver, but the rate of dispersal from this organ was rapid. In contrast, the concentration in the adipose tissue was initially low, but rose markedly after 12 hr.

Tulliez et al. (1975) fed male and female rats a diet containing 25 % of dried *Spirulina* algae. The algae contained n-alkanes, predominantly heptadecane, at up to 0.2 % dry weight and the final diet contained 280 mg heptadecane per kg feed. The experiment was carried out over one year. The amount of heptadecane retained in the whole animal rose steadily for about 4 months, then it levelled off. At the end of the experiment, concentrations of heptadecane measured in adipose tissue were 80 and 272 µg/kg in males and females respectively, whereas in liver the levels were 2.5 and 1.9 µg/kg, respectively.

Tulliez and Bories (1975a) measured the concentration of eicosane in various tissues of rats fed a diet containing 0.1 % of this alkane for one week. Three days after the end of the experiment the animals were euthanised. GC analyses of tissue and carcass extracts indicate that 7.2 % of the total amount of ingested eicosane was found in the whole animal, and that the highest concentration was in the adipose tissue (317 µg/g). The levels found in the liver were below 3 µg/g. The experiment was repeated under the same conditions but for a 6 month exposure period. Analysis of the tissues and remaining carcasses at the end of the experiment showed that 6.6 % of the total amount of eicosane ingested was present in the whole animal. The concentrations found in the liver and the adipose tissue were 17.2 and 1 172 µg/g, respectively, indicating an accumulation of eicosane in the adipose tissue. At the end of the 6 month exposure period, although the steady state was not reached, a proportion of the animals was returned to an alkane-free diet for an additional 4 months. At the end of this 4-month period, only two third of the total amount of eicosane accumulated in the animal was eliminated, whereas the concentration in adipose tissue decreased to 738 µg/g.

A similar protocol was used to investigate the accumulation of dodecylcyclohexane in rats, except that the duration of the exposure period was limited to 3 months before a return to a cycloalkane-free diet for 5 months (Tulliez and Bories, 1975b). The total amount of dodecylcyclohexane in the whole animal increased linearly with time, reaching a steady state after 2 months, representing approximately 7 % of the total amount of ingested cycloalkane. At the end of the exposure period, the levels in liver and adipose tissue were 30 and 1 134 µg/g, respectively. The concentration in adipose tissue fell to 638 µg/g at the end of the depuration period (5 months).

The same authors investigated the distribution of radioactivity in rats 24 hr after administration of a single oral dose (100 mg/kg b.w.) of ^{3}H-dodecylcyclohexane (Tulliez and Bories, 1979). The concentration found in adipose tissue was 4-5 fold higher than the levels found in liver, kidney or lung, and 100 times those observed in blood.

The distribution of another radiolabelled cycloalkane, ^{14}C-eicosanylcyclohexane (^{14}C-EICO), was investigated by Halladay et al. (2002) in Fischer 344 and Sprague Dawley female rats (see experimental conditions in Section 7.1.1). At the end of the experiment (96 hr postdose) approximately 3 % of administered radioactivity was present in the liver of Fischer 344 rats, whereas only 0.1 % remained in the liver of Sprague Dawley rats. Regarding mesenteric lymph nodes, approximately 0.02 % of the administered radioactivity was retained in the Fischer 344 rat strain compared to 0.009 % in Sprague Dawley rats. In Fischer 344 rats, less than 1% of administered radioactivity was found in other organs, i.e. kidney (0.2 %), lung (0.1%), heart (0.03 %) spleen (0.04 %) and subcutaneous fat (0.5%). When the retention of ^{14}C-EICO was computed as a percentage

of administered radioactivity per gram of tissue, the concentration of ^{14}C-EICO in each respective strain was similar in both liver and mesenteric lymph nodes.

Data on the disposition of branched-alkanes are limited to pristane. Le Bon et al. (1988) investigated the distribution of radioactivity in tissues 7 days after oral administration of a single dose (0.5 mg/kg b.w.) of ^{3}H-pristane to male Wistar rats. The highest concentrations were found in liver and adipose tissue, followed by spleen, kidney, heart and lung. The liver retained approximately 0.5 % of administered dose. Janz and Shacter (1995) described the disposition of intraperitoneally injected pristane that would conventionally be used for a tumour induction protocol in genetically susceptible BALB/c mice. The distribution of radioactivity in various tissues was monitored by liquid scintillation counting at different times after injection of 0.5 mL ^{3}H-pristane per animal (weighing about 20 g). At the end of the experiment (4 months post injection), the highest concentrations of radioactive material were found in gall bladder and lung. The lungs began to accumulate pristane only late in the time course (i.e. 1 month).

7.1.2.2. MOAH

The disposition of radiolabelled 1,6-dimethylnaphthalene was investigated following intraperitoneal (i.p.) administration of a single dose (20 mg/kg b.w.) to rats (Kilanowicz et al. 2002). In organs and tissues, the highest concentration during the first hours after administration was detected in fat, liver, spleen and kidney. After 72 hr, the highest concentrations were found in spleen and brain, corresponding approximately to 2 ng 1,6-dimethylnaphthalene equivalents per g tissue.

The distribution of radioactivity in the tissues of rats after a single oral administration of ^{3}H-7,12-dimethylbenz[*a*]anthracene (DMBA) (approximately 150 mg/kg b.w.) was investigated by Flesher (1967). As observed for PAHs (IPCS, 1998), highest levels were found in all lipid-rich tissues. Of the tissues examined (perirenal adipose tissue, kidney, liver, mammary gland, adrenal, uterus, blood), adipose tissue had the greatest concentrations of radioactivity, which was equivalent to approximately 40 µg 7,12- DMBA equivalents per g of adipose tissue, 24 hr after administration. However, 72 hr after administration, the highest values were found in kidney and liver. Furthermore, studies in pregnant mice have shown that 7,12-DMBA and 3-methylcholanthrene were detected in foetuses, showing that they crossed the placenta (IPCS, 1998). It may be assumed that other alkyl-substituted PAHs having similar molecular weight show similar properties.

No data were identified on multibranched- and long chain-alkylated MOAH or for sulphur containing MOAH.

7.1.3. Metabolism

7.1.3.1. MOSH

Alkanes are metabolised to the corresponding fatty alcohols and then fatty acids (Figure 27) by both the small intestine (Mitchell and Hübscher, 1968; Ichihara et al., 1981) and the liver (McCarthy, 1964; Kusunose et al., 1969; Perdu-Durand and Tulliez, 1985). The oxidative metabolism of alkanes shows species differences and is mediated through the cytochrome P450 system (Ichihara et al., 1981; Perdu-Durand and Tulliez, 1985). The oxidation rates of radiolabelled heptadecane were investigated for three different rat strains (Sprague Dawley, Wistar and Fischer 344) using liver microsome incubations (Cravedi et al., 2011). Heptadecane hepatic hydroxylation occurred at higher rates in Wistar rats than in Sprague Dawley rats, the latter strain being more active than the Fischer 344 strain. No difference was observed between males and females. In a follow up study, Cravedi and Perdu (2012) studied the biotransformation of radiolabelled n-heptadecane, pristane and dodecylcyclohexane by hepatic microsomes from the three rat strains and from human donors of both sexes. While the difference for n-heptadecane among the three rat strains was not observed in this study, the biotransformation rate by microsomes from human female donors was statistically significantly higher than that by microsomes from female Fischer 344 rats. No measurable biotransformation was observed

for pristane and dodecylcyclohexane when incubated with microsomes from any of the rat strains or human donors (Cravedi and Perdu, 2012).

The oxidised metabolites of normal alkanes can undergo the same metabolic pathways as fatty acids which include integration into the lipid fraction as phospholipids and neutral lipids such as triglycerides, and incorporation into lipoproteins (Kolattukudy and Hankin, 1966; Albro and Thomas, 1974; Tulliez and Bories, 1978, 1979).

Similar products have been identified from the oxidative metabolism of branched-alkanes (Le Bon et al., 1988). These authors identified four metabolites of ^{3}H-pristane in the liver and carcass of rats given by gavage a single dose of this hydrocarbon (1 000 mg/kg b.w.): pristan-1-ol, pristan-2-ol, pristanic acid, and 4,8,12-trimethyltridecanoic acid. The presence of these metabolites in different tissues indicates that pristane undergoes terminal (ω) or subterminal (ω-1) oxidation. Whereas the terminal hydroxylation is followed by the classical β-oxidation, the subterminal hydroxylation leads to pristan-2-ol which is considered by the authors as a metabolic dead end because hydroxylation on a tertiary carbon would prevent any further oxidative step and may therefore explain the substantial amounts of this metabolite in tissues of rat. Incorporation of metabolites in phospholipids and more precisely in the phosphatidylserine fraction was also reported (Le Bon et al., 1988).

Regarding cyclo-alkanes, Tulliez and Bories (1979) investigated the metabolism of a monocycloalkane in rat. Animals received a single dose (100 or 1 000 mg per kg b.w.) of ^{3}H-dodecylcyclohexane incorporated into the diet and the metabolites present in the lipid extracts from liver and adipose tissue were analysed. The evidence for ω-oxidation, previously demonstrated by Tulliez and Peleran (1977), was confirmed by the presence of cyclohexyldodecanoic acid in neutral lipids and phospholipids. Then the alkyl chain undergoes the classical fatty acid degradation pathway as shown by the identification of cyclohexyldecanoic acid. The cyclohexyl ring is subsequently eliminated in urine as cyclohexylacetic acid (Tulliez, 1986). Metabolic utilisation of the labelled acetyl-CoA produced by β-oxidation resulted in the formation of radiolabelled phospholipids and fatty acids. Tulliez (1986) reported that ring hydroxylation may occur, resulting in the formation of cyclanols and subsequently to the production of free and conjugated hydroxylated cyclohexyl acids eliminated in urine (Figure 26).

Halladay et al. (2002) identified 12-cyclohexyldodecanoic acid and 10-cyclohexyldecanoic acid as the main metabolites in the urine of female Fischer 344 and Sprague Dawley rats administered a single dose of 1.8 g/kg b.w. eicosanylcyclohexane by gavage, confirming the ω-oxidation of the alkyl chain previous observed by Tulliez and Bories (1979) with dodecylcyclohexane.

The metabolic pathways of cis- and trans-decalin in rats was reported by Olson et al. (1986) and by Dill et al. (2003a). Both isomers were found to be metabolised differently in males and females. While the main metabolites identified in the urine of male rats treated with cis-decalin were cis,cis-2-decalol (major) , cis,trans-1-decalol, and cis,cis-1-decalol (minor), only cis,cis-2-decalol and cis,trans-1-decalol were found in females. In the case of trans-decalin, trans,cis-2-decalol was the main metabolite observed in male urine and trans,trans-1-decalol was found as a minor biotransformation compound, whereas only trans,cis-2-decalol was present in female urine. These hydroxylated urinary metabolites were primarily found as glucuronic acid or sulfate conjugates. Extracts of kidney samples from male rats treated with cis- and trans-decalin yielded cis-2-decalone and trans-2-decalone, respectively. There was no trace of decalin metabolites in female kidney extracts (Olson et al. 1986). Dill et al. (2003a) showed that the retention of decalin and decalone in male rat kidney was linked with the accumulation of α_{2u}-globulin in this organ occurring subsequently to the production of this protein in the liver. This accumulation, which is considered to be responsible for the nephropathy of decalin in rodents (Ridder et al., 1990; Dill et al., 2003b), occurs in male but not in female rats.

No data were identified on either cyclopentane alkylated compounds or polycyclic alkanes other than decalin.

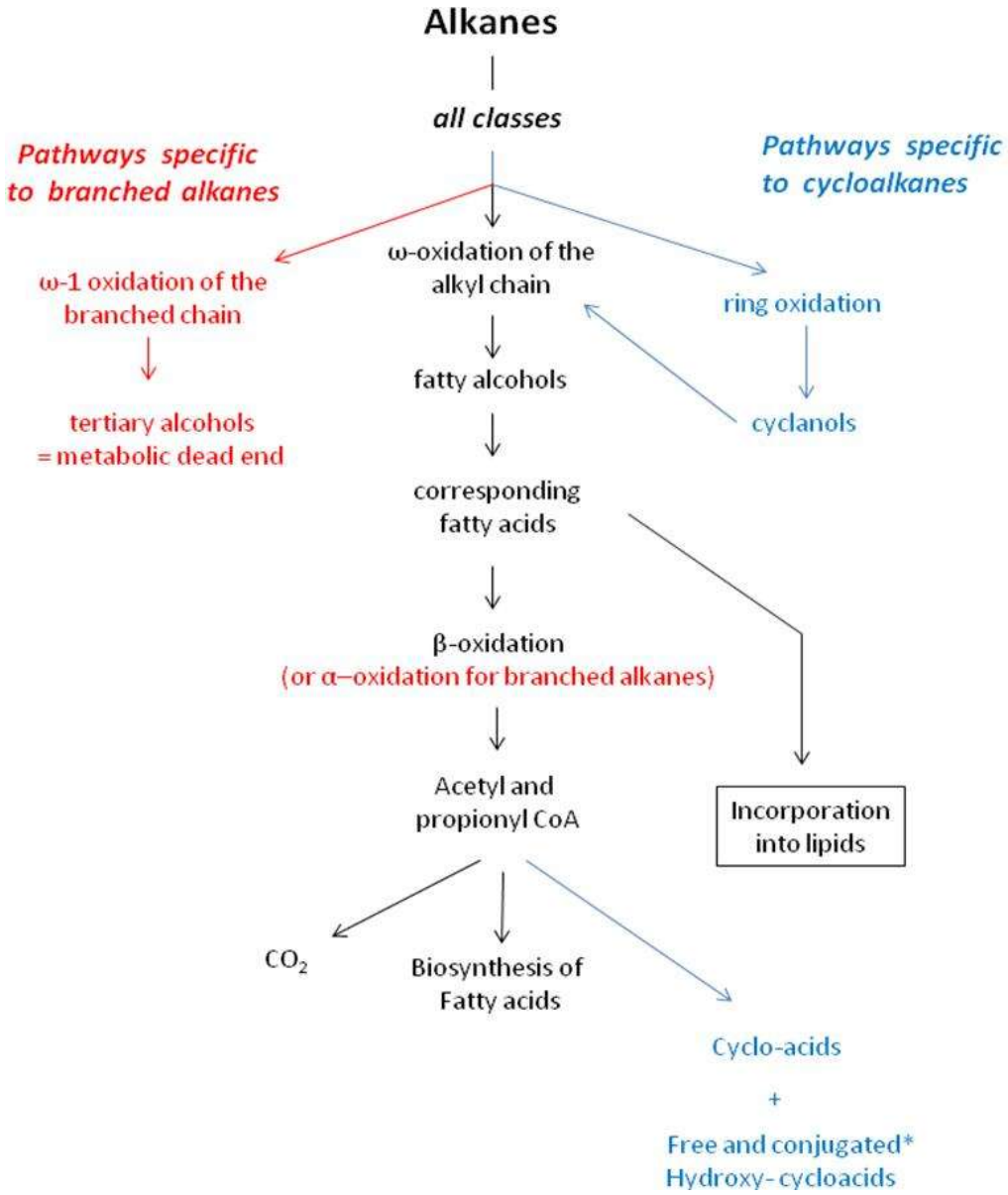


Figure 26: Metabolic pathways of alkanes in mammals. The data in black are common to all classes (n-, branched-, and cyclo-alkanes) whereas indications in red and blue are specific to branched- and cyclo- alkanes, respectively.

7.1.3.2. MOAH

The metabolism of diisopropylnaphthalene in rodents was found to proceed almost exclusively via oxidation of the isopropyl side chain, presumably mediated by the cytochrome P450 system, followed by the conjugation of the hydroxylated metabolites, mainly to glucuronic acid (Höke and Zellerhoff, 1998). The absence of ring epoxidation supports the view that oxidative metabolic pathways involved in the biotransformation of MOAH having multiple branched alkyl side chains as it is the case for diisopropylnaphthalene has to be considered as a detoxification process. In contrast, the oxidative metabolism of MNAP and dimethylnaphtalene by cytochrome P450 dependent-enzymes leads to intermediate epoxides and subsequently to corresponding phenols and diols or to glutathione conjugates (Höke and Zellerhoff, 1998; Kilanowicz et al., 2002; Lin et al., 2009). These intermediates are a key step in the toxicity of aromatic hydrocarbons.

Studies conducted in rodents with 7,12-DMBA indicate the formation of various hydroxymethyl metabolites, but also of the 3,4-dihydrodiol, a proximate carcinogenic metabolite (Wislocki et al., 1980; Chou et al., 1981; Vater et al., 1991). The major part of these compounds has been found in excreta, mainly in bile, as glucuronide or sulphate conjugates (Levine, 1974; Khanduja et al., 1981). The 7-hydroxymethyl sulphate ester was identified by Watabe et al. (1982) as a reactive metabolite of 7,12-DMBA, suggesting that ring oxidation is not the only pathway involved in the genotoxicity of 7,12-DMBA.

No data were identified on multibranched- and long chain-alkylated MOAH.

Sulphur compounds

Urine of rats injected intraperitoneally with tritiated thiophene was found to contain mostly the mercapturic acid conjugate of the dihydrothiophene sulfoxide (Dansette et al., 1992), suggesting the S-oxidation of thiophene as a primary step followed by the addition of glutathione to the reactive thiophene-S-oxide.

The metabolism of thioarenes (sulphur-containing PAHs) such as dibenzothiophene, benzo(b)naphtho(2,1-*d*)thiophene or benzo(b)phenanthro(2,3-*d*)thiophene has been investigated after oral administration to rats (Ambaye et al., 1961) or using rodent liver subcellular fractions (Murphy et al., 1992; Yuan et al., 2003). The *in vivo* experiment conducted by Ambaye and co-workers on dibenzothiophene showed that this compound is eliminated through urine as 1-hydroxydibenzothiophene-5,5-dioxide, indicating that oxidation occurred on the sulphur atom and on the benzene ring. The *in vitro* studies provide evidence that thioarenes are metabolised by both ring oxidation and sulphur oxidation, leading to active metabolites such as the dihydrodiol (and subsequently diol epoxide) and the sulphoxide derivatives. They also indicate that CYP1A1, 1B1, 2B1, 3A1 (and/or 3A2) are responsible for these oxidations.

7.1.4. Excretion

7.1.4.1. MOSH

The metabolic balance reported by Tulliez and Bories (1978) in rats administered single oral doses of ^{14}C-heptadecane (5 or 1 000 mg/kg b.w.) showed that urine collected during one week following intake contained 1.1 and 0.5 % of the ingested radioactivity, for the low and high dose, respectively. The corresponding values for faeces were 1.0 and 28.0 %, respectively. Residual radioactivity present in rat carcasses at the end of the experiment (7 days) represented approximately 37 and 25 % of the ingested radioactivity. The high amount of unaccounted radioactivity (approximately 61 and 46 %) suggested extensive $^{14}CO_2$ exhalation (not measured).

The elimination of pristane and related metabolites was investigated in rats given a single oral dose (0.5 mg/kg b.w.) of ^{3}H-pristane (Le Bon et al., 1988). Whereas after one week about 8 % of the ingested radioactivity was still retained in the carcass, faecal and urinary elimination amounted to 66 and 15 % of the ingested dose, respectively. No trace of unchanged pristane was found in urine.

In rats administered a single oral dose (100 mg per kg b.w.) of ^{3}H-dodecylcyclohexane, approximately 60 % of the radioactivity was eliminated in urine within one week (Tulliez and Bories, 1979). During the same period, faecal excretion accounted for approximately 10 % and the remaining radioactivity was found in the animals at the end of the experiment (7 days). No trace of unchanged dodecylcyclohexane was found in urine.

The excretion profile of ([^{14}C]-EICO) was studied in female Fischer 344 and Sprague Dawley rats by Halladay et al. (2002). Rats were administered a single dose of 0.18 or 1.8 g/kg b.w. ([^{14}C]-EICO) by gavage. Faecal excretion of total radioactivity was the main route of elimination for both strains. Although similar recoveries were observed 96 hours after the administration in Fischer 344 rats (92 %

and 76 % after high and low dose, respectively) and Sprague Dawley rats (88 % and 70 % in after high and low dose, respectively), a more rapid faecal elimination profile was observed in the Sprague Dawley strain. Similarly, radioactivity excretion was observed in the urine with a dose-dependent fashion in both strains (11 % at high dose and 22 % at low dose in Fischer 344 rats, and 11 % at high dose and 27 % at low dose in Sprague Dawley rats) on 96 hours after the exposure, but a more rapid excretion was noted in the latter.

The half lives of MOSH in blood and liver were investigated in female Fischer 344 and female Sprague Dawley rats dosed by gavage a single dose (200 or 1 500 mg/kg b.w.) P15 white oil (Cnubben and van Stee, 2010). The calculated terminal half life in blood was between 47 and 59 hours for Fischer rats and between 23 and 47 hours for Sprague Dawley rats. In liver, half lives ranging from 46 to 69 hours were reported for both strains. It must be noted that these half life calculations were based on total MOSH analyses; very likely some components of the tested white oil have a slower clearance rate than the total mixture.

Unmetabolised MOSH can be eliminated through the milk, but the extent of this route of elimination has not been quantified (see Section 6.1.8.1).

7.1.4.2. MOAH

Iwahara (1974) reported that the excretion of the radioactivity in the urine and faeces of rats treated with ^{3}H-diisopropylnaphthalene was approximately 26 and 71 % of the dose administered, respectively. One day after a single oral administration of diisopropylnaphthalene to rats (240 mg/kg b.w.), the total urinary excretion of metabolites was about 23 % of the dose (Kojima et al., 1982).

No study was identified concerning the elimination of MOAH into milk. However, the presence of traces of PAHs in cow and human milk (EFSA, 1998) and the fact that BaP, phenanthrene (PHEN) and pyrene (PYR) and/or corresponding metabolites were found in the milk of goats exposed to these PAHs (Grova et al., 2002) suggests that the same type of transfer may occur for MOAH.

Sulphur-containing aromatic compounds

Bray et al. (1971) found that thiophene (200-300 mg/kg b.w.) administered by gavage to rats was partly (approximately 30 % of the dose) excreted unchanged in expired air and partly (approximately 40 % of the dose) eliminated as mercapturic acids in urine. Less than 1% of the dose was found in faeces.

7.1.5. Conclusions

In spite of the limited number of individual compounds investigated given the complexity of mineral oil composition, and in spite of some discrepancies existing between studies, mainly due to the different doses administered, the vehicles used, the animal species or strains investigated, the toxicokinetic data on MOSH in rodents indicate that n-alkanes and cycloalkanes are well absorbed when ingested at low levels. In rats, the absorption of these hydrocarbons can be estimated to vary from 25 % (for C_{26}-C_{29}) to 90 % for carbon numbers between C_{14} and C_{18}. The available information on branched alkanes suggests that uptake of this category of compounds occurred at a somewhat lower extent as compared with n-alkanes or straight chain cyclo-alkanes of similar molecular weight.

Although ingested alkanes of low molecular mass ($\leq C_{16}$) may be transported via the portal blood, the major part of MOSH are absorbed through the lymphatic system. In rats, significant toxicokinetics differences were observed between strains, particularly between Fischer 344 and Sprague Dawley.

There are no data from animal testing to explain the upper limit of the molecular mass of MOSH accumulated in humans. On the one hand, high (75 %) absorption of n-C_{29} was determined in rats. On the other hand, little MOSH are found in human body fat beyond n-C_{30}, particularly when considering

the relatively high exposure to these oils. However, there is new information indicating that accumulation in human liver reaches up to at least n-C_{35} (Grob, unpublished results).

Animal experiments provide evidence that MOSH accumulate in various tissues, including liver, mesenteric lymph nodes and fat. Studies carried out with individual compounds indicate that naphthenes (e.g. dodecylcyclohexane) and paraffins (e.g. eicosane) have similar accumulation rates.

The observed presence of MOSH in human tissues demonstrates that accumulation also occurs in humans. The MOH in body fat are centred on n-C_{23}/n-C_{24} and range from n-C_{16} to about n-C_{30}. The molecular mass distribution does not resemble a mineral oil product humans are exposed to, indicating selectivity of the accumulation with regard to molecular mass. Further, no mineral n-alkanes are observed, supporting that bioaccumulative MOSH are mainly branched and cyclo-alkanes. Considering the amounts of MOH found in human tissue and the estimated daily intake via food, it is likely that a part of the MOH deposited might persist for decades in humans.

The metabolic studies performed with a few hydrocarbons (n-alkanes, pristane, n-alkyl cyclohexane) do not support conclusions on the nature of the MOSH accumulated by humans or on the half life of these compounds. In particular it cannot be concluded whether certain hydrocarbons are retained over the lifetime. Such information would be important for the translation of results from animal testing to accumulation in humans. Nevertheless, recent *in vitro* studies suggest that humans are more efficient than Fischer 344 rats in oxidizing n-alkanes (heptadecane) and confirm that branched- and cyclo-alkanes of comparable molecular mass are more resistant to biotransformation than the linear alkanes.

Although limited studies exist on MOAH toxicokinetics, available data suggest that these compounds are well absorbed, extensively biotransformed and do not bioaccumulate in mammals. The fate of highly substituted MOAH is unknown.

7.2. Toxicity

7.2.1. Acute toxicity

Available information on aromatic and aliphatic hydrocarbons shows generally low to moderate acute oral toxicity in laboratory animals. LD_{50} data for some representative MOSH and MOAH mixtures and individual substances are listed in Table 15.

Table 15: List of LD_{50}s for representative hydrocarbons.

MOSH				
Substance	**LD_{50} (mg/kg)**	**Species, strain, sex**	**Observation period**	**Reference**
Mixture C_9-C_{13} (MOAH approximately 0.5 % v/v)	> 10 000	Rats, (strain and sex not reported)	Not reported	Amoruso et al. (2008)
Mixture C_9-C_{13} (MOAH approximately 5 % v/v)	> 20 000[1] – > 64 000[1]	Rats, (strain and sex not reported)	Not reported	Amoruso et al. (2008)
Mixture C_9-C_{13} (MOAH approximately 15-17 % v/v)	> 5 000 – 6 000[1]	Rats, (strain and sex not reported)	Not reported	Amoruso et al. (2008)
White mineral oil (C_{15}-C_{50}, highly refined)	> 5 000	Sprague Dawley rats, 5 males and 5 females	14 days	ECHA (2012a)
Light paraffinic distillate (C_{15}-C_{30}, viscosity < 19 mm^2/s)	> 5 000	Sprague Dawley rats, 5 males and 5 females	14 days	ECHA (2012b)
Distillates (petroleum), solvent-refined heavy paraffinic (C_{20}-C_{50}, viscosity ≥ 19 mm^2/s)	> 5000	Sprague Dawley rats, 5 males and 5 females	14 days	ECHA (2012c)
MOAH (including sulphur containing aromatics)				
Naphthalene	2 200-2 400	Sherman rats, male and female	14 days	Gaines (1969)
	533-710	CD-1 mice, male and female	14 days	Shopp et al. (1984)
Tetralin	2 860	Sherman rats, 10 males/group	5 days	OECD (2004)
Dibenzothiophene	470	CD-1 mice, male and female	14 days	Leighton (1989)
Aromatic distillate extract (MOAH 61 % w/w, C_{15}-C_{50})	> 5 000	Sprague Dawley rats, 5 males and 5 females	14 days	API (2003)

[1]calculated based on LD_{50} expressed in ml/kg b.w., by considering an approximate density of 0.8 g/ml.

7.2.2. Sub-chronic and chronic toxicity

7.2.2.1. MOSH

Mixtures

Exxon Biomedical Sciences (1991a) carried out an oral subchronic study in Sprague Dawley rats exposed by gavage to a **mixture of n-, iso- and cyclo-alkanes with carbon number included between C_{10} and C_{13} (containing 0.5% of MOAH).** Male and female rats were exposed to 500, 2 500 or 5 000 mg/kg b.w. per day of the mixture for 13 weeks. The main observation was the occurrence of α_{2u}-globulin-mediated nephrotoxicity in male rats at all treatment doses. Increased liver weight was observed in males at 2 500 and 5 000 mg/kg b.w. per day and hepatocellular hypertrophy was observed in both sexes at all doses. Signs of irritation such as thickening of the stomach mucosa were observed at 2 500 and 5 000 mg/kg b.w. per day. As the nephrotoxic effects observed in male rats are known not to be relevant for humans (as described in Section 7.3), they were not considered for the setting of the no effect level of the study by the CONTAM Panel. However, a lowest-observed-effect level (LOEL) of 500 mg/kg b.w. per day was identified based on the incidence of hepatic hypertrophy.

In a subsequent study performed by Exxon Biomedical Sciences (1991b), male and female Sprague Dawley rats were exposed by gavage to 0, 100, 500 or 1 000 mg/kg b.w. per day of a **mixture of n-, iso- and cyclo-alkanes with carbon number included between C_{10} and C_{14} (<2 % of MOAH)** in a 13-week study. $Alpha_{2u}$-globulin-mediated nephrotoxicity was observed in male rats at all treatment doses. A statistically significant increase in absolute liver weight was observed only in females

exposed to the high dose. Minimal to slight centrilobular hepatic hypertrophy was observed in males exposed to the high dose and in females exposed to the mid and high doses. A full recovery from the hepatic changes was observed following a 4-week recovery period. In line with the previous study a no-observed-effect level (NOEL) of 100 mg/kg/day was identified. The CONTAM Panel noted that the observed hepatic effects are likely adaptative changes, possibly of no or minimal adversity.

Mattie et al. (1995) observed the occurrence of α_{2u}-globulin-mediated nephrotoxicity in male rats exposed for 90 days to JP-8 fuel at doses of 750, 1500 and 3000 mg/kg b.w. by gavage. No changes were observed in liver weight and histopathology, but elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were recorded without a clear dose-response relationship in rats exposed to JP-8.

The repeated dose toxicity of highly refined paraffinic and naphthenic mineral oils and waxes has been exhaustively evaluated in previous assessments (See Section 3). Briefly, all the tested mineral oils and waxes, with the exception of the microcrystalline wax, were observed to accumulate in a dose-related fashion in the liver and mesenteric lymph node (MLN) of Fischer 344 rats (with female rats being more sensitive than male rats) following (sub)chronic exposure via gavage, as described in Section 7.1.2. For some grades (identified as Class II and III low- and medium-viscosity mineral oils by FAO/WHO, see Table 1) mineral oil accumulation led to histopathological changes mainly in liver and MLN. In the liver, the histopathological changes were classified as granulomas or microgranulomas, consisting of focal aggregations of macrophages surrounded of inflammatory cells and occasionally necrotic cells and fibrosis. On the other hand, histiocytosis (also classified as microgranulomas) consisting of macrophage accumulation similar to that observed in the liver, but not presenting the inflammatory cascade and the necrotic and fibrotic tissues, was observed in the MLN (Smith et al., 1996; Fleming et al., 1998; Carlton et al., 2001). For all grades where both the described effects were reported, MLN histiocytosis occurred at lower doses than hepatic microgranuloma. However, MLN histiocytosis has recently been considered as a non specific, adaptative effect observed with high molecular weight and poorly absorbed materials and not progressing to more severe pathological effects following long-term exposure (Carlton et al., 2001; EFSA 2009; FAO/WHO, 2009). The effects observed in Fischer 344 rats were not observed in other species and other rat strains (Shubik, 1962; Bird et al., 1990; Firriolo et al., 1995). Recently, Griffis et al. (2010) reported a comparative study carried out with the most active grade evaluated in earlier studies (low melting point wax) in female Fischer 344 and Sprague Dawley rats administered 0, 2 000 or 20 000 mg/kg in the diet for 90 consecutive days. The results in Fischer rats confirmed the effects previously observed with low melting point waxes (Smith et al 1996; Scotter et al., 2003). On the other hand, Sprague Dawley rats were less sensitive regarding MLN and hepatic effects. The different sensitivity was attributed to the higher absorption and retention of the substance by Fischer 344 in comparison to Sprague Dawley rats and possibly to a generally exacerbated immunomediated response occurring in the former strain (Miller et al., 1996; Griffis et al., 2010). The recent comparative study in Fischer and Sprague Dawley rats confirms that differences in the pharmacokinetic profiles could play a role in the sensitivity of the two strains regarding the effects caused by mineral oils and waxes (Cnubben and van Stee, 2010, see Section 7.1.1.1). The NOAEL values observed in female Fischer 344 rats exposed to white mineral oils and waxes and based on the occurrence of liver microgranulomas and MLN histiocytosis are reported in Tables 16 and 17.

Table 16: NOAELs observed in female Fischer 344 rats exposed to white mineral oils, based on MLN histiocytosis and liver micrograniulomas.

Test item identification: P indicates a paraffinic white oil, mainly containing branched alkanes, no or minor amounts of aromatics. N indicates a naphthenic white oil, mainly containing cyclo alkanes, no or minor amounts of aromatics. The following number indicates the approximate viscosity (expressed in mm^2/s) at 40 °C. The letter between brackets indicates the refining method applied (A: acid treatment; H: hydrogenation treatment). OTWO: oleum treated white oil (acid treatment), containing alkanes and cyclo alkanes, minor amounts of aromatics. HTWO: hydrotreated white oil, containing alkanes and cyclo alkanes (cyclo-alkanes in higher proportions than OTWO), no or minor amounts of aromatics.

Test item	Physico-chemical properties	Duration	Concentration in diet (mg/kg)	Dose (mg/kg b.w. per day)	NOAEL (mg/kg)		Reference
					MLN histiocytosis	Liver granulomas	
			Oils				
N10(A)	Viscosity at 40 °C (mm^2/s): 13.3 Viscosity at 100 °C (mm^2/s): 3.1 Average MW: 320, C number range: 15-30	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
N15(H)	Viscosity at 40 °C (mm^2/s): 16.6 Viscosity at 100 °C (mm^2/s): 3.4 Average MW: 330 C number range: 17-30	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	< 2	190	Smith et al, 1996
P15(H)	Viscosity at 40 °C (mm^2/s): 15.0 Viscosity at 100 °C (mm^2/s): 3.5 Average MW: 350 C number range: 18-30	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
	Viscosity at 40 °C (mm^2/s): 14.8	90 days	2000, 20 000	161, 1582	< 161	< 161	Firriolo et al., 1995
OTWO	Viscosity at 40 °C (mm^2/s): 26	90 days	10, 100, 500, 5 000, 10 000, 20 000	0.93, 9.3, 46, 440, 940, 1 800[1]	0.93	46[2]	Baldwin et al. 1992
N70(A)	Viscosity at 40 °C (mm^2/s): 76.4 Viscosity at 100 °C (mm^2/s): 7.9 Average MW: 410 C number range: 21-35	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
N70(H)	Viscosity at 40 °C (mm^2/s): 68.0 Viscosity at 100 °C (mm^2/s): 7.6 Average MW: 420 C number range: 22-37	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	2	190	Smith et al, 1996
HTWO	Viscosity at 40 °C (mm^2/s): 69	90 days	10, 100, 500, 5 000, 10 000, 20 000	0.93, 9.0, 45, 450, 940, 1 800[1]	45	45[2]	Baldwin, 1992

Table 16: Continued.

Test item	Physico-chemical properties	Duration	Concentration in diet (mg/kg)	Dose (mg/kg b.w. per day)	NOAEL (mg/kg)		Reference
					MLN histiocytosis	Liver granulomas	
P70(H)	Viscosity at 40 °C (mm^2/s): 69.5 Viscosity at 100 °C (mm^2/s): 8.6 Average MW: 485 C number range: 27-43	90 days	20, 200, 2000, 20 000	2, 19, 190, 1 951	19[3]	1 951	Smith et al, 1996
	Viscosity at 100 °C (mm^2/s): 8.97 Average MW: nr C number range: nr	2 years	-	60, 120, 240, 1 200	1 200[4]	1 200[4]	Trimmer, 2004
P100(H)	Viscosity at 40 °C (mm^2/s): 99.8 Viscosity at 100 °C (mm^2/s): 11.0 Average MW: 510 C number range: 28-45	90 days	20, 200, 2000, 20000	2, 19, 190, 1 951	1 951	1 951	Smith et al, 1996
	Viscosity at 100 °C (mm^2/s): 11.3 Average MW: nr C number range: nr	2 years	-	60, 120, 240, 1 200	1 200[2]	1 200[2]	Trimmer, 2004

b.w.: body weight; NOAEL: no-observed-adverse-effect level; MLN: mesenteric lymph node.

1: Average doses calculated from the daily intake range calculated by Baldwin et al. (1992).

2: Baldwin et al. (1992) identified hepatic NOELs based on the incidence of slight to moderate Kupffer cell hypertophy, observed in female rats at ≥ 100 mg/kg and ≥ 500 mg/kg for OTWO and HTWO, respectively. The CONTAM Panel considered these changes as non adverse and based the hepatic NOAELs on the incidence of liver lipogranulomas (i.e. liver microganulomas).

3: NOAEL based on the presence of pigmented macrophages at 20 000 mg/kg, an effect of possibly low biological significance.

4: No hepatic microgranulomas or MLN histiocytosis observed. Minor changes were observed in MLN (infiltrating histiocytes at all doses, with slight increased severity in groups exposed to MOH and dose-related increase in MLN weight) and in liver (vacuoles in liver portal areas observed with increased incidence and severity) of rats treated with the oil. Those changes were judged of no toxicological relevance but indicative of chronic exposure to mineral oil.

Table 17: NOAEL observed in female Fischer 344 rats exposed to waxes, based on MLN histiocytosis and liver microgranulomas.

Test item[1]	Physico-chemical properties	Duration	Concentration in diet (mg/kg)	Dose (mg/kg b.w per day)	NOAEL (mg/kg b.w. per day)		Reference
					MLN histiocytosis	Liver microgranulomas	
Waxes							
LMPW	Viscosity at 40 °C (mm^2/s): solid Viscosity at 100 °C (mm^2/s): 3.3 Average MW: 375 C number range: 19-42	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	< 2	19	Smith et al, 1996
IMPW	Viscosity at 40 °C (mm^2/s): solid Viscosity at 100 °C (mm^2/s): 6.3 Average MW: 450 C number range: 21-49	90 days	200, 2 000, 20 000	19, 190, 1 951	< 19	19	Smith et al, 1996
HMPW	Viscosity at 40 °C (mm^2/s): solid Viscosity at 100 °C (mm^2/s): 15.4 Average MW: 630 C number range: 22-80	90 days	20, 200, 2 000, 20 000	2, 19, 190, 1 951	1 951	1 951	Smith et al, 1996

[1]LMPW: Low melting point wax; IMPW: Intermediate melting point wax; HMPW: high melting point wax.

Linear alkanes

n-Undecane was tested in a combined repeated dose/reproduction and developmental toxicity test by the Japanese Ministry of Health and Welfare (MHW, 1996). Groups of 12 male and 12 female Sprague Dawley rats were exposed by gavage to n-undecane (99 % purity) at doses of 0, 100, 300 or 1 000 mg/kg b.w. per day. Males were exposed for 47 days and females were exposed from 2 weeks before the mating period through the mating and gestation periods up to day 3 of the lactation period. Decreased body weight gain and food consumption were observed in males at 1 000 mg/kg b.w. per day and at ≥ 300 mg/kg b.w. per day, respectively. Changes in haematology (decreased haemoglobin concentration and increased white blood cell count) and in blood chemistry (decreased albumin levels, increased in α_{2u}-globulin, ALT, cholinesterase and total cholesterol levels) were observed in males administered 1 000 mg/kg b.w. per day. Increased relative liver weights and absolute and relative thymus weight were observed in males administered 1 000 mg/kg b.w. per day. Absolute and relative liver weights were increased in females given 1 000 mg/kg b.w. No changes were detected in the gross pathology or histopathology examinations. A NOAEL of 300 mg/kg b.w. per day related to the repeated dose toxicity was identified by the CONTAM Panel.

Branched alkanes

Several low molecular weight branched alkanes (C_8-C_{14}) have been tested by gavage for their potential to cause α_{2u}-globulin-mediated nephrotoxicity in male rats (Amoruso et al., 2008). No further information is reported, as this effect is known to be not relevant for humans (see Section 7.4.1).

Several studies have been performed on pristane (2,6,10,14-tetramethylpentadecane) by i.p. injection, as it is used as a model to induce plasmacytoma and autoimmune diseases in rodents. An overview of those studies is given in Section 7.2.6.

Cyclic alkanes

Decalin (decahydronaphthalene) was tested by inhalation by the U.S. National Toxicology Programme (NTP) in a 13-week and a 2-year study in male and female Fischer 344 rats, and male and female B6C3F1 mice (NTP, 2005). Subchronic exposure to 25 - 400 ppm (141 – 2 482 mg/m^3) decalin caused typical nephropathy associated with α_{2u}-globulin accumulation in male rats (Dill et al., 2003b). Similarly, nephrotoxicity progressing to renal tubule adenoma and carcinoma was observed in male rats exposed to the same concentrations for 105 weeks (see Section 7.2.5). In mice, centrilobular hepatic cytomegaly associated with increased liver weight was observed in male mice exposed to 50-400 ppm (282 – 2 482 mg/m^3) decalin. A statistically significant decrease in the absolute spermatid count was recorded at the highest concentration tested in male mice in the 90-day study. Chronic exposure to 400 ppm (2 482 mg/m^3) decalin caused histological changes in the liver of male mice, including centrilubolar hypertrophy, necrosis and syncytial alteration, Equivocal results were observed in the occurrence of neoplastic incidences in the uterus and liver of female mice (See Section 7.2.5).

7.2.2.2. MOAH

Mixtures

The results of a 13-week study in rats exposed to a petroleum distillate aromatic extract are summarised in the US Environmental Protection Agency (US EPA) High Production Volume (HPV) dossier on aromatic Extracts (API, 2003) and in the information published by ECHA for several REACH[17] Registration dossiers (e.g. ECHA, 2012c). Briefly, a **heavy paraffinic distillate aromatic**

[17] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals

extract (reported composition: 22.3% total non-aromatics, 77.7 % total aromatics, of which 37.2 % < 3 ring PAH, 23 % 3-5 ring PAH, 12.8 % sulphur containing-PAC, 2.3 % nitrogen containing-PAC, and 1.6 non-basic) was administered by oral gavage to male Sprague Dawley rats (10 rats/group) for 13 weeks (5 days per week) at doses of 0, 125 or 500 mg/kg b.w. per day. In parallel, the same mixture was administered dermally to 10 rats/sex per group for 13 weeks (5 days per week) at doses of 0, 30, 125, 500 or 1 250 mg/kg b.w. per day. In the oral study, rats showed clinical signs of toxicity during the exposure period at both doses. A significantly lower body weight gain was observed at 500 mg/kg b.w. per day during the study period and four out of ten rats exposed at this dose were sacrificed or found dead prior to schedule. Red blood cell count, haemoglobin concentration and haematocrit were statistically significantly decreased at 125 and 500 mg/kg b.w. per day. White blood cell count and platelet count were statistically significantly decreased at 500 mg/kg b.w. per day. Changes in organ weight were observed at both treatment doses. Namely, a statistically significant increase in relative liver weight, and statistically significant decrease in relative prostate weight, relative thymus weight and relative seminal vesicles weight were observed at both doses in comparison to controls. Histopathology showed several dose related effects occurring at both doses, including atrophy in the thymus, prostate and seminal vesicles, fibrosis and decreased cellularity in the bone marrow, hepatocyte necrosis and vacuolation. Similar results were obtained in the dermal study. The CONTAM Panel did not identify a NOAEL for the oral study and a lowest-observed-adverse-effect level (LOAEL) of 125 mg/kg b.w. per day was identified.

In another study, Smith et al. (1999) exposed female Sprague Dawley rats and male C57BL/6 mice to the **aromatic extract of a jet fuel** (**aromatic HC in the carbon number range C_9-C_{16}**). 15 rats/group and 15 mice/group were administered the extract at the doses of 0, 20, 100 and 500 mg/kg b.w. per day by gavage for 13 consecutive weeks. No treatment-related deaths were recorded during the exposure period. Clinical signs of discomfort such as hunched posture and lethargy were observed in all mouse groups treated with the aromatic extract, with frequency increasing with the dose. In rats, clinical signs including salivation and lethargy were observed at 100 and 500 mg/kg b.w. per day. Slight but statistically significant decreases in mean haemoglobin, haematocrit and red blood cell count were observed in rats exposed to 100 and 500 mg/b.w. per day. In mice, haematological analyses did not show treatment related changes. Statistically significant increases were recorded in absolute and relative liver weights in rats exposed to 500 mg/kg b.w. per day in comparison to controls. Mice exposed to the same dose also showed increased liver weight, without achieving statistical significance. Relative kidney weight was also observed as statistically significantly increased in rats exposed to the highest tested dose. Gross pathology examination revealed enlarged livers in rats exposed to 500 mg/kg b.w. per day. No treatment-related changes were recorded in the histopathological examinations. The CONTAM Panel identified an NOAEL of 20 mg/kg b.w. per day for rats, based on haematological changes. At this dose, mice showed only minimal clinical signs (lethargy in 5/15 animals), and no other treatment-related changes.

Non alkylated-aromatic hydrocarbons

In a limited study Shopp et al. (1984) tested **naphthalene** (NAP) in CD-1 mice. Male and female mice were exposed by gavage to 5.3, 53 and 133 mg/kg b.w. per day for 90 days. A vehicle control group of 112 male and 76 female mice, low and mid dose groups of 76 male and 40 female mice and a high treatment group of 96 male mice and 60 female mice were included. No treatment-related mortality or body weight changes were observed in either sex. Statistically significant decreases in absolute spleen, liver and brain weight and relative spleen weight were observed in female mice exposed to the highest dose (133 mg/kg b.w. per day). Blood chemistry analyses showed a statistically

Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1-849.

significant decrease in blood urea levels and serum proteins at 53 and 133 mg/kg b.w. per day. No histopathological examinations were conducted.

The results of an unpublished 90-day study on NAP in Fischer 344 rats is reported by US EPA (EPA, 1998). Groups of 10 rats/sex were exposed by gavage to NAP (> 99 % pure) at dose levels of 0, 25, 50, 100, 200, or 400 mg/kg b.w. per day for 5 days per week. Clinical signs of toxicity were observed in both sexes at the highest treatment dose. Two male rats from this dose level were found dead. Decreased terminal body weight was recorded in males at 200 and 400 mg/kg b.w. per day and in females at 400 mg/kg b.w. per day in comparison to controls. Neutrophils were found to increase in males and females at the dose level of 400 mg/kg b.w. Decreased lymphocyte count was observed in male rats exposed to 400 mg/kg b.w. per day. Only marginal effects were observed during the histopathological examination in the kidney of males exposed to 200 and 400 mg/kg b.w. per day and in the thymus of females exposed to the highest dose level only. EPA identified an NOAEL of 100 mg/kg b.w. per day.

Development of haemolytic anaemia is the most commonly reported effect in humans following acute ingestion of high doses of NAP (ECB, 2003; ATSDR, 2005). Children and subjects deficient in glucose-6-phosphate dehydrogenase are more susceptible to the haematological effects of NAP. In laboratory animals, haemolytic anaemia was reported in a dog fed a diet containing 263 mg/kg b.w. for a seven day period (Zuelzer and Apt, 1949).

NAP is also applied as a model to induce cataract formation in animal models. Acute or short term repeated ingestion of high doses of NAP (e.g. 5 days of exposure to 500 or 1 000 mg/kg b.w. per day in rats (Yamauchi et al., 1986) or 28 days of exposure to 1000 mg/ kg b.w. per day in rabbits (Van Heyningen and Pirie, 1976).

Fluorene (FLUO) was tested in a 13-week study in CD-1 mice (unpublished study, reported by US EPA, 1990). In the study, 25 female and 25 male mice/group were exposed to 0, 125, 250, or 500 mg/kg b.w. per day by gavage for 13 weeks. Clinical signs were observed in all the treated groups, with severity increasing with the dose. A statistically significant decrease in red blood cell count and packed cell volume was observed at 500 mg/kg b.w. per day in males and at ≥ 250 mg/kg b.w. per day in females. At the top dose, increased serum bilirubin levels and decreased haemoglobin concentration were also observed. Statistically significant increases were observed in absolute and relative liver weight at ≥ 250 mg/kg b.w. per day, and in absolute and relative kidney and spleen weights in females exposed at ≥ 250 mg/kg b.w. per day and in males at 500 mg/kg b.w. per day. In line with changes in haematology and organ weight, increased haemosiderin levels in the spleen and liver cells were observed at 500 mg/kg b.w. per day. EPA identified an NOAEL of 125 mg/kg b.w. based on the reported haematological effects (EPA, 1990).

In a similar unpublished study, **PYR** was tested in male and female CD-1 mice (EPA, 1993). 20 mice/sex/group were exposed by gavage to 0, 75, 125, or 250 mg/kg b.w. per day for 13 weeks. Decreased absolute and relative kidney weights were recorded in both sexes exposed to 250 mg/kg b.w. per day. In the histopathology examinations, minimal or mild severity lesions, consisting of multiple regenerative foci accompanied by interstitial lymphocytic infiltrates and fibrosis, were observed in the kidney tubules of males and females exposed to 250 mg/kg b.w. per day and ≥ 125 mg/kg b.w. per day, respectively. EPA identified an NOAEL of 75 mg/kg b.w. per day.

Alkylated aromatic hydrocarbons

Information was only found for short chain-alkylated aromatics.

Murata et al. (1993, 1997) tested **1-MNAP** and **2-MNAP** by exposing B6C3F1 mice (50/sex per group) to the 2 substances at dietary concentrations of 750, or 1 500 mg/kg for 81 weeks. A shared control group of 50 male and 50 female mice was included.

For 1-MNAP, average daily intakes of 0, 71.6 and 140.2 mg/kg b.w. per day for males, and 0, 75.1 and 143.7 mg/kg b.w. per day for females were reported for the dietary concentrations of 750 and 1 500 mg/kg, respectively. A statistically significant increase in pulmonary alveolar proteinosis was observed at the end of the exposure period (incidences of 4/49, 23/50 and 19/50 in males and 5/50, 23/50 and 17/49 in females exposed to 0, 750 and 1 500 mg/kg, respectively). Histopathologically, pulmonary alveolar proteinosis was characterised by the filling of alveolar lumens with cholesterol crystals, foamy cells, and an amorphous acidophilic material. No other non-neoplastic effects were reported. Increased incidence of lung adenoma and carcinoma was also observed in the treated males in comparison to controls, as reported in Section 7.2.5. (Murata et al., 1993). No NOAEL can be identified for this study.

For 2-MNAP, average daily intakes of 54.3 or 113.8 mg/kg b.w. per day for males and 50.3, or 107.6 mg/kg b.w. per day for females were reported for the dietary concentrations of 750 and 1 500 mg/kg, respectively. A statistically significant increase in the incidence of pulmonary alveolar proteinosis, described by the authors as similar to the changes observed in the mice exposed to the structural isomer, was observed in males (21/49 and 23/49) and females (27/49 and 22/48) exposed to 2-MNAP at 750 and 1 500 mg/kg. Changes in haematology (decrease in neutrophil count and increase in lymphocyte count in females) and blood chemistry (non statistically significant increases in neutral fat, total lipid and phospholipid levels in males and females) were observed in treated mice in comparison to controls, but no data were shown in the publication (Murata et al., 1997). No NOAEL can be identified for this study.

2,6-diisopropylnaphthalene has been assessed by US EPA for its use as pesticide (US EPA, 2003). In the technical report, the results of an unpublished 90-day feeding study in rats are briefly reported. Rats were administered 2,6-diisopropylnaphthalene at levels of 0, 750, 1 500 and 3 000 mg/kg in the diet (corresponding to doses of 0, 53.9, 104 and 208 mg/kg b.w. per day in males and 0, 61.8, 121 and 245 mg/kg b.w. per day in females, respectively). A NOAEL of 104 mg/kg b.w. per day was reported by US EPA, based on lower body weight and food consumption, higher adrenal weight and increased incidence of adrenal cortical hypertrophy and renal tubule necrosis observed at higher doses.

Partially hydrogenated aromatic hydrocarbons

Tetralin (1,2,3,4-tetrahydronaphthalene) caused nephropathy likely associated to α_{2u}-globulin accumulation in male Fischer 344 rats exposed by gavage to 485 mg/kg b.w./day on alternate days over a 14 day period (Servé et al., 1989).

However, no nephrotoxicity was observed in a more recent unpublished study reported in the OECD SIDS dossier for the substance (OECD, 2004). In this study, groups of 5 Wistar rats per sex were exposed to tetralin by gavage at doses of 0, 15, 50 or 150 mg/kg b.w. per day for 28 days. Two further groups were exposed to 0 or 150 mg/kg b.w. per day for 28 days and allowed for a two week recovery period. Reduced bodyweight was observed in males of the 150 mg/kg b.w. per day group at the end of the exposure period. Haematology analysis showed a decreased red blood cell count in males and increased reticulocyte and eosinophil count in females from the high dose group. Statistically significant changes were observed in urinalysis in females (increased urine volume in the high dose group; decreased urine pH combined with presence of glucose and increased ketone bodies in the 150 mg/kg b.w. per day group and, non statistically significant, in one female from the 50 mg/kg b.w. per day group) and in males (increased oxalate levels in the 150 mg/kg b.w. per day group, with an increased trend, non statistically significant, in the 50 mg/kg b.w. per day group; increased triphosphate levels and decreased erythrocytes in the 150 mg/kg b.w. per day group). Relative spleen weight was found to increase in intermediate and high dose males, achieving statistical significance in the high dose group only. No treatment-related changes were observed in gross pathology and histopathology analyses. An NOAEL of 15 mg/kg b.w. per day was identified for this study in the SIDS dossier (OECD, 2004). Due to the limited information reported in the dossier, the CONTAM

Panel could not evaluate the adverseness of the changes in urinalysis observed at 50 mg/kg b.w. per day.

US EPA (1994) reported the results of an unpublished subchronic study on **acenaphthene** (1,2-dihydroacenaphthylene). Groups of 20 male and 20 female CD-1 mice were exposed by gavage to acenaphthene 0, 175, 350, or 700 mg/kg b.w. per day for 90 days. It was reported that no effects were observed on mortality, clinical signs, body weight changes, total food intake, and ophthalmological alterations. Increased liver weight accompanied by hepatocellular hypertrophy was observed at ≥ 350 mg/kg b.w. per day in both sexes. At those doses, a statistically significant increase in cholesterol levels was observed in females, while it was limited to the high dose group in males. Although liver weight was found to increase also at the lowest tested dose, it was not accompanied by hepatocellular hypertrophy or change in cholesterol levels and was thus considered as an adaptative and non adverse change by US EPA, that identified an NOAEL of 175 mg/kg b.w. per day.

Sulfur-containing aromatic compounds

Benzo[*b*]thiophene has been investigated by Poon et al. (1997, 1998) in Sprague Dawley rats by oral exposure.

In a short-term study, groups of 5 male rats were exposed to benzo[*b*]thiophene either via gavage or via the diet (Poon et al., 1997). In the gavage study, rats were exposed to 0, 2, 20 or 200 mg/kg b.w. per day benzo[*b*]thiophene for 21 days. During the exposure period, rats exposed to 200 mg/kg b.w. per day showed reduced food consumption and a depressed growth rate, resulting in an average body weight gain 53 % lower than that of the control group. A statistically significant increase in the overnight urine output was observed at 200 mg/kg b.w. per day during the overall study period, resulting in a mean urine volume about 4.5 fold higher at day 21 than that measured in the same group at the beginning of the experiment. A lower but statistically significant increase in urine volume was also observed at 20 mg/kg b.w. per day. A statistically significant increase in relative liver weight and relative kidney weight was observed at 200 mg/kg b.w. per day group in comparison to controls. Both the absolute and relative thymus weights were statistically significantly decreased in the 200 mg/kg b.w. per day in comparison to controls. Blood chemistry analysis revealed a statistically significant decrease in uric acid levels and increase in g- glutamyltransferase activity at 200 mg/kg b.w. per day. Several statistically significant changes in hepatic enzyme activities were observed in the high dose group in comparison to controls (increased aniline hydroxylase, aminopyrine-N-demethylase, UDP-glucuronosyltransferase, glutathione-S-transferase and pentoxyresorufin O-dealkylase). No histopathological changes were observed in liver and kidney.

In the feeding study, rats were exposed to dietary concentrations of 0, 100 and 500 mg/kg benzo[*b*]thiophene (corresponding to calculated doses of 0, 8 and 44 mg/kg b.w. per day) for 28 days. No significant changes were observed in food consumption, body weight gain, and overnight urine output in comparison to controls. A low but statistically significant increase in absolute and relative liver weight was observed at 500 mg/kg b.w. per day. Rats exposed to 500 mg/kg b.w. per day had a statistically significant increase in blood glucose, blood urea nitrogen, and alkaline phosphatase activity and a statistically significant reduction in red blood cell count. Similarly to the gavage study a statistically significant increase was observed in the activity of aniline hydroxylase, aminopyrine-N-demethylase and glutathione-S-transferase in the high exposure group only in comparison to controls.

In a follow-up study, male and female Sprague Dawley rats (10 rats/sex per group) were exposed to dietary concentrations of 0, 0.5, 5, 50 and 500 mg/kg benzo[*b*]thiophene for 13 consecutive weeks (Poon et al., 1998). Average daily doses of 0, 0.04, 0.34, 3.51 and 34.13 mg/kg b.w. per day for males and 0, 0.04, 0.39, 4.18 and 38.73 mg/kg b.w. per day for females were calculated. No clinical signs were observed during the exposure period. No treatment-related differences were observed in growth rates, food and water consumption, overnight urine output and organ weights between the treatment groups and the control group. The performance of a battery of neurobehavioural screening tests did

not reveal any treatment related effect. No significant changes were observed in the activity of hepatic enzymes observed in the short term studies. Histological changes were observed with higher severity in female rats. In particular peribiliary fibrosis in a mild degree of severity, combined in some case with prominent biliary duct, was observed in all females of the 50 and 500 mg/kg groups. In the kidney, epithelial hyperplasia of the pelvis was observed in females exposed to 5 mg/kg and above. In males, an increased incidence of binucleated hepatocytes was observed at 5 mg/kg and above, and increased incidence of mild thickening of basement membrane of the kidney cortex was observed at 500 mg/kg. In view of the histopathological effects in female kidney, the CONTAM Panel identified a NOAEL of 0.04 mg/kg b.w.

7.2.3. Genotoxicity

7.2.3.1. Mineral oil mixtures

Most studies on the genotoxicity of mineral oil and mineral oil fractions have been performed in the *Salmonella typhimurium* mutagenicity assay (Blackburn et al., 1984, 1986; IARC, 1984/1987; Roy et al., 1988; Granella and Clonfero, 1991; Brooks et al., 1995; Mackerer et al., 2003). With the exception of highly purified oil varieties consisting of alkanes and naphthenes, all mineral oils are mutagenic in the *Salmonella typhimurium* assay (IARC, 1984; Roy et al., 1988; Mackerer et al., 2003). It is the aromatics, including alkylated PAHs, that cause the mutagenicity of mineral oils and this fraction is also responsible for the formation of DNA adduct in mice skin following skin painting (Granella and Clonfero, 1991; Ingram et al., 2000). Even non-mutagenic refined mineral oils may become mutagenic after use. High temperature processes, e.g. > 800 ^{o}C can convert non-mutagenic mineral oil components into mutagenic PAHs (Granella and Clonfero, 1991) and motor oils in addition to thermal decomposition may also pick up engine combustion products such that after use they may contain several orders of magnitude higher concentrations of PAH.

Generally, upon testing in the *Salmonella typhimurium* assay, in spite of their carcinogenic activity, whole mineral oils often show weak genotoxic activity (Brooks et al., 1995). Several approaches have therefore been used to enhance the sensitivity of the bacterial tests. These modified methods include prior fractionation by solvent extraction (with DMSO alone or in combination with cyclohexane) in order to concentrate the total polycyclic aromatic hydrocarbon fraction. Also chromatographic separation has been employed for testing of individual components (Epler et al., 1979; Brooks et al., 1995; Mackerer et al., 2003). Since the mineral hydrocarbons are not soluble in water, procedures involving emulsification of oils have also been used (Brooks et al., 1995). A modified *Salmonella typhimurium* assay for testing was developed by Blackburn et al. (1984, 1986) using DMSO extracts of mineral oils in a test system with increased concentration of rat or hamster liver S-9 activating system as well as co-factors. Although the *Salmonella typhimurium* assay is not considered to give quantitative results, in this test the mutagenic index (MI), a semi-quantitative measure of mutagenic potency, was calculated. The MI represents the slope of a plot of dose versus number of revertants and samples giving a double number of revertants above background are considered positive (Blackburn et al 1986; Reddy et al., 1997; Brandt et al., 1999). Later, Brooks and co-workers (1995) found that testing a range of whole mineral oils with a method using washed rat liver microsomes correlated well with the results obtained with the modified extraction method by Blackburn and co-workers (1984, 1986). Brooks and co-workers (1995) in their paper were not clear about the rationale for using washed microsomes, but suggested that enzymes in the supernatant could inhibit the response. A possible increase in microsomal enzyme activity in this assay could be in line with the use of increased amounts of S-9 by Blackburn and co-workers. The modified Ames assay developed by Blackburn et al. (1986) has later been recognised by the American Standardization for Testing and Materials (ASTM) International (ASTM, E 1687-95).

In the oils, which are complex mixtures, the genotoxic activity is the combined effect of the simultaneous presence of all of the MOAH, which individually on a molecular level might express additivity, synergy or antagonism (e.g. BaP may be inhibited by less active MOAH) (Brooks et al.,

1995; Booth et al., 1998; Mackerer et al., 2003). Hence, it is not possible to sum up the activity of a number of single fractions. It is mainly the alkylated and non-alkylated (usually low amounts from oil, but they may accumulate at high temperatures) aromatics of 3-7 fused rings including sulphur containing aromatics that causes genotoxicity (mutagenicity and DNA-adduct formation) (and carcinogenicity) of mineral oils (Roy et al., 1988; Mackerer et al., 2003). Because of this also two other tests in addition to the modified Ames assay have been used for classification of mineral oils; the measurement of 3-7-ring aromatics by extraction and chromatography (Mobil method) and the gravimetric IP 346 assay based on extractions and the weight of the MOAH fraction after removal of solvent (Mackerer et al., 2003). The IP 346 test has been approved for printing ink mineral oils by the Institute of Petroleum and is accepted by the European Chemical Agency (ECHA) for carcinogenicity classification and labelling of mineral oils. For extracts with a PAH (3-7 ring PAHs) content of less than 0.7 weight percent of the oil the MI was zero (Roy et al., 1988). The extraction tests as well as the Salmonella mutagenicity test appear to correlate quite well with the ability of a series of tested oils to cause skin tumours (papillomas) in mice following skin painting (Mackerer et al., 2003).

7.2.3.2. MOSH

Low molecular weight MOSH mixture solvents

Information on unpublished genotoxicity studies on low molecular MOSH solvents were submitted to EFSA by the study owners. Information on the composition of the products was retrieved from datasheets and similar information on the internet.

Shell carried out genotoxicity studies on three products, Shellsol TD, Shellsol D60 and Shellsol D70. Shellsol TD, which is a faster evaporating hydrocarbon solvent consisting of isoparaffinic components (> 98 %) and low in naphtenes (< 2 %), as well as impurities such as sulphur, benzene and aromatics (Shell Chemicals, 2007a). Shellsol D60 consists predominantly of C_{10}- C_{12} paraffins and naphthenes with a very low aromatic content (Shell Chemicals, 2007b). ShellSol D70 consists predominantly of C_{11}-C_{14} paraffins and naphthenes with a very low aromatic content (Shell Chemicals, 2007c). Shellsol TD and Shellsol D60 were tested in *S. Typhimurium* strains TA1535, TA1537, TA98, TA100 and TA 102 (Shell, 1998a; Shell, 1999). None of the strains showed a positive response to Shellsol TD or Shellsol D60 in any tester strain neither in the presence nor in the absence of S9 mix. The ability of Shellsol D70 to cause chromosome aberrations was examined in cultured human lymphocytes with and without metabolic activation (Shell, 1998b). Several experiments were performed and the conclusion was that Shellsol D70 did not induce chromosome aberrations in this system when tested to its limits of toxicity neither in the absence nor in the presence of S9 mix.

Information on Soltrol 130, which consists of C_{10}-C_{13} isoalkanes, was available from Chevron Phillips Chemical Company (Chevron Phillips, 2010). Soltrol 130 was tested for its ability to induce sister chromatide exchange in Chinese Hamster Ovary cells in the absence and presence of metabolic activation, and it did not exhibit a positive response in these systems (Hazleton Laboratories, 1983).

Exxon submitted information on MRD-89-582 (Exxon Biomedical Sciences, 1991c), which is an aliphatic hydrocarbon solvent (light petroleum distillate, hydrotreated). It was tested for its ability to induce micronuclei in bone marrow cells in mice *in vivo*. Test doses were 5.0, 2.5 and 1.25 g/kg b.w. by oral gavage. The test was considered negative and no bone marrow toxicity was reported.

It can be concluded from these studies that alkane mixture solvents are not genotoxic, neither *in vitro* nor *in vivo*.

n-Alkanes and branched-alkanes (n-paraffins and iso-paraffins)

There are hardly any genotoxicity data on single alkanes neither from bacterial nor from other genotoxicity tests. An exception is a series of studies by Felix and co-workers (Felix et al., 1997, 1998, 1999) on the iso-alkane pristane, 2,6,10,14-tetramethylpentadecane, which by unknown

mechanisms induces a distinct form of B-cell-derived malignant lymphoma. They reported that pristane significantly increased *lacI* mutations *in vivo* in lymphoid tissues of congenic BALB/c.lLIZ N5 mice as well as *in vitro* in LPS-stimulated B lymphoblasts and rat 2λlacI fibroblasts. Mutations were observed both in mice with and without plasmacytomas. The mechanism of mutagenesis is unknown, but the authors suggest that it might be linked to lymphoblast activation and oxidative stress (Felix et al., 1999).

With regard to alkane mixtures, generally, white highly refined paraffinic mineral oils with a very low content of aromatics are not mutagenic in the *Salmonella typhimurium* mutagenicity tests without or with metabolic activation (Granella and Clonfero, 1991; Mackerer et al., 2003).

A number of alkanes were tested for their ability to induce morphological transformation and inhibit intercellular communication in primary Syrian hamster embryo cells in culture. Neither of the following compounds, nonane, decane, undecane, dodecane, tridecane, 2-methylheptane, induced morphological transformation. Tridecane, 2-methyloctane and 2-methylnonane reduced intercellular communication (Rivedal et al., 1992).

Cycloalkanes (naphthenes)

No mutagenicity data on single cycloalkanes have been identified. With regard to cyclohexane mixtures, generally, highly refined naphthenic mineral oils with very low content of aromatics are not mutagenic in *Salmonella thyphimurium* mutagenicity tests without or with metabolic activation and do not produce DNA-adducts upon painting of mouse skin (Granella and Clonfero, 1991; Ingram et al., 2000; Mackerer et al., 2003).

7.2.3.3. MOAH

Mineral oil products high in the concentration of 3-7 ring PAHs, such as unrefined and acid- or hydro-treated oils, are mutagenic (Mackerer et al., 2003).

Whereas most of these aromatics are alkylated, this fraction may also contain non-alkylated PAHs. Further review of genotoxicity and risk assessment of PAH is contained in the Opinion on PAH in food by the EFSA Panel on Contaminants (EFSA, 2008d).

Ingram and co-workers (2000) investigated some unsubstituted- and methylated PAHs as well as various mineral oils with respect to their ability to produce DNA adducts in mouse skin exposed by painting. PAHs which were neither mutagenic in bacterial assays nor were initiators or carcinogens failed to induce DNA adducts, whereas BaP, 5-methylchrysene (5-MCH), 1,4-dimethylphenantrene produced adducts. Highly refined white N11 oil 'with a very low content of aromatics' did not produce adducts; whereas there was clear evidence of adduct formation with both a non solvent-refined oil and with a solvent-extracted (but not hydrogenated) oil. The level of adduct formation with the extracted oil, however, was much lower than with the non-extracted oil. Upon fractionation and examination of adducts formed by the 2-3-ring and 4-6-ring aromatic fractions, it appeared that the main adduct spots produced by the non-extracted oil could be attributed to the 2-3-ring aromatic components of the oil. The authors suggested that the activity of non-extracted oils was largely caused by substituted 3- and 4-ring polycyclic aromatic compounds. It should be noted that physicochemical properties of the oil can influence the polyaromatic compounds' ability to penetrate the skin and interact with the epidermal cells of the skin (Ingram and Philips, 1993).

Previous studies indicate that for (un-substituted) PAHs carcinogenic activity requires four or more aromatic rings and that activity is dependent on the position of the aromatic rings (Ingram et al., 2000). Indications that both genotoxic as well as carcinogenic activity of MOAH are mainly related to 3- and 4-ring aromatics (Ingram et al., 2000; Mackerer et al., 2003) point to the potential carcinogenicity of alkyl-substituted aromatics. However, very little systematic information on biological activity is available on the many MOAH, virtually all of which to some extent are alkyl

substituted (Mackerer et al., 2003). The enhancing or inducing effect of alkylation on the activity is dependent on both size and location of the substituents. Whilst methyl substitution at aromatics with few rings might enhance biological activity, particularly bulky ring substitutions would tend to prevent bio-activation and intercalation in DNA.

1,2-dimethylbenzene, 1,2,4-trimethyl benzene and tert-butylbenzene did not induce morphological transformation or inhibit intercellular communication in primary Syrian hamster embryo cells in culture (Rivedal et al., 1992).

Sulfur-containing aromatics (condensed thiophenes)

Several of the condensed thiophenes show mutagenic activity in the *Salmonella typhimurium* assay with metabolic activation (Kropp and Fedorak, 1998). A number of un-substituted 3- and 4-ring thiophenes were tested by Pelroy and co-workers (1983) and McFall et al. (1984, cited in Jacob, 1990) for mutagenic activity in *S. typhimurium* strains TA 98 and TA100. Of the 3-ring thiophenes only naphtho[1,2-*b*]thiophene was mutagenic whereas its isomers naphtho[2,1-*b*]thiophene and naphtho[2,3-*b*]thiophene and dibenzothiophene were not. Seven of thirteen 4-ring thiophenes were mutagenic with phenanthro[3,4-*b*]thiophene as the most potent and equipotent with BaP, whilst its isomer phenanthro[4,3-*b*]thiophene had only low activity. Also anthra(1,2-*b*)thiophene, anthra(2,1-*b*)thiophene and anthra(2,3-*b*)thiophene were mutagenic. Swartz and co-workers (2009) tested phenanthro[3,4-*b*]thiophene and phenanthro[4,3-*b*]thiophene as well as their sulfone and dihydrodiol derivatives. Several of these were mutagenic and produced DNA adducts. Benzo[*b*]phenanthro[2,3-*d*]thiophene and particularly its sulfoxide derivative were strongly mutagenic in *S. typhimurium* and caused CG→AT transversion based on the results in Tester Strain TA 7005 (Kumar et al., 2004). McFall and co-workers (1984) examined methyl-substituted 4-ring thiophene derivatives and five of these exhibited mutagenic activity, 1-methylbenzo[*b*]naphtho[1,2-*d*]dithiophene, 3-methylbenzo[*b*]naphtho[1,2-*d*]dithiophene, 1-methylbenzo[*b*]naphtha[2,1-*d*]thiophene, 6-methylbenzo[*b*]naphtha[2,1-*d*]thiophene and 4-methylbenzo[*b*]naphtho[2,3-*d*]dithiophene. The most potent of these was 6-methylbenzo[*b*]naphtha[2,1-*d*]thiophene, which is isosteric to the potent mutagen 5-MCH (Kropp and Fedorak, 1998). In addition, for several dimethylsubstituted condensed thiophenes, which were carcinogenic in rodents, information on mutagenicity were not identified (Jacob, 1990). Jacob (1990) noted that in general there is no simple correlation between the genotoxicity of thioarenes and their carbocyclic isosteres.

7.2.4. Reproductive toxicity

There are limited reproductive toxicology data on non-food grade mineral oil fractions and substances therein by the oral route. In the absence of such information, reproductive toxicity studies by other routes have been considered to provide some indication of biological activity.

7.2.4.1. MOSH

Mixtures

A number of studies on developmental toxicity of white spirits (C_9-C_{13}, aromatic content ranging from 0.4 % to 24 % w/w) in rats exposed by inhalation were reviewed by Amoruso et al. (2008). In all cases, no developmental toxicity was observed.

Groups of pregnant rats were exposed to clean air or air-containing white spirit for 6h/day on days 6-15 of gestation (237, 482 or 953 ppm) or on days 3-20 of gestation (950 ppm). In the two high dose groups (950 and 953 ppm) signs of maternal toxicity were observed. No effects on reproduction data or the incidence of skeletal or visceral anomalies or malformations were observed, but in the groups exposed on days 3-20 of gestation mean fetal body weight was lower (14 %) and signs of delayed ossification and the number of fetuses with extra ribs were significantly increased (Jakobsen et al., 1986).

In a developmental toxicity study, a sample of white mineral oil (no details available) was administered to female Sprague-Dawley rats (20 rats/group) at dose levels of 0 or 5000 mg/kg b.w. per day from days 6 through 19 of gestation. No maternal or fetal toxicity were observed (ECHA, 2012a).

In information published by ECHA for several REACH Registration dossiers (e.g. ECHA, 2012c) heavy paraffinic distillate extract was administered by oral gavage, 1 000 mg/kg/day, to male and female Sprague Dawley rats (12 rats/group) in a screening reproductive and development toxicity study. The extract is described as a complex combination of hydrocarbons obtained as the raffinate from a solvent extraction process. It consists predominantly of saturated hydrocarbons having carbon numbers predominantly in the range of C_{20} through C_{50} and produces a finished oil with a viscosity of less than 19 mm^2/s at 40 °C. Males were dosed for at least 14 days prior to mating, continuing for a total dosing period of 30 days. Females were dosed for a minimum of 14 days prior to mating and continuing until the day prior to the scheduled necropsy on lactation day 4 (total dosing period of 39 days). The number and sex of pups, stillbirths, live births, physical or behavioural abnormalities, weights (lactation days 1 and 4), and presence of gross anomalies were measured in the F_1 animals. There were no treatment-related effects on pup body weights, sex ratios, live litter sizes, viability indices, and general physical conditions. Parental animals were necropsied and organ weights and histopathological tissue evaluation was performed. Body weight gain for treated males was significantly decreased throughout the study period in comparison to controls. Female body weights and body weight gains were not affected throughout the treatment period including gestation and lactation period. There were no other treatment-related effects.

n-Alkanes

Reproductive/developmental effects were studied by administering n-undecane orally at doses of 0, 100, 300 and 1 000 mg/kg b.w. per day, in males for 46 days and in females from 14 days before mating to day 3 of lactation (Ministry of Health and Welfare (MHW) Japan. 1996). No effects were detected with regard to reproductive ability, reproductive organ weights, autopsy or histopathology findings in either sex, and there was no observable effect on pregnancy outcome in the dams. Body weight gain was decreased in the male and female offspring in the 1 000 mg/kg b.w. per day dose group. No effects were noted in terms of viability, general condition or autopsy findings of the offspring. From this experiment the authors concluded the NOELs for both reproduction and development to be 300 mg/kg b.w. per day.

7.2.4.2. MOAH

Mixtures

The developmental toxicity of a heavy paraffinic distillate aromatic extract (CAS No. 64742-04-7, 318 Isthmus furfural extract), was tested by dermal exposure in Sprague Dawley rats. Fifteen pregnant rats/group were exposed to the distillate aromatic extract at doses of 0, 8, 30, and 125 mg/kg b.w per day on gestation days 0 to 19. Two additional groups were treated at 500 mg/kg b.w. per day on gestation days 0 to 16 and at 1000 mg/kg b.w. per day on gestation days 10 to 12. Signs of maternal toxicity, including red vaginal discharge in all exposed groups, decreased body weight gain at > 30 mg/kg b.w. per day, and haematological changes, were observed. Maternal toxicity was also observed with respect to reproductive parameters (statistically significantly higher incidence of dams with no viable offspring at the end of the gestation period and lower litter size were observed at > 30 mg/kg b.w). Fetal toxicity, including a significant decrease in fetal body weight and incompletely ossified skull bones, was observed at 125 mg/kg b.w. per day in the prenatal study. Furthermore, fetal resorption was observed to increase in the 30 mg/kg b.w. per day group, without achieving statistical significance. A significant increase of defects in costal cartilage development and the occurrence of cleft palate in 2/114 pups exposed to 1 000 mg/kg b.w. per day were observed (ECHA, 2011c).

Partially hydrogenated aromatic hydrocarbons

In an unpublished 28-day study reported in the OECD SIDS dossier for tetralin (OECD, 2004), four groups of 24 mated female Sprague-Dawley rats received tetralin by daily oral administration (gavage) at 0 (sesame oil = control), 15, 45 and 135 mg/kg b.w. per day from day 6 to day 19 post-coitum inclusive. On day 20 post-coitum, the dams were sacrificed and subjected to macroscopic examination. The study was designed according to OECD TG 414 (2001). There was no treatment-related death in any of the dams. Clinical signs were not observed. Mean absolute and relative food consumption was distinctly to slightly decreased in high dose animals as compared to the controls, attaining statistical significance on study days 6 – 18. The terminal body weight (gestation day 20) was decreased significantly (-5 %) for high dose females compared to controls. A significantly lower body weight gain was recorded for the whole treatment period.

No specific studies have been performed on the toxicity of tetralin on reproduction. No adverse effects on vaginal cytology, sperm and on reproductive organs were observed in a 13-week inhalation study on rats. In mice, no effects on vaginal cytology and sperm were noted in the 13-week inhalation study, but uterus atrophy and atrophy of the ovary were found in the absence of systemic toxicity. Therefore, in the SIDS dossier it is concluded that an effect of the chemical on reproduction cannot fully be excluded (OECD, 2004).

7.2.5. Carcinogenicity

There are only limited data on the carcinogenicity of non-food grade mineral oil fractions and substances therein by the oral route. In the absence of such information, carcinogenicity studies by other routes have been summarised to provide some indication of biological activity. Studies using the dermal route have been of particular value in discriminating genotoxic and non-genotoxic effects.

7.2.5.1. Evaluation of carcinogenicity studies on mineral oils and fractions thereof (MOSH)

Mixtures

A number of studies have been performed on the carcinogenicity of mineral oils and fractions thereof following dermal application to mice, i.e. so-called skin painting experiments. Some of these were designed to investigate methods that might be used to reduce the carcinogenicity of mineral oils during their production and others to study the two-stage initiation/promotion model of carcinogenesis. In this model, tumours are initiated by a single dermal treatment with a genotoxic compound and are then promoted by dermal treatment for a prolonged period, of weeks or months, with a tumour promoting agent. Compounds can act as initiators, promoters or both and the model can be used to determine this for a given substance, by using well-established initiators or promoters, as appropriate. In general, these studies date from several decades ago or even longer. Only a brief summary is provided here, as the mineral oil fractions used were often ill-defined and classified by methods that are now out-dated.

Many preparations of mineral oil and their sub-fractions administered repeatedly to the shaved skin of mice resulted in local irritation and epidermal hyperplasia. In general, preparations containing PAH, with greater than 3 rings, were carcinogenic, resulting in the appearance of cutaneous epithelial tumours (papillomas, squamous cell carcinomas or sebaceous adenomas). Mineral oil preparations low in, or lacking, polycyclic aromatic hydrocarbons but containing longer chain aliphatic compounds, could act as tumour promoters in mouse skin, following initiation by a single treatment with a genotoxic compound, such as a polycyclic aromatic hydrocarbon (e.g. benzo[*a*]anthracene (BaA) or 7,12-DMBA) (Fisher et al., 1951; Smith et al., 1951; Shubik and Saffiotti, 1955; Horton et al., 1957; Bingham and Barkley, 1979; Doak et al., 1983; Bingham, 1988; McKee et al., 1989). Studies on jute batching oil reached similar conclusions, i.e. that the carcinogenicity of such mineral oil preparations to mouse skin required the presence of polycyclic aromatic hydrocarbons with 4 or more rings (Mehrota and Saxena, 1979; Agarwal et al., 1986, 1988).

Groups of fifty 6-8 week old male and female Sprague-Dawley rats were fed diets containing 10 % ground wax (petrolatum; 5 samples) for two years. Untreated controls comprised 140 males and 157 females. The waxes used reflected the range of polycyclic aromatic hydrocarbon content of waxes in commercial use at the time, which was low for those PAHs analysed. The tumour incidences of rats administered the test diets did not differ from those observed in control animals (Shubik et al., 1962). There was no evidence for deposition in the reticuloendothelial system or hepatic granulomas in treated rats in this study (FAO/WHO, 2002). Administration of solutions of the waxes in benzene repeatedly to the shaved skin of mice resulted in local irritation, with some desquamation and epilation, with histological evidence of moderate hyperplasia in both the treated and solvent control groups. There was no evidence of any difference in the incidences of either skin or systemic tumours in any of the wax-treated groups compared to the controls. (Shubik et al., 1962).

In a series of studies conducted over a period of 15 years from 1955 (Wrigley Co, 1959; Davidsohn and Stern, 1960), reported in FAO/WHO (1993), 4 week old rats were fed various hydrocarbon waxes in a chewing gum base for 6 months. In all, there were 16 control groups (120 males and 120 females) and 27 test groups (186 males and 195 females) of animals. The wax varied from 2 to 57 % of the gum base, giving a calculated wax intake from 0.16 % to 4.75 % of the diet. Test animals received diets comprising 75 % basal diet, 8.3 % gum base, and 16.67 % wood flour. A total of 78 control rats and 109 treated rats were maintained up to 19 months of age after cessation of feeding the test diets. These animals were observed until they died.

It was noted that an outbreak of influenza in the animal colony in the early part of 1957 resulted in an increased number of pulmonary lesions and inter-current deaths in test animals. There was no statistically significant increase in the incidence of any tumour type in the treated groups compared with the controls. The incidence of malignant tumours in the test animals was 4/381, compared with 5/241 in the controls. No compound-related differences were found between the groups for any of the endpoints investigated.

Dalbey and Biles (2003) have summarised published work prior to 1985 on inhalation studies of mineral base oils, i.e. a range of lubricant base oils prepared from naturally occurring crude petroleum oils. The base oils used comprised a mixture predominantly of branched paraffins (isoalkanes) and naphthenes (cycloparaffins). They varied in carbon number, from > 15 and their boiling points were in the range 300-600 °C.

Wagner et al. (1964, cited in Dalbey and Biles, 2003) exposed various species to an aerosolised light mineral oil containing 85 % naphthenes and 5 % paraffins, at a concentration in air of 100 mg/m^3 for up to 26 months (Saybolt viscosity = 85 – 95, cf viscosity of a light motor oil). No consistent difference in tumour incidence was observed in any of the species studied.

Stula and Kwon (1978, cited in Dalbey and Biles, 2003) studied the effects of inhalational exposure of mice and dogs to a fibre-finish oil with 70% unspecified paraffinic mineral base oil (Saybolt viscosity = ~50 at 38 °C) and other ingredients, including surfactants. Groups of 27 CAF$_1$/JAX mice were exposed to aerosolised oil at 100 mg/m^3 together with 1 000 ppm acetone, to simulate conditions in acetate fibre production for 12 months.

Yevich (1965, cited in Dalbey and Biles, 2003) exposed dogs to 100 mg/m^3 of mineral oil (no further information provided) for up to 26 months. These authors also exposed rats to 100 mg/m^3 of the oil for up to 16 months.

Lushbaugh et al. (1950, cited in Dalbey and Biles, 2003) exposed groups of rats and mice to motor oil (SAE No. 10) at a concentration of 132 mg/m^3 for 3.5 months and to diesel oil (SGF No. 1) at a concentration of 63 mg/m^3 for 12 months.

Shoshkes et al. (1950, cited in Dalbey and Biles, 2003) exposed mice to petrolatum at 4 500 mg/m^3 or motor oil (SAE No. 10) at 4330 mg/m^3 for one month.

Dalbey and Biles (2003) concluded that the effects of inhalation of mineral oils observed in these studies were concentration-dependent, restricted largely to the lungs, and comprised progressive accumulation of oil-containing (vacuolated or foamy) macrophages in the alveoli, terminal bronchioles, or lymph nodes. In some instances there was also evidence of an inflammatory response or microgranuloma formation. In general, the rat was the most sensitive species, followed by the dog, while other species were relatively insensitive. Overall, although somewhat limited, these studies did not indicate any carcinogenic potential of the mineral oils tested on inhalation.

Groups of 50 Fischer 344 (CDF® (Fischer 344)/CrlBR rats per sex group were administered white mineral oil in their diet for two years (Trimmer et al., 2004). The test mineral oils used met the United States Pharmacopoeia (USP) and Food and Drug Administration (FDA) criteria for food and food contact uses. The oils tested were P70(H), i.e a medium and low viscosity class I oil, and P100(H), a high viscosity oil. Diets were adjusted at regular intervals to provide intakes of 60, 120, 240, and 1 200 mg/kg b.w. per day. There was a slight, but statistically significant decrease in survival in the high dose P100(H) females ($P \leq 0.05$). Both body weight and food consumption were slightly, and statistically significantly, elevated in high dose P70(H) and P100(H) males and females. There was no difference in food conversion efficiency. No neoplastic lesions attributable to treatment with either of the mineral oils were observed in this study. The most common tumour types were mononuclear leukemia and pituitary/pars distalis adenoma (tumour incidences reported in Table 18).

Table 18: Tumour incidences in rats exposed to P70(H) and P100(H) (Trimmer et al., 2004).

P70(H)		Doses (mg/kg b.w. per day)				
		0	60	120	240	1 200
Mononuclear cell leukemia	Males	13/50	17/50	11/50	10/50	15/50
	Females	7/50	15/50	12/49	19/49	14/48
Pituitary/pars distalis adenoma	Males	27/48	7/17	5/15	6/24	25/50
	Females	26/49	8/16	6/13	7/12	29/50

P100(H)		Doses (mg/kg b.w. per day)				
		0	60	120	240	1 200
Mononuclear cell leukemia	Males	16/50	16/50	13/50	12/49	14/50
	Females	8/50	8/50	8/49	6/50	11/50
Pituitary/pars distalis adenoma	Males	19/50	5/19	5/18	9/21	20/50
	Females	21/50	2/9	7/14	4/10	30/50*

*Statistically significant from the control group at $P \leq 0.05$ level.[18]

The incidences of mononuclear leukemia in female rats at 240 mg/kg P70(H) and of pituitary/pars distalis adenoma in high dose females (1200 mg/kg) receiving P100(H) were within the respective historical control ranges for the strain of rat used and are considered incidental, and not treatment related.

The carcinogenicity of dietary administration of a mixture of eight medium-viscosity liquid paraffin oils meeting Japanese food additive and Japanese Pharmacopoeia standards was investigated in Fischer 344/DuCrj rats (Shoda et al., 1997). Groups of 4 week old Fischer 344 rats, 50 per sex per dose group, were fed 0, 2.5 or 5 % of this oil in their diets for 2 years. Control animals (0 % liquid paraffin) received only basal diet. There were no differences in survival between the groups. Body weight gain in high dose (5 %) liquid paraffin fed animals was greater than in the other groups (b.w.

[18] The statistical analyses and the historical range values reported in this Section were cited from the original references.

at 104 weeks cf controls: females – 2.5 % liquid paraffin = + 1.4 %; 5 % liquid paraffin = + 2.7 %; males – 2.5 % liquid paraffin = + 0.9 %; 5 % liquid paraffin = + 5 %). Food consumption was statistically significantly elevated in high dose males (total mean food consumption 104 weeks in the 5 % liquid paraffin group cf controls = + 11 %; 2.5 % liquid paraffin = + 6 %). In females the change in food consumption was not statistically significant (total mean food consumption 104 weeks in the 5 % liquid paraffin group cf controls = + 8 %; 2.5 % liquid paraffin = + 5 %). There were no statistically significant differences in the incidences of any tumour type between the test groups and the control animals. The most common sites of tumours in males were testes, pituitary gland, adrenals, haematopoietic organs, thyroid gland, mammary gland and pancreas. The testicular tumours were all benign interstitial cell type. The most common sites of tumours in females were pituitary gland, uterus, haematopoietic organs and mammary gland (Table 19).

Table 19: Tumour incidences in Fischer rats exposed to liquid paraffins (Shoda et al., 1997).

		Doses (% in feed)		
		0	**2.5**	**5**
Pituitary adenomas	Males	8/50	15/50	11/50
	Females	8/48	16/50	10/49
Thyroid C-cell adenoma	Males	7/50	6/50	7/50
	Females	1/48	3/50	4/49
Adrenal - benign pheochromocytoma	Males	11/50	5/50	7/50
	Females	1/48	1/50	1/49
Haematopoietic organs - large granular lymphocytic leukemia	Males	9/50	9/50	7/50
	Females	6/48	11/50	12/49
Testis - interstitial cell tumour	Males	50/50	49/50	48/50
Prostate adenoma	Males	9/50	4/50	3/50
Uterus - endometrial stromal polyp	Females	13/48	13/50	10/49
Mammary gland – fibroma	Males	8/50	4/50	1/50
	Females	1/48	0/50	0/49
Mammary gland – fibroadenoma	Males	7/50	7/50	7/50
	Females	9/48	10/50	8/49
Lung - alveolar/bronchiolar adenoma	Males	6/50	7/50	2/50
	Females	1/48	2/50	2/49

In general, where petroleum-derived materials can serve as carcinogens, this appears to be due to the presence of polycyclic aromatic hydrocarbons (PAHs) containing three/four or more aromatic rings.

n-Alkanes

Horton et al (1976) tested a range of n-alkanes from C_{12} to C_{28}, in a two stage mouse skin tumour model for their promoting effects. Groups of 9- 15 male C3H mice were initiated with BaP, applied to the interscapular region and then treated with solutions of the test aliphatic hydrocarbons three times per week. The time to appearance of papillomas was determined. Amongst C_{12}, C_{16}, C_{18} and C_{20} n-alkanes, octadecane (C_{18}) and eicosane (C_{20}) produced tumours more rapidly than the other two compounds. The C_{18}, C_{20} and C_{28} n-alkanes had similar relative promoting activity on a molar basis, whereas the C_{30} olefin squalene had only marginal activity. In this model, the branched-chain C_{19} alkane pristane was more potent than C_{18} n-alkane. The C_{22} (docosane) and C_{24} (tetracosane) n-alkanes had similar activity to that the C_{19} compound, octadecane.

Groups of 30 (initiated) or 50 (non-initiated) female Swiss mice were treated topically with a range of n-alkanes and alkanols (Sicé, 1966). All compounds were purified prior to use. Animals were initiated or not by treatment with a single dose of DMBA in the interscapular region, followed by repeated administration of the one of the test substances. Animals were observed until the appearance of papillomas. Treatment had no effect on survival of the mice (~50-70 %). The various preparations all caused cutaneous irritation and this was most marked with decane, dodecane, tetradecane, decanol and dodecanol. Dodecane, tetradecane and decanol were tumour promoters in this model. Decane, decanol and tetradecanol were less potent tumour promoters. Hexadecane, octadecane, eicosane, octanol, hexadecanol and octadecanol showed some evidence of tumour promoter activity (1 tumour-bearing animal per group). Hexane, octane and hexanol were negative in this model. None of the compounds was carcinogenic in the absence of initiation (1 mouse in the tetradecane group developed a papilloma at 37 weeks). There was some relationship with tumour promoting activity and carbon chain length (Figure 27).

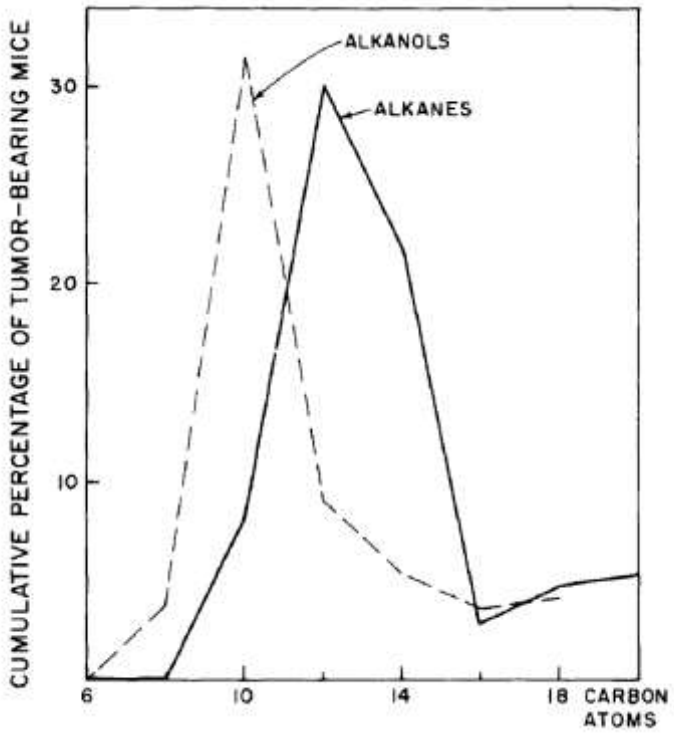


Figure 27: Tumour-promoting activity and chain length of n-alkanes (Sicé, 1966) (Note that the authors also studied 1-alkanols, which can arise as metabolites of n-alkanes).

A number of other studies have also shown that n-alkanes can serve as tumour promoters in mouse skin models, but are not carcinogenic themselves (Horton et al., 1957; Baxter and Miller, 1987).

Branched alkanes

Early studies on pristane showed that i.p. injection of mice resulted in the induction of plasma cell tumours (Anderson and Potter, 1969). This is now a widely used model of plasmacytoma and there are a number of publications on the molecular biology of the tumours.

Thirty female BALB/cAnN mice were treated with pristane (97 - 99 pure), 0.5 ml by i.p. injections, every 2 months for 6 months (i.e. 3 injections). Nineteen of the mice developed plasma cell tumours (plasmacytosis) over a period of 1 year (9/30 after 9 months). One of the animals developed thymic lymphocytic leukemia. Mice showed a widespread peritoneal oil granulomatous reaction, similar to

that observed after injection of white mineral oils. There were no control animals in this study, but the tumours seen do not normally occur in BALB/c mice (Madison et al., 2008).

Groups of male C3H mice were initiated with a single topical administration of DMBA to the interscapular region, followed by repeated administration of pristane of an n-alkane in cyclohexane as vehicle (Horton et al., 1981). Animals were observed for the appearance of papillomas. Pristane was a more potent tumour promoter in this system than C_{18} n-alkane, one of the most potent of the n-alkanes for tumour promoting activity in this model. n-Tetracosane (C_{24}) was negative.

Cyclic alkanes

Groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 25, 100, or 400 ppm decalin ($C_{10}H_{18}$, decahydronaphthalene, CAS #91-17-8) vapour by whole body inhalation for 6 h plus T90 per day (T90 ≈12 min), 5 days per week 5 days per week for 105 weeks. Decalin in these studies comprised 42:58 cis:trans with a purity of > 99 %. Survival of mice in the exposed groups was similar to that of the controls and was > 50 % in all groups. There were no differences in body weight or body weight gain between the groups. Exposure to decalin resulted in hepatic changes indicative of toxicity in high dose males. These animals showed increased incidences of centrilobular hypertrophy, necrosis and syncytial alteration. The incidence of eosinophilic foci was also increased in this group. Exposure to decalin had no effect on the incidence of either hepatocellular adenoma or carcinoma in male mice. There was no evidence of non-neoplastic liver toxicity in females exposed to decalin. The incidence of hepatocellular adenoma was statistically significantly increased in high dose females, and exceeded that of the historical controls. The incidence of hepatic carcinoma was unaffected by exposure to decalin in females. There was no statistically significant difference in the incidence of uterine stromal polyps in any of the exposed groups of female mice compared to the controls, but there was a statistically significant positive trend with dose (Table 20).

Table 20: Tumour incidence in female B6C3F1 mice exposed by inhalation to decalin (NTP, 2005).

	Exposure concentration (ppm)				Poly-3 test
	0	25	100	400	P value (trend test)
Liver					
Hepatocellular adenoma[1]	7/49 (14 %)	13/50 (26 %)	8/50 (16 %)	17/50* (34 %)	0.024
Hepatocellular carcinoma[2]	4/49 (8 %)	16/50* (32 %)	6/50 (12 %)	5/50 (10 %)	0.115 (negative trend)
Hepatocellular adenoma or carcinoma[3]	11/49 (22 %)	27/50** (54 %)	14/50 (28 %)	20/50 (40 %)	0.339
Uterus					
Stromal polyp[4]	0/49 (0 %)	0/50 (0 %)	2/50 (4 %)	3/50 (6%)	0.049

1: Historical controls: 144/954 (15.9 ± 6.1 %), range: 7 - 28 %;
2: Historical controls: 69/954 (7.8 ± 4.4 %), range 3 - 16 %;
3: Historical controls: 203/954 (22.6 ± 9.1 %), range 9 - 40 %;
4: Historical controls: 15/959 (1.6 ± 2.2 %), range 0 - 6 %;
*P < 0.05; **P < 0.001

There was equivocal evidence that exposure to decalin at high concentrations could increase the incidence of hepatocellular adenomas in female mice. Given the nature of this lesion and evidence that decalin can cause non-neoplastic effects in the liver, it is concluded that this finding is not of toxicological concern at potential levels of human exposure.

Groups of ~6 week old Fischer F344/N rats, 50/sex/group (except 20 males in the 400 ppm group) were exposed by whole body inhalation to decalin, at concentrations of 0, 25, 50, 100 or 400 ppm for

males and 0, 25, 100 or 400 ppm for females, for 6 h plus T90/day (T90 ≈12 min), 5 days per week (other than for holidays) for 2 years. Decalin in these studies comprised 42:58 *cis*:*trans* and had a purity of -101% (Dill et al., 2003b; NTP, 2005).

Survival in the exposed males was slightly lower than that in the controls (40-47 % cf 56 % in controls) other than in the 400 ppm group (70 %). For females, other than in the 400 ppm group (56 % cf 64 % in the controls), survival in the exposed groups was similar to that in the controls. There was little difference in body weight between the groups, other than a slight reduction in 400 ppm males from week 33 onwards (~3-7 % lower cf controls). There were no clinical signs associated with exposure to decalin in either males or females.

Exposure to decalin resulted in a range of effects in the kidneys of male rats, both non neoplastic (Table 21) and neoplastic (Table 22). The incidences of renal tubular adenoma and adenoma or carcinoma (combined) were statistically significantly increased in males exposed to decalin at 50 ppm or greater (Table 22). The incidences of adenoma and adenoma or carcinoma (combined) exceeded the historical control ranges in all exposed groups of males (data from NIEHS, 2002). There was also a slight, not statistically significant, increase in the incidence of renal tubular carcinoma in all exposure groups of males, which nevertheless exceeded the historical control range. In two animals these tumours metastasised to the lung, one in the 25 ppm group and one in the 100 ppm group. Exposure to decalin had no effect on the incidence of renal tumours in female rats. There were no statistically significant differences in the incidences of tumours in any non-renal tissue in either male or female rats exposed to decalin.

All groups of exposed males had increased incidences of renal tubular hyperplasia. Nephropathy was also more severe in the exposed males than in the controls. This was characterised by tubular dilation, proteinaceous tubular casts, atrophy, regeneration and hypertrophy of tubular epithelium, thickening of tubular and glomerular basement membranes, interstitial fibrosis, varying numbers and aggregates of mononuclear inflammatory cells within the interstitium, an increase in the incidence and severity of mineralisation of the renal papilla and an increased incidence of relatively mild hyperplasia of the transitional epithelium lining of the renal pelvis. The incidence of hyaline droplets was increased in exposed groups, reaching statistical significance in the 25 and 100 ppm groups.

Table 21: Non neoplastic changes in male Fischer 344/N rats exposed by inhalation to decalin (NTP, 2005).

	Exposure concentration (ppm)				
	0	**25**	**50**	**100**	**400**
Kidney					
Renal tubule hyperplasia	0/50	11/50**	11/49**	15/50**	5/20**
Renal tubule hyaline droplet accumulation	2/50	9/50*	7/49	11/50*	2/20
Chronic nephropathy, severity	1.4	2.3	2.6	2.3	3.0
Papilla mineralisation	1/50	34/50**	41/49**	43/50**	17/20**
(severity)	(1.0)	(2.4)	(2.9)	(3.1)	(3.3)
Pelvis, transitional	1/50	8/50*	8/49*	10/50**	5/20**
epithelium, hyperplasia	(1.0)	(1.5)	(2.1)	(2.4)	(1.6)
(severity)					

*P < 0.05; **P < 0.001

Table 22: Tumour incidence in male Fischer 344/N rats exposed by inhalation to decalin (NTP, 2005).

	Exposure concentration (ppm)					Poly-3 test
	0	**25**	**50**	**100**	**400**	**P value (trend test)**
Kidney						
Renal tubule adenoma[1]	1/50 (2 %)	2/50 (4 %)	6/49[a] (12 %)	9/50[b] (18 %)	5/20[c] (25 %)	0.05
Renal tubule carcinoma[2]	0/50 (0 %)	1/50 (2 %)	1/49 (2 %)	4/50 (8 %)	1/20 (5 %)	-
Renal tubule adenoma or carcinoma[1]	1/50 (2 %)	3/50 (6 %)	7/49*49[d] (14 %)	12/50**50[e] (24 %)	6/20**20[e] (30 %)	0.002

1: Historical controls: 3/906 (0.9 ± 6.1 %), range: 0-2 %.
2: Historical controls: 0/906.
a: P = 0.058;
b: P = 0.007;
c: P = 0.004;
*P <[d]P = 0.05; **P031; [e]P < 0.001.

There was a significant increase in the incidence of chronic nephropathy in the 100 ppm group females, although given the background incidence of this lesion, the small increase is not considered to be of toxicological relevance [0 ppm decalin, 44/50 (1.6); 25 ppm, 45/46 (1.6); 100 ppm, 48/49* (1.9); 400 ppm, 48/50 (1.6)].

Based on the findings in this study and in an accompanying 13 week study (see Section 7.2.2), it was concluded that the carcinogenic effects of decalin on the renal cortical epithelium of male rats were secondary to increased epithelial cell turnover, resulting from cytotoxicity induced by α_{2u}-globulin accumulation in the cells.

7.2.5.2. Evaluation of carcinogenicity studies of MOAH

PAHs (non-alkylated polycyclic aromatic hydrocarbons)

There is an extensive literature of the carcinogenicity of PAHs, following exposure mainly by the dermal or inhalation route, with more limited information following oral exposure. A number of PAHs, and PAH-containing mixtures are genotoxic and carcinogenic, and there is good evidence that the mode of action for much of their carcinogenicity involves their genotoxic effects. Mineral oils containing PAHs are also genotoxic and carcinogenic. The literature on PAHs has not been reviewed here. Rather, reference to recent reviews by EFSA, JECFA and IARC are provided, together with the most recent IARC classifications (EFSA, 2008d; FAO/WHO, 2006; IARC, 2010).

There is sufficient evidence in experimental animals for the carcinogenicity of BaA, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[k]fluoranthene, BaP, chrysene (CHR), cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene (IP) and (5-MCH).

There is limited evidence in experimental animals for the carcinogenicity of anthanthrene, benz[*j*]aceanthrylene, benzo[*c*]fluorene, benzo[*c*]phenanthrene, dibenz[*a,c*]anthracene, dibenz[*a,j*]anthracene, dibenzo[*a,e*]fluoranthene, 13H-dibenzo[*a,g*]fluorene, dibenzo[*a,e*]pyrene, fluoranthene (FA), 2-methylchrysene (MCH), 3-MCH, 4-MCH, 6-MCH, 2-methylfluoranthene (MFA) and picene.

There is inadequate evidence in experimental animals for the carcinogenicity of acenaphthene, acepyrene (3,4-dihydrocyclopenta[*cd*]pyrene), anthracene, 11H- benz[*bc*]aceanthrylene, benz[*l*]aceanthrylene, benzo[*b*]chrysene, benzo[*g*]chrysene, benzo[*a*]fluoranthene,

benzo[*ghi*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*ghi*]perylene, benzo[*e*]pyrene, coronene, 4H-cyclopenta[*def*]chrysene, 5,6- cyclopenteno-1,2-benzanthracene, dibenzo[*h,rst*]pentaphene, dibenzo[*e,l*]pyrene, 1,2- dihydroaceanthrylene, 1,4-dimethylphenanthrene, FLUO, 1-MCH), 3-MFA, 1-methylphenanthrene, naphtho[1,2-b]fluoranthene, naphtho[2,1-*a*]fluoranthene, naphtho[2,3-*e*]pyrene, perylene, PHEN, PYR and triphenylene.

The NTP has investigated the carcinogenicity of NAP in both rats and mice, following inhalational exposure (Abdo et al., 1992).

Groups of 75 or 150 (high dose) 6-7 week old male and female B6C3F$_1$ mice were exposed to atmospheres containing 0, 10 or 30 ppm NAP (> 99 % pure) for 6 h per day, 5 days per week, for 2 years (103 weeks). Growth and final weights of the exposed mice were very similar to those of the control animals. Survival in control males (38 %) was statistically significantly lower ($P < 0.001$) than in the treated groups (10 ppm, 75 %; 30 ppm, 83 %). This was attributed to less fighting in the exposed animals. Survival in the female groups was 75 – 90 %, and there were no significant differences amongst the groups.

There were no significant differences on the incidences of any tumour type in male mice exposed to NAP. There was a marginal (statistically non-significant) increase in the incidence of alveolar/bronchiolar lesions in exposed males, which might have been a consequence of their better survival compared to controls (alveolar/bronchiolar adenoma: 0 ppm, 7/69 (10 %); 10 ppm, 15/69 (22 %); 30 ppm, 27/135 (20 %); alveolar/bronchiolar carcinoma: 0 ppm, 0/69 (0 %); 10 ppm, 3/69 (4 %); 30 ppm, 7/135 (5 %)). Exposed males showed a statistically significant increase in the incidence of alveolar epithelial hyperplasia. There was a statistically significant increase in the incidence of alveolar/bronchiolar adenomas in females exposed to 30 ppm ($P < 0.01$). There was no difference in the incidence of alveolar/bronchiolar carcinoma in exposed females (alveolar/bronchiolar adenoma: 0 ppm, 5/68 (7 %); 10 ppm, 2/64 (3 %); 30 ppm, 28/134 (21 %); alveolar/bronchiolar carcinoma: 0 ppm, 0/68 (0 %); 10 ppm, 0/64 (0 %); 30 ppm, 1/134 (1 %)). The incidence of alveolar epithelial hyperplasia was slightly increased in exposed females.

The incidence and severity of chronic inflammation was increased in the lungs of male and female mice exposed to NAP. Group of 49 male and female Fischer 344/N rats (4 weeks old) were exposed to atmospheres containing 0, 10, 30 or 60 ppm NAP (> 99 % pure) for 6 h per day, 5 days per week, for 2 years (105 weeks). Growth of exposed males was slightly less than that of the controls, throughout most of the study period. Growth and final body weights of the exposed female groups were very similar to those of the control animals. Survival of all groups was similar, ~ 45-55 %, and there were no significant differences amongst the groups (Abdo et al., 2001).

The incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose were statistically significantly increased in both male and female rats exposed to NAP. The incidences of respiratory epithelial adenoma were: Males - 0 ppm, 0/49 (0 %); 10 ppm, 6/49 (12 %); 30 ppm, 8/48 (17 %); 60 ppm, 15/48 (31 %) [P=0.001 for trend; significantly increased in all exposed groups]; Females - 0 ppm, 0/49 (0 %); 10 ppm, 0/49 (0 %); 30 ppm, 4/49 (8 %); 60 ppm, 2/49 (4 %) [trend not statistically significant; historical control incidence = 0 %]. The incidences of olfactory epithelial neuroblastoma were: Males - 0 ppm, 0/49 (0 %); 10 ppm, 0/49 (0 %); 30 ppm, 4/48 (8 %); 60 ppm, 3/48 (6 %) [P=0.027 for trend; incidences in exposed groups cf controls not statistically significant]; Females - 0 ppm, 0/49 (0 %); 10 ppm, 2/49 (4 %); 30 ppm, 3/49 (6 %); 60 ppm, 12/49 (24 %) [P=0.001 trend; 60 ppm group cf controls, $P < 0.01$].

In general, the incidences and severity of hyperplasia of the respiratory epithelium and chronic inflammation of the lung were significantly increased in rats exposed to NAP. Even at the lowest concentration tested, the incidences were almost 100 %, compared with a negligible incidence in the controls.

Alkylated aromatic hydrocarbons

Groups of 6 week old B6C3F1 mice, 50 per sex per group, were exposed 1-MNAP (purity > 97 %) in their diet for 81 weeks, at concentrations of 0, 750 and 1 500 mg/kg (Murata et al., 1993). Body weight gain in both high dose males and females was reduced slightly compared to controls after week 10, but had recovered by the end of the study. Exposure to 1-MNAP had no effect on food consumption. Only two animals died during the course of the study, one female from the 0.15 % group and one male from the control group, both from leukemia.

There was a statistically significant increase in the incidences of lung adenomas in males exposed to either 750 mg/kg (13/50 cf 2/49 in controls [$P < 0.05$]) or 1 500 mg/kg (12/50 [$P < 0.05$]) 1-MNAP in their diet. There was a slight, non-significant increase in the incidence of adenocarcinoma in the high concentration group (0 mg/kg 1-MNAP, 0/49; 750 mg/kg, 0/50; 1 500 mg/kg, 3/50). The incidences of these tumours in females were not influenced by treatment. All lung tumours were bronchiolar/alveolar adenomas or carcinomas. It was noted that there was no dose-dependency for the effect on adenomas in males and that the incidence of carcinomas was not significantly affected.

There were no increases in the incidences of any other tumour type in either males or females exposed to 1-MNAP in their diet. If anything, the incidences of hepatic adenoma and carcinoma were lower in both groups of treated males compared to the controls, but the differences were not statistically significant.

Groups of 6 week old B6C3F1 mice, 50 per sex per group, were exposed 2-MNAP (purity > 97 %) in their diet for 81 weeks, at concentrations of 0, 750 mg/kg and 1 500 mg/kg (Murata et al., 1997). Body weight gain in both high dose males (- 7.5 % cf controls) and females (- 4.5 %) was reduced slightly compared to controls after week 10, and was still lower in males, but not females, at the end of the study ($P < 0.001$). Exposure to 2-MNAP had no effect on food consumption. Exposure to the test article had no effect on survival of the animals. Overall mortality throughout the study was very low.

The incidences of lung adenomas and of lung adenocarcinomas in the treated groups were not statistically significantly different from those in the control groups. (Adenomas – males: 0 mg/kg 2-MNAP, 2/49; 750 mg/kg, 9/49; 1 500 mg/kg, 5/49. Females: 0 mg/kg 2-MNAP, 4/50; 750 mg/kg, 4/49; 1 500 mg/kg, 5/48. Adenocarcinomas – males: 0 mg/kg 2-MNAP, 0/49; 750 mg/kg, 1/49; 1 500 mg/kg, 1/49. Females: 0 mg/kg 2-MNAP, 1/50; 750 mg/kg, 0/49; 1 500 mg/kg, 1/48). However, the combined incidences of lung adenomas and adenocarcinomas were statistically significantly increased in male mice exposed to 750 mg/kg 2-MNAP in the diet (Adenomas plus adenocarcinomas – males: 0 mg/kg 2-MNAP, 2/49; 750 mg/kg, 10/49 [$P<0.03$]; 1 500 mg/kg, 6/49. Females: 0 mg/kg 2-MNAP, 5/50; 750 mg/kg, 4/49; 1500 mg/kg, 6/48). All lung tumours were bronchiolar/alveolar adenomas or carcinomas.

There were no increases in the incidences of any other tumour type in either males or females exposed to 2-MNAP in their diet. The incidences of hepatic adenoma and carcinoma in males were lower in both groups treated with 2-MNAP compared to the controls, but the differences were not statistically significant.

Groups of 50 - 55 day old female outbred Crl:CD/l (ICR)BR mice, 20 per group, were treated topically with a range of methylated derivatives of PHEN in acetone, followed by promotion with 12 O-tetradecanoylphorbol-13-acetate (TPA). Mice received 10 doses of the hydrocarbons, once every other day followed, 10 days later, by thrice weekly doses of TPA for 20 weeks (LaVoie et al., 1981, 1982).

1,4-Dimethylphenanthrene was a relatively potent tumour initiator in this model. 4,10-Dimethylphenanthrene was also carcinogenic, but was less potent than

1,4-dimethylphenanthrene. 1,9-Dimethylphenanthrene, 2,7-dimethylphenanthrene, 3,6-dimethylphenanthrene, 4,5-dimethylphenanthrene, 4,9-dimethylphenanthrene, 2,4,5,7-tetramethylphenanthrene and 3,4,5,6-tetramethylphenanthrene were all non-carcinogenic in this study. No cutaneous tumours were found in mice treated with any of the monomethylphenanthrene derivatives, i.e. 1-, 2-, 3-, 4- or 9-methylphenanthrene.

Groups of 7-week old female CD-1 mice, 30 per group, were treated topically with various doses of a range of monomethylated derivatives of anthracene in acetone, followed by promotion with TPA. Mice received a single dose of the hydrocarbons followed, 1 week later, by twice weekly doses of TPA for 21 weeks (Wislocki et al., 1982).

In this model, DMBA was a strong initiator, producing multiple tumours in almost all of the mice. In comparison, 7-methylbenzanthracene (MBA) was a moderate initiator. 8-MBA, 12-MBA, 6-MBA and 9-MBA were weak initiators, the 8- and 12- analogues being slightly more potent than the other two. BaA and 1-MBA were very weak initiators. Although the incidences of papillomas in mice treated with 2-, 3-, 4-, 5-, 10- and 11-MBA were greater than that in the solvent controls, these did not reach statistical significance suggesting that if these compounds can act as initiators, there are of very low potency in comparison to the other compounds tested. In this model, the incidence of papillomas in mice treated with 9,10-dimethylanthracene (diMA) was statistically significantly different from that in the controls (controls, 23 %, 0.23 tumours/mouse; 9,10-diMA 400 nmol, 27 %, 0.33 tumours/mouse; 9,10-diMA 1000 nmol, 30 %, 0.33 tumours/mouse).

In another study by LaVoie et al. (1985), the same protocol as used to investigate the tumour initiating effects of dimethylated phenanthrenes (LaVoie et al., 1982) was used to study the carcinogenicity of methylated derivatives of anthracene.

The incidences of cutaneous tumours in mice treated with anthracene and its mono-methyl derivatives (1-, 2- and 9-methylanthracene (MA)) were not significantly different from that in the controls. (control (repeated studies): 10 %, 5 %; anthracene: 15 %; 1-MA: 0 %; 2-MA: 10 %; 9-MA: 20 %). Treatment with 9,10-diMA, 2,9,10-triMA and 2,3,9,10-tetramethylanthracene (tetraMA) resulted in statistically significant increases in the incidence of cutaneous tumours in mice (9,10-diMA, 50 %; repeat with n = 40, 35 %; 2,9,10-triMA, 56 %; 2,3,9,10-tetraMA, 24 %). Tumour multiplicity was also increased by all of these treatments. The incidence of tumours in mice treated with 2,9-diMA (12 %) was similar to that in controls. It was noted that in this model, BaP was an order of magnitude more potent than 9,10-diMA.

The authors point out that in the previous study of Wislocki et al. (1982), in which 9,10-diMA was not carcinogenic, mice received only a single, lower dose of 9,10-diMA than in the present study.

A commercial preparation of dodecylbenzene comprising C_{10}-C_{15} (predominantly C_{12}) branched chain alkylbenzenes, provided by Exxon Chemicals, Belgium (Iversen, 1989), identification No. MRDE-7, was administered topically to groups of 28 male and 28 female hairless hr/hr Oslo strain mice, 60-90 days of age, twice per week for 80 weeks at concentrations of 16 and 80 % in acetone. Groups of 24 males and 24 females were treated with a single dose of 51.2 µg DMBA as a positive control and then either received no further treatment or were treated, starting one week later, with 40 % dodecylbenzene, twice per week. Administration was to the middle of the back. A concurrent negative control group of 28 male and 28 female mice was treated with acetone alone.

Survival in all groups after 80 weeks was 70-90 %, the slightly lower survival rates being seen in the groups that received 7,12-DMBA. No information was provided on the growth or on the terminal body weights of the animals.

In the experience of the authors, the background incidence of skin tumours in the strain of mouse used was zero. Treatment with 16 % dodecylbenzene had no statistically significant effect on the incidence

either of malignant skin tumours or of all skin tumours. The group painted with 80 % dodecylbenzene showed no statistically significant difference in the incidence of malignant tumours in comparison to control, but there was a statistically significant increase in the incidence of all skin tumours in this group. Animals treated with 7,12-DMBA had a markedly greater incidence of both malignant and all skin tumours, compared with the vehicle treated controls. There was some indication, not statistically significant, that administration of 40 % dodecylbenzene for 78 weeks enhanced the tumourigenicity of 7,12-DMBA to the skin. Tumour latency, both malignant and total, was markedly reduced in the groups treated with DMBA.

Incidences of all skin tumours (relative to a group size of 56) (no of tumours/no of affected animals):

- Acetone (negative control): 3/2; 16 % dodecylbenzene: 4/4; 80 % dodecylbenzene: 13/9 ($P < 0.05$); 7,12-DMBA: 62/27 ($P < 0.001$); 7,12-DMBA + 40 % dodecylbenzene: 79/26 ($P < 0.01$) [7,12-DMBA cf 7,12-DMBA + dodecylbenzene, $P > 0.05$]

Incidences of all skin malignancies (relative to a group size of 56) (no of tumours/no of affected animals):

- Acetone: 0; 16 % dodecylbenzene: 2/2; 80 % dodecylbenzene: 1/1; 7,12-DMBA: 6/6 ($P < 0.01$); DMBA + 40 % dodecylbenzene: 13/13 ($P < 0.01$) [7,12-DMBA cf 7,12-DMBA + dodecylbenzene, $P > 0.05$]

There were no statistically significant differences in tumour incidences at any other site in the animals, following either 7,12-DMBA or dodecylbenzene.

Treatment of mice with dodecylbenzene caused dermal hyperplasia, that was more pronounced in the groups treated with 40 % or 80 %. In these groups, there were also signs of skin ulceration. 7,12-DMBA alone had no such effects.

Partially hydrogenated aromatic hydrocarbons

Groups of 50 male and 50 female B6C3F1 mice, 5-6 weeks of age at the start of the study, were exposed to tetralin (> 98 % purity) by whole body inhalation at concentrations of 0, 30, 60 and 120 ppm, 6 h + T90/day (T90 = 12 min), 5 days per week for 105 weeks (NTP, 2009). Survival in all groups was > 50 % at the end of the study, that in the 60 and 120 ppm females being statistically significantly greater than in the controls (84 and 86 %, respectively compared with 62 %). Exposure to tetralin had no consistent effect on body weight or body weight gain in males. In the 60 and 120 ppm groups there was a slight retardation in weight gain in females, although there was no difference in terminal weights. There was a statistically significant trend ($P<0.05$) for an increase in the incidence of hemangiosarcomas of the spleen in female mice. The incidence was elevated only in the high dose group (4/50 compared to 1/50 in controls) and although this was not statistically significant ($P = 0.239$) it did exceed the historical control values (mean ± SD: 1.5 % ± 1.4 %, range 0-4 %, calculated by NTP from previous studies). The incidences of all other tumour types were similar in mice exposed to tetralin to those in the controls.

Groups of 50 male and 50 female Fischer 344/N rats, 5-6 weeks of age at the start of the study, were exposed to tetralin (> 98 % purity) by whole body inhalation at concentrations of 0, 30, 60 and 120 ppm, 6 h + T90/day (T90 = 12 min), 5 days per week for 105 weeks (NTP, 2009). Survival in all groups was similar, and was ≥50 % except in male controls (40 %). Body weight and body weight gain in exposed groups were similar to those in the controls, other than in high dose females where there was a reduction in body weight of between 5-10 % from week 29. There was a statistically significant trend ($P < 0.02$) for an increased incidence of renal cortical adenomas in male rats. The overall incidences (survival-adjusted rates reported in parentheses) were: 0/50 (0 %) in control, 3/50 (7.0 %) at 30 ppm [P=0.134], 2/50 (4.9 %) at 60 ppm [P=0.0244], 6/50 (13.9 %) at 120 ppm

[P=0.020]. The incidence at 120 ppm exceeded the historical control values (mean ± SD: 0.6 % ± 1.0 %; range: 0 %-2 %). The occurrence of adenomas in the high dose group was accompanied by an increased incidence of renal cortical hyperplasia.

There was a statistically significant trend (P = 0.01) for an increased incidence of renal cortical adenomas in female rats. The overall incidences in controls, 30 ppm and 60 ppm groups were: 0/50 (adjusted rate 0 %). At 120 ppm the overall incidence was 3/50 (6.5 %) [P=0.131]. The incidence in the high dose group exceeded the historical control values (0 %, 0/350).

There was a statistically significant trend (P < 0.01) for an increased incidence of uterine stromyl polyps in female rats. The overall incidences (survival-adjusted rates reported in parentheses) were: 6/50 (13.8 %) in controls, 10/50 (22.2 %) at 30 ppm [P=0.228], 9/50 (19.8 %) at 60 ppm [P=0.318], 17/50 (36.5 %) at 120 ppm [P=0.011]. The incidence in the high dose group exceeded the historical control values (mean ± SD: 18.9 % ± 5.9 %, range: 12 %-26 %, calculated by NTP from previous studies).

Sulphur-containing compounds

Jacob (1990) has reviewed early work on the carcinogenicity of a series of 4- and 5-ring thioarenes. Most of these studies were on compounds that were isosteric to 7,12-DMBA, DBahA, 7,14-dimethylbenz[*a*,*h*]anthracene, dibenz[*a*,*j*]anthracene, 7,14-dimethylbenz[*a*,*j*]anthracene, BcP, CHR and benzo[*c*]chrysene. Very few methodological details are provided, but it is apparent that the compounds were administered to mice, mostly by dermal application. No absolute potencies are provided, but rather potency of the thioarenes is compared to that of the corresponding hydrocarbons.

A summary of the observations follows (from Jacob, 1990):

Hydrocarbon	Potency	Thioarene isostere	Potency
7,12-Dimethylbenz[*a*]anthracene	+++	7,11-dimethylphenanthro[2,3-*b*]thiophene	++
		6,11-dimethylanthra[1,2-*b*]thiophene	++
		6,11-dimethylbenzo[*b*[naphtha[2,3-*d*]thiophene	+
Dibenz[*a*,*h*]anthracene	+++	benzo[*b*]phenanthro[2,3-*d*]thiophene	-
		benzo[1,2-*b*: 4,5-*b′*]bis[1]benzothiophene	-
7,14-Dimethylbenz[*a*,*h*]anthracene	+	7,13-dimethylbenzo[*b*]phenanthro[2,3-*d*]thiophene	+
		6,12-dimethylbenzo[1,2-*b*: 4,5-*b′*] bis[1]benzothiophene	++
Dibenz[*a*,*j*]anthracene	++	dinaphtho[2,1-*b*: 1′,2′-*d*]thiophene	+
		benzo[*b*]phenanthro[3,2-*d*]thiophene	?
7,14-Dimethylbenz[*a*,*j*]anthracene	+	7,13-dimethylbenzo[*b*]phenanthro[3,2-*d*]thiophene	++
		6,12-dimethylbenzo[1,2-*b*: 4,5-*b′*]bis[1]benzothiophene	++

Benzo[*c*]phenanthrene	+/++	naphtho[2,1-*b*; 7,8- *b′*]dithiophene	-
		benzo[*b*]naphtho[1,2-*d*]thiophene	-
		[1]benzothieno[2,3-*b*][1]benzothiophene	-
Chrysene	+	[1]benzothieno[3,2-*b*][1]benzothiophene	-
		benzo[*b*]naphtho[2,1-*d*]thiophene	-/+
		naphtho[2,1-*b*; 6,5- *b′*]dithiophene	-
Benzo[*c*]chrysene	++	dinaphtho[1,2-*b*: 1′,2′-*d*]thiophene	+
		benzo[*b*]phenanthro[3,4-*d*]thiophene	++

Notes: +++ = very potent; ++ = potent; + = weak of moderately potent; - = inactive; more than one symbol indicates results from contradictory studies

The carcinogenicity of benzo[*b*]naphtho[2,1-*d*]thiophene, an isostere of the weak carcinogen chrysene, in mouse skin painting experiments was equivocal. In another study, the potencies of the compounds were comparable when injected into the lungs of rats (unpublished results from Brune and Deutsch-Wenzel, 1986, cited in Jacob, 1990).

Croisy et al. (1984) synthesised a series of thiophene analogues of carcinogenic polycyclic aromatic hydrocarbons and determined their carcinogenic potential. Very few methodological details are provided. Groups of XVII nc/Z strain mice (number not stated) were administered 0.6 mg of the test substances in 0.2 ml neutral olive oil by subcutaneous injection at 4-weekly intervals. Animals were terminated on the appearance of large tumours or after 700-880 days, whichever came sooner.

Benzo[*b*]naphtha[2,1-*d*]thiophene, benzo[*b*]phenanthro[3,4-*b*]thiophene, benzo[*b*]phenanthro[3,2-*d*]thiophene and benzo[*b*]phenthanthro[2,3-*d*]thiophene were more carcinogenic to mouse skin than the analogous hydrocarbons, respectively CHR, benzo[*e*]chrysene, dibenz[*a*,*j*]anthracene and DBahA. Indeed, benzo[*b*]phenanthro[3,4-*b*]thiophene was as potent as BaP. These results are not entirely consistent with those cited by Jacob (1990).

Croisy et al. (1984) synthesised a number of other thiophenes, i.e. 2-[2-methyl-1-naphthoyl]benzo[*a*]thiophene, 3-[2-methylbenzoyl]benzo[*b*]thiophene, 3-[2-methyl-1-naphthoyl]benzo[*b*]thiophene, benzo[*b*]naphtha[2,3-*d*]thiophene, 6-hydroxybenzo[*b*]naphtha[2,3-*d*]thiophene, 1-hydroxybenzo[*b*]naphtha[2,3-*d*]thiophene and benzo[*b*]phenanthro[2,1-*d*]thiophene. It is not clear whether these were tested for carcinogenicity and were negative or were not tested at all.

Jacob (1990) noted that in general there is no simple correlation between the carcinogenic potential of thioarenes and their carbocyclic isosteres.

7.2.6. Immunotoxicity

Animal experiments

No validated animal models for assessing or identifying chemicals that induce or exacerbate autoimmune diseases are currently available. Reports on an autoimmune potential of high dose mineral oil exposures to animals, mainly by the injection route, are shortly summarised below.

A series of experiments in mice have demonstrated that one single dose of 0.5 ml pristane (C_{19}) administered intraperitoneally induce lipogranulomas, and also autoantibodies and clinical signs relevant for human systemic lupus erythematosus (SLE) (reviewed by Reeves et al., 2009). Months (> 6) after exposure, female mice are reported to have increased production of autoantibodies to nuclear components (like anti-Sm, -RNP, -dsDNA, -ssDNA, -chromatin, -Su and -ribosomal P).

Furthermore, clinical manifestations of SLE, including arthritis, immune complex-mediated glomerulonephritis with proteinuria and pulmonary capillaritis had developed in Balb/c and SLJ mice. Although there are strain differences, both autoantibodies and clinical signs of lupus have been reported in several mouse strains not prone for autoimmunity, like Balb/c, SJL and weaker in C57BL/6 (Shaheen et al., 1999; Summers et al., 2010). Under the same experimental conditions, a number of mineral oils acted as adjuvants by promoting elevated concentrations of autoantibodies. While injection of 0.5 ml n-hexadecane, squalene or incomplete Freund's adjuvant induced autoantibody responses to nuclear antigens in female Balb/c mice, medicinal mineral oils (MO-HT, MO-S (C_{20}-C_{40}), MO-F (C_{15}-C_{40})) induced lipogranulomas, but only selected anti-nuclear autoantibodies and no clinical manifestations (Satoh, 2003; Kuroda et al., 2004, Nacionales et al., 2006).

Another autoimmune endpoint, i.e. joint-specific arthritis, has been reported to be induced in Dark Agouti (DA) rats by intradermal injection of 0.1 - 0.5 ml of mineral oils (i.e. squalene, pristane, medicinal white oils, as well as baby oils, cosmetic products containing mineral oils, incomplete Freund's adjuvant) or i.p. injection of 0.5 ml of pristane or incomplete Freund's adjuvant (Sverdrup et al., 1998; Holmdahl et al., 2001; Holm et al., 2002; Laragione et al., 2011; Zhu et al., 2011). When one of the intradermally potent arthritis-inducing oils was administered percutaneously, 5 of 10 DA rats (and 0 of 10 control animals) showed a mild and transient arthritis. On the other hand, 5 daily gavages of 500 µl of an intradermally potent white mineral oil did not give any arthritic reactions (Sverdrup et al., 1998). The oil-induced arthritis appear not to develop due to local irritation or oil accumulation in joints, but rather through effects on draining lymph node T-cells, cells which can induce arthritis after adaptive transfer to naïve rats. Lymph node histology after intradermal injection with a baby oil showed increased number of cells, diffuse germinal centres and large round vesicles (probably oil droplets) in the marginal zone (Sverdrup et al., 1998). The amount in the lymph nodes, however, was not closely linked to the appearance of pathogenic cells (Holm et al., 2002). Only DA rats are susceptible to oil-induced arthritis (Cannon et al., 1993; Carlson et al., 2000), and this genetic susceptibility have been associated with a leucocyte-expressed gene complex, the corresponding gene complex in humans may be associated with rheumatoid arthritis (Lorenzen et al., 2007). Fourteen of 27 strains of mice were demonstrated to be susceptible to pristane-induced (0.5 ml, i.p. injection) arthritis and rheumatoid factor seropositivity (Wooley et al., 1989; Carlson et al., 2000).

Major limitations for these reports on autoimmunity induced by mineral oils in animal models are the application of high doses with a lack of dose-response studies, via injection and percutaneous routes. It is not known whether the results of these studies have relevance for chronic exposure to mineral oils via the oral route. Although short term peroral feeding did not give any arthritic reactions in DA rats, the authors speculate that oral administration of adjuvants in conjunction with inflammation of the gut caused by some other agent could parallel the arthritic effects of percutaneous exposure on abraded skin (Sverdrup et al., 1998). Incomplete Freund's adjuvant (containing pristane and n-hexadecane), which is a known adjuvant previously used in human and animal injection vaccines (Cox and Coulter, 1997), have been reported to not demonstrate any significant adjuvant effect after oral co-administration with antigen (Silin et al., 2007).

Due to uncertainty about the long-term significance of the histiocytosis (microgranulomas) observed in lymph nodes after orally administered mineral oils to Fischer 344 rats, a few studies have also included immune-related outcomes. After 90 days of dietary exposure to LMPW, increased serum concentrations of IgA, IgM and IgG2c, but reduced concentrations of IgG and IgG2a in female Fischer 344 (but not Sprague Dawley) rats were reported (Burleson et al., 2001). Changes in non-specific antibody levels alone are generally equivocal evidence of immunotoxicity. Immune function was investigated after dietary exposure to P15(H) for 90 days. Exposure did not result in any effect on serum levels of specific IgM and IgG antibodies specific after immunisation with dinitrophenol-human serum albumin in Fischer 344 rats. After sheep-red-blood-cell (SRBC) injection, the number of SRBC-specific IgM-producing splenocytes were dose-dependently reduced by up to 40 % when expressed per million cells (in Fischer 344 but not in Sprague Dawley). However, since no effect was

seen when expressed on the basis of total spleen activity due to the increased spleen cell numbers, primary immune response to SRBC was considered non-affected in this study. As stated by WHO/IPCS (2003), data on spleen cell types or histopathology examinations, which is lacking in this study, could alter the interpretation (ImmunoTox, 2001).

Overall, there is inadequate evidence in humans and in animal models for the promoting or exacerbating effect of relevant mineral oil exposure on autoimmune diseases, and so far no indications of altered immune function or autoimmunity after peroral exposures. The presence, however, of epidemiological studies, as well as the demonstration of autoimmunity-promoting properties of mineral oils after injection (and percutaneous administration on abraded skin) in various animal species and strains, may raise a concern that mineral oil ingestion can contribute to immune modulation and subsequent autoimmune responses in susceptible humans. Further human and animal studies should address exposures via the oral route of various mineral oils and the putative association with systemic autoimmune diseases, also investigating the dose-response relationship.

Human studies

No studies in humans investigating immunotoxic effects of oral exposure to mineral oils have been identified.

A few epidemiological studies have, however, reported an association between exposure to high doses of mineral oils and increased risk to develop autoimmune diseases. First, a population-based case-control study of incident cases of rheumatoid arthritis (RA) was performed in Sweden during 1996-2003 (Sverdrup et al., 2005). The 1419 cases and the 1674 age-, gender- and residential area-matched controls answered a questionnaire regarding life style factors and occupational exposures, including different types of mineral oils (cutting fluid, motor oil, hydraulic oil, form oil, asphalt, any mineral oil). Sera were investigated for rheumatoid factor (RF) and anti-citrulline containing peptides (anti-CP) antibodies. Only men reported substantial occupational exposure to mineral oils (n=135 cases and 132 controls, exposure concentrations not stated). Such occupational exposure is primarily via inhalation and skin exposures. Among men, exposure to any mineral oil was associated with an increased relative risk of developing RA (risk ratio (RR) = 1.4, 95 % confidence interval (CI) = 1.0 - 1.7). When stratifying for RF^+/RF^- or anti-CP^+/anti-CP^- cases, increased risk was only significant for the RF positive or anti-CP positive populations: RF + RA (RR = 1.4, CI = 1.0 - 2.0 any mineral oil, RR = 1.5, CI = 1.0 - 2.3 hydrolic oil) or anti-CP + RA (RR = 1.6, CI = 1.1 - 2.2 any mineral oil, RR = 1.7, CI = 1.1 - 2.6 hydraulic oil).

In a community comparison study, the population living near an oil field waste site had a significantly higher prevalence of RA and SLE than an unexposed community population (odds ratio OR=10.78 and 19.33, respectively; Dahlgren et al., 2007). The study design, recruitment of participants involved in litigations, limited characterisation of exposures and the impossibility to distinguish the possible effects of mineral oils versus mercury, however, limits the impact of the study. The exposed community residents were reported to have higher concentrations of pristane in house dust, higher ambient air mercury concentrations, and a higher proportion of individuals having detectable blood concentrations of pristane, phytane and/or pristanic acid. All subjects with detectable concentrations of pristane or phytane compounds had diagnosis of lupus or common symptoms associated with immune system disorders. Also immunological blood parameters differed significantly between control and exposed subjects.

To what degree occupational exposures, mainly via inhalation and skin, are of relevance for ingestion of mineral oils is not known.

7.3. Observations in humans

Epidemiological studies

Sections of over 200 livers and 4 000 spleens were subjected to histological examination for the presence of oil droplets by Boitnott and Margolis (1970). No information on possible exposure to mineral oil hydrocarbons was given in the publication. Focal collections of vacuoles of diameter ranging from several μm to over 100 μm were frequently observed. The authors reported that often the smaller vacuoles were found to be within macrophages, whereas multinuclear giant cells were sometimes associated with large vacuoles. In the spleen, the vacuoles were generally observed near the periphery of the splenic follicles and close to trabeculae and blood vessels. In the liver, the portal areas were mainly interested by the presence of vacuoles. In several cases, clusters of vacuoles and macrophages were observed in the hepatic parenchyma. As described in Section 7.1.2.1, analytical determination of the MOH content was undertaken in the spleen and the livers of 63 subjects (4 children and 59 adults) at the time of autopsy. A correlation between the MOSH content and the extent of oil droplets observed in the histological examinations was observed both for liver and spleen. In 26 subjects for which both organs were analysed, a good correlation was also showed for the MOSH content in liver and spleen.

Dincsoy et al. (1982) reported the result of the evaluation of hepatic lipogranulomas in 824 liver biopsies carried out between 1978 and 1980. In total, 76 granulomas were observed of which 48 were lipogranulomas. 38 of the 48 lipogranulomas were observed in non-fatty livers, indicating a lack of association between these lipogranulomas and liver steatosis. A review of 240 biopsies carried out in the period 1952-53 showed a significantly lower incidence of lipogranulomas in comparison with 1978-80 (1.7 % versus 4.6 %). Several lipogranulomas were also observed in six autopsy livers (range of 1-12 lipogranulomas/liver, with an average number of four). Twenty-six out of 44 cases showing lipogranulomas in non-fatty livers (38 biopsies and 6 autopsies) presented a series of different liver diseases, without a clear correlation with the incidence of lipogranulomas. Extracts of the autopsy livers were also analysed by means of thin layer chromatography (TLC) and gas-liquid chromatography (GLC), as previously done by Boitnott and Margolis (1970), and the presence of MOSH of similar composition with commercial white oil grades was observed. The authors concluded that the incidence of lipogranulomas in non fatty livers is likely associated to the exposure to MOSH. As only one of the 38 patients with lipogranulomas in non-fatty livers declared the use of MOSH as laxatives and no other possible sources of exposure were identified, the authors explained the exposure with the occurrence in food and correlated the lower incidence of lipogranulomas observed in 1952-53 with the use of mineral oils by the food industry, permitted only after 1962.

Cruickshank (1984a) analysed the spleens obtained from more than 500 autopsies performed on 1970-71 in Toronto, Canada, and from 74 centres in 41 different countries. Moreover, spleen samples obtained from autopsies of white men performed in Toronto and Richmond (Virginia, US) in 1946, 1955 and 1970 were compared. The spleen samples were analysed for the occurrence of follicular lipidosis (i.e. presence of large lipidic droplets with predominant site in the layer of small lymphocytes of follicles). Spleen content of mineral oils was analysed in six samples by TLC and GLC. The mineral oil content was lower in three samples scored negative for follicular lipidosis (0.1 - 0.3 mg/g tissue) in comparison to that in three samples scored with high severity follicular lipidosis (3.4 – 4.2 mg/g tissue). Although the presence of mineral oils was confirmed only on those samples the author, based on existing literature, assumed that the presence of mineral oils was responsible for the presence of follicular lipidosis. The occurrence of follicular lipidosis was correlated with the age and the geographic origin of the samples. The results indicated an incidence of 75 % follicular lipidosis in subjects older than 11 year. A significantly higher incidence in each age class was observed in males in comparison to females. The medicinal use of mineral oil was confirmed in a minority of subjects indicating a low probability to represent the cause of the follicular lipidosis incidence. Increasing trends in the incidence of follicular lipidosis was observed both in the Canadian and US samples from 1970, 1955 and 1946 respectively. Finally, the study indicated higher

incidences in North America and Australia in comparison to UK and continental Europe. In a subsequent publication, Cruickshank (1984b) examined samples of liver, spleen bone marrow and lymph nodes from eight anatomic sites obtained from 100 autopsies performed on 1970-71. Follicular lipidosis was observed in the spleen, with an incidence similar to the previous study (approximately 80 % over all cases). In lymph nodes, lipidosis was observed at high incidence and severity in the porta hepatis, mesenteric and coeliac sites, whereas lower incidences were observed in the mediastinal and para-aortic, cervical and interior iliac lymph nodes. A lower incidence of lipidosis was found to occur in the liver (approximately 40 % over total cases) and bone marrow (approximately 25 % over total cases) in comparison to spleen.

Wanless and Geddie (1985) reported the results of 465 autopsies performed between 1974 and 1976. Lipogranunlomas were observed in liver and spleen, at an incidence of 48 % and 46 % of all cases, respectively. A strong association was found between the presence of lipogranulomas in liver and spleen of the same subjects. A correlation of the incidence and severity of lipogranulomas with the age was observed in the liver but not in the spleen. No correlation was found between the incidence of lipogranulomas and the occurrence of liver steatosis, diabetes mellitus or obesity. No correlation was observed between the incidence of lipogranulomas and any liver disease.

The incidence of hepatic lipogranulomas in patients with chronic liver diseases was recently studied by Zhu et al. (2010). The authors reviewed 376 liver biopsy samples from patients affected by different diseases including hepatitis C virus, hepatitis B virus, fatty liver, primary biliary cirrhosis and autoimmune hepatitis. Lipogranulomas were identified in 15.4 % of the patients and resulted significantly higher in patients with hepatitis C and fatty liver disease in comparison to controls (2.4 - 5 %, reported from Dellatesima et al., 1987 and from Scheuer and Lefkowitch, 2006).

Lagana et al. (2010) recently reviewed the occurrence and pathology of granulomas in human liver. They concluded that the underlying incidence of granulomas, including those caused by mineral oil, in unselected liver biopsies was 4%, which is lower than the incidence reported in some earlier studies. The underlying causes for the majority (90-95%) of these granulomas have been established. The most common cause in developed countries is noninfectious immunological insult. The CONTAM Panel concluded that whilst dietary exposure to MOH could result in the formation of lipogranulomas in the liver and other tissues of humans, the current incidence is very low and that these do not appear to have any adverse consequences.

Cases of intoxication associated with intake of MOSH

The liver of a 33 year old male patient who had undergone a vagotomy operation was found to be enlarged and irregular (Blewitt et al., 1977). The patient had an anamnesis including a treatment against dyspepsia with one tablespoon of liquid paraffin per day, possibly over a period of approximately two months in the year before the operation, and presented no liver dysfunction at the time of the operation. Two liver biopsies and an enlarged lymph node were taken from the porta hepatis. Both liver biopsies revealed pale areas. At microscopical examination, the pale area in one biopsy revealed a fibrotic portal area characterised by an enlarged bile duct and cellular inflammation and proliferation in the blood vessel endothelium (endarteritis obliterans). Other portal areas in both biopsies revealed cystic spaces and vacuolated hystiocytes infiltrated by chronic inflammatory cells and showing increased fibrous tissues and bile ductule proliferation. Cystic spaces and vacuolated hystiocytes had similar morphology and staining (i.e. no or low staining with osmium tetroxide, a specific staining agent for lipids) to those reported by Boitnott and Margolis (1970) and associated with the presence of mineral oil hydrocarbons. The microscopical examination of the enlarged lymph node revealed the presence of lymphoid follicles with prominent germinal centres. Presence of hystiocytes, erythrocytes and lymphocytes and deposits of haemosiderin were noted in the dilated medullary sinuses. Cystic spaces associated with vacuolated hystiocytes were observed in the cortex of the lymph node. The lipids extracted from the liver biopsies were analysed by TLC and produced one spot with similar retention factor as liquid paraffin. The authors noted that beside the

pharmacological treatment, other sources of exposure, including the dietary and occupational exposure, may have contributed to the presence of MOH in the liver of the patient.

Salvayre et al. (1988) reported a case of a 55 year old man who died because of ventricular fibrillation. The authors reported that the man used to eat approximately one kilogram of unpeeled apples every day, which would correspond to an intake of about 10 mg of natural long chain n-alkanes per day. Chest X-ray radiography showed the presence of micronodules in the axillary and apical lung areas. At autopsy, histological examination revealed the presence of granulomas with giant macrophagic cells containing crystalline inclusions of material which dissolved with organic solvent (methanol/chloroform 2:1 v/v) in the lung micronodules of the patient. Lipid analyses of various tissues indicated high concentrations of C_{29}-C_{33} n-alkanes (see Section 7.1.2.1). The authors concluded that dietary intake was responsible for the presence of n-alkanes in the body, most likely due to some inborn error.

In another publication, Nochomovitz et al. (1975) reported the case of a 54 year old man who had ingested large amount of liquid paraffins over many years for laxative purposes. The subject, described by the authors as having "obscure, irrational dietary habits", had a medical history characterised by repeated basal respiratory infections, was found in a comatose status and died after 24 hours of hospitalisation. At the autopsy, severe changes were observed in the liver, spleen, small intestine, abdominal lymph nodes and lungs. The liver was described as small, pale and rubbery at macroscopic examination. Histological analysis revealed an extensive disruption of the hepatic architecture by deposition of oil droplets of variable size and shape. A negligible inflammatory reaction to the oil droplets was generally observed, with the exclusion of the areas in which the droplets caused severe tissue lesions. In those areas, scattered lymphocytic infiltration and irregular fibrotic response were observed. Similar lesions with minor inflammatory responses were noted also in the spleen, small intestine and mesenteric and porta hepatic lymph nodes. A wider variety of lesions were observed in the lungs, including extensive lipid deposition. Those consisted in generally more severe inflammation related changes, including granulomatous hystiocytes typical of lipid pneumonia and presence of fibrous tissues. In addition, presence of caseous tuberculosis, bronchiectasis and emphysematous changes were observed in the lungs. The analysis of sampled tissues showed the presence of material with IR spectrum and GLC profiles similar to those of a commercially available sample of liquid paraffins. The CONTAM Panel concluded that the inflammatory reaction of the lung cannot be ultimately related to the presence of liquid paraffins, in view of the concurrent presence of lesions likely to have been caused by previous lung infections.

Trivalle et al. (1991) described the case of a 69 year old woman hospitalised for chronic fever. The patient had an anamnesis including a treatment against constipation consisting of ingestion of three tablespoons of liquid paraffins per meal for a period of 10 years. Clinical examinations revealed an important inflammatory syndrome not related to bacterial infections or haematological or neoplastic causes. Liver biopsy revealed the presence of inflammatory infiltrations in the portal areas, constituted by the presence of giant cells and activated macrophages (epithelioid histiocytes). Histiocytic granulomas were vacuolated at histological examination, suggesting the presence of lipophilic deposition. According to the authors, the morphology of the lipid inclusions examined by electronic microscopy was similar to that observed in lipidic pneumonia caused by inhalation of liquid paraffins. Following the interruption of the treatment with liquid paraffins, the woman progressively recovered from the chronic fever symptoms and a second liver biopsy taken ten months after the interruption of the treatment showed an evident improvement with the rare presence of granulomas surrounded by post-inflammatory fibrotic tissue.

7.4. Modes of action

7.4.1. Non neoplastic effects

Lipogranulomas

As mentioned in Section 7.2.2.1, lipogranulomas are observed in the rat liver and MLN after repeated oral administration of mineral oils and waxes, and appear to be linked to MOSH bioaccumulation. However, for the most part this effect was found to be unique to Fischer 344 rats (Baldwin et al., 1992; Firriolo et al., 1995; Smith et al., 1996; Griffis et al., 2010). The liver microgranulomas induced in Fischer 344 rats are associated with T and B lymphocyte influx and sometimes with central necrosis and fibrosis. In MLN, exposure to mineral oils increase microgranulomas, but these have not been associated with inflammation or necrosis (Carlton et al., 2001; Griffis et al., 2010). Reduced T and B lymphocyte number in the MLNs due to spatial displacement by macrophages have been reported, but not quantified (Griffis et al., 2010). Although MLN microgranulomas were also observed in Sprague-Dawley rats, and in control Fischer 344 rats in long term studies, these responses were of lower incidence and severity. Greater mineral oil component deposition in macrophages and hepatocytes and more activated Kupffer cells has been suggested to cause granuloma formation in the Fischer-433 rat in contrast to other strains (Griffis et al., 2010).

I.p. injection of pristane (and paraffin oils in the range of C_{14}-C_{19}) in mice led to a chronic inflammatory response and the formation of lipogranulomas on peritoneal surfaces (reviewed by Potter, 2003). The lipogranulomas are formed after phagocytosis of oil droplets by myeloid cells/macrophages, and adherence to larger oil droplets. These structures adhere to the peritoneal wall, and are invaded by inflammatory cells like neutrophils and lymphocytes. Plasma cells can be found scattered around, entering from the peritoneal space but also from the blood vessels. Oil injection lipogranulomas are developed in several strains of mice (e.g. C57BL/6. DBA/2, Balb/cA), and resembles a typical foreign body-type response (Shaheen et al., 1999). Cells in the granulomas produce pro-inflammatory cytokines, like IL-6 and IL-12. Aspiration of mineral oil in humans causes "lipid pneumonia", and inflammatory lesion of the human lung closely resembling murine pristane-induced lipogranulomas (Reeves et al., 2009).

In humans, lipogranulomas are observed in liver, spleen, lymph nodes and other organs, attributed to intestinal absorption of mineral oils (Section 7.3). The clinical significance of the lipogranulomas is not known, but their presence had not been associated with inflammatory responses, and not reported to be associated with clinical abnormalities. A pathology workshop in 2001 reviewed published and unpublished reports and histological slides dealing with rat studies of various white mineral oils and waxes, as well as mineral oil-induced alterations in tissues of human patients (liver, hepatic lymph node and spleen) (Carlton et al., 2001). The authors concluded that the changes in humans are usually found incidentally in tissues taken at biopsy or autopsy and that MOSH-induced lesions can be considered incidental and inconsequential in humans. With regard to the hepatic and MLN microgranulomas in Fischer 433 rats, they concluded that these were unlike the lesions observed in human tissues. The MLN microgranulomas were considered not to be biologically significant to the rat (Carlton et al., 2001). Therefore, the MLN microgranulomas in Fischer 433 rats have been considered a non specific, adaptive effect not progressing to more severe pathological effects following long-term exposure (Carlton et al., 2001; EFSA, 2009). No experimental animal data suggest that MOH-induced histiocytosis in MLN after oral exposure progress to adverse lesions, inflammatory lesions, nor that MOH induce a clear effect on immune function. In support, other high molecular weight, poorly soluble materials giving histiocytosis in MLN after oral exposures, like calcium lignosulfonate, polypentosan sulphate and copovidone, are reported not to progress to pathological lesions nor affect immune function, thus MLN histiocytosis after calcium lignosulfonate exposures have recently been considered non-adverse effect (FAO/WHO, 2009). Although Carlton et al. (2001) concluded that the liver microgranulomas in the Fischer 433 rat were of potential toxicological significance, they dismissed these as being relevant for humans as they were considered

specific to this strain of rat. The CONTAM Panel is of the view that there is insufficient information to reach such a conclusion at this time. Therefore, the CONTAM Panel concluded that this endpoint in the Fischer 344 rat should be considered relevant for the risk assessment of MOSH.

Adjuvant effects

Hydrocarbons can induce inflammation and act as adjuvants by enhancing the immune responsiveness, and for instance incomplete Freund's adjuvant and squalene have previously been used in human and animal injection vaccines (Cox and Coulter, 1997). Although the pathways leading from adjuvant injection or percutaneous administration to arthritis remain unclear, studies of mineral oil- induced arthritis in rats propose that critical activation events take place in the lymph nodes, and adaptive transfer of activated T-cells from the draining lymph node to naïve rats was able to transfer arthritis (Holm et al., 2002). As expected due to a strong genetic component in autoimmune disease development in humans and animals, the presence of the adjuvant squalene and hyperplasia in the lymph nodes was not sufficient for arthritis induction, since similar distribution was observed in arthritis-resistant rat strains, neither were the squalene amount in the draining lymph nodes directly linked to the development of pathogenic cells (Holm et al., 2002).

Type I interferons (IFN) are increasingly recognised as a key mediator of SLE in humans, with elevated serum concentrations of IFN type I and over-expression of IFN type I-stimulated genes associated with disease severity, nephritis and the lupus-specific autoantibodies against dsDNA, Sm or ribosomal P (Reeves et al., 2009). Mechanistic studies in mice suggest that the pathogenesis of pristane-induced lupus-associated autoantibodies and glomerulonephritis is IFN type I-dependent. The formation of ectopic lymphoid tissue in conjunction with lipogranulomas contain monocytes producing IFN type I and IL-12 (Nacionales et al., 2006). Expression of type-I interferon-inducible genes was greatly increased in pristane-induced versus medicinal mineral oil-induced lipogranulomas, and thus correlated with the induction of SLE-like symptoms and autoantibodies also in mice. Both Toll-like Receptor (TLR)7, TLR9 and TLR4 have been reported to be required for development of autoimmunity and nephritis in pristane-induced lupus in mice, and since germ-free mice also develop disease, endogenous TLR7-ligands are probably involved (Reeves et al., 2009; Summers et al., 2010). Further, pristane has been reported to induce apoptosis both *in vitro* (dose-dependent) and *in vivo*, in peritoneal cells 48 hours after exposure to B6, Balb/cJ and Balb/cAn mice (Calvani et al., 2005). With regard to autoimmune events, nuclear autoantigens created by pristane-induced apoptosis of lymphoid cells in the setting of a profoundly altered cytokine milieu has been suggested to be the initiating event (Calvani et al., 2005). The CONTAM Panel concluded that this mechanism is not relevant for the MLN histiocytosis observed in rats after oral exposures, since no inflammation or formation of neo-lymphatic tissue is reported.

Plasmacytomas

Like other agents inducing plasmacytomas, pristane is poorly metabolised and is difficult to remove. As described in Section 7.2.5.1, in Balb/cA mice the pristane-induced lipogranulomas may develop into plasmacytomas (overgrowth of the granulomas by plasma cells). Multiple (> 6) focal plasma cell proliferation appears to be pre-neoplastic lesions in the plasmacytoma formation. These foci can be observed already at 25 days after pristane injection, while the latent period for plasmacytoma formation is on average 215 days. The major contribution from the lipogranulomas in plasmacytoma development appears to be the production of IL-6 (Potter, 2003).

After i.p. pristane injections, 11 % and 61 % of Balb/cJ and Balb/cAn mice, respectively, developed plasmacytomas (Potter and Wax, 1981). In contrast, the respective percentages of mice developing arthritis were 70 % and 20 %, and pristane-induced lupus is induced in most mouse strains tested, suggesting that the mode of action and susceptibility for development of plasmacytomas and autoimmune disease differ. In agreement, the enhancement of plasmacytomas in Balb/c mice by pristane was reported not to be due to immunosuppressive properties (Ruiz-Bravo et al., 1995).

7.4.2. Neoplastic effects

Long chain MOSH and highly alkylated MOAH are not genotoxic and are not themselves carcinogenic, i.e. they are not carcinogenic in the absence of prior initiation. However, a number can serve as promoters following initiation with a genotoxic compound, such as a polycyclic aromatic hydrocarbon, particularly in a 2-stage mouse skin model. The mode of action appears to involve stimulation of cells to proliferate, often secondary to local irritation. However, tumour promotion is a complex process and despite many years of research, the exact mechanisms and processes involved remain to be determined (Rundhaug and Fischer, 2010).

The male rat specific nephrocarcinogenicity of **some aliphatic hydrocarbons, such as decalin**, is due to their tight, but reversible physical association with the protein α_{2u}-globulin. This 18.7 kD protein is synthesised in the liver and enters the systemic circulation from where it is readily filtered in the glomerulus. It is taken up by renal proximal tubular cells and rapidly degraded. However, complexes with hydrocarbons such as decalin are resistant to degradation and hence accumulate in the renal tubular cells. This accumulation occurs specifically in sexually mature male rats, which have much higher α_{2u}-globulin urinary concentrations in comparison to female rats or to other species (Hard et al., 1993). The accumulated protein is cytotoxic and cell destruction stimulates surviving cells to proliferate. As described below for compounds such as NAP, this would increase the probability of selection or occurrence of initiated cells and hence of cancer (Warnasuriya et al., 2010). This mode of action is not relevant to humans, as there is no protein equivalent to α_{2u}-globulin present at urinary concentrations anywhere near comparable with those of α_{2u}-globulin in male rats (IARC, 1993).

Simple MOAH such as NAP are carcinogenic by a non-genotoxic mode of action. These can undergo target tissue metabolism into cytotoxic products which cause necrosis of cells in which they are produced and possibly in nearby cells as well. The reduction in cell number serves as a proliferative stimulus, mediated by growth factors, to the remaining cell population. The increase in cell turnover can give rise to the selection and/or spontaneous occurrence of initiated cells, which may progress to malignancy (IARC, 2002).

Carcinogenic **three or more ring MOAH with no or a low degree of alkylation, and heterocyclic-containing analogues** are metabolised by P450 enzymes into chemically reactive electrophilic products. These covalently bind to nucleophilic centres in DNA, such as N2 of guanine, resulting in mutation during DNA replication. Mutations at critical sites in oncogenes or tumour suppressor genes result in tumour initiation. Continuing exposure leads to further genetic changes and ultimately to the emergence of malignantly transformed cells, which give rise to tumours (Croisy et al., 1984; IARC, 2010). It is also possible that carcinogenic effects of PAHs are due, at least in part, to a non-genotoxic mode of action by stimulation of the aryl hydrocarbon receptor (Baird et al., 2005; Bock and Köhle, 2006).

7.5. Hazard characterisation

7.5.1. Dose-response considerations and critical effects

MOH composition varies considerably with the origin of the oil. The mixtures to which humans are exposed in their diets are extremely complex, it is not possible to separate them into individual compounds and there is limited information on the chemical composition of the mixtures. It is not meaningful to base the hazard characterisation on single compounds or indicator substances. It is possible to distinguish and quantify certain fractions of MOH, based either on aromaticity, in particular MOSH and MOAH, or molecular mass. Therefore, particularly a distinction between MOSH and MOAH mixtures will be made in the hazard characterisation.

Both human and animal data were considered for the hazard characterisation of MOH. However, data on toxicity in humans were generally not of a quantitative nature that could be used for the hazard characterisation.

Acute toxicity

Since MOSH and MOAH in general have low acute toxicity by ingestion, acute toxicity, given the nature of current human exposure via food, was not considered relevant and therefore not further discussed.

MOAH

Unless MOH are treated specifically to remove MOAH, MOH derived from crude oil are mutagenic. The mutagenicity of MOH is caused mainly by 3-7 ring MOAH, including alkylated PAHs and non-alkylated PAHs of which the latter is a minor fraction and mainly formed by the heating of the oil. Some of these non-alkylated PAHs are covered by monitoring programmes in food. The genotoxicity, carcinogenicity and risk assessment of the monitored PAHs is addressed in the Opinion on PAH in food by the EFSA Panel on Contaminants (EFSA, 2008d). Three to seven ring MOAH may form DNA adducts and may be carcinogenic. The MOAH fraction of MOH is a complex mixture of thousands of compounds, which only to some extent can be resolved into very crude fractions and not single compounds. There are no indicator compounds that can adequately characterise a particular MOAH mixture. Because of their mutagenic nature no thresholds of carcinogenicity for MOAH mixtures can be assumed. Normally, a margin of exposure (MOE) approach would be employed in the risk characterisation of MOAH, but there are no dose-response data on carcinogenicity of such mixtures for establishing an RP upon which to base an MOE calculation.

Some highly alkylated MOAH can act as tumour promoters. Some simple MOAH, such as naphthalene, are carcinogenic by a non-genotoxic mode of action, involving cytotoxicity and proliferative regeneration. Generally, no dose response data are available for the tumourigenicity or for the toxicological effects that preceed tumour formation for these classes of compounds.

Hence, it is not possible to further characterise the hazards associated with the MOAH fraction of MOH.

MOSH

MOSH are neither mutagenic nor carcinogenic. Some long-chain MOSH can at high doses act as tumour promoters, but there is little dose response information available.

In rodents, MOSH mixtures with a distribution of carbon numbers in the range of C_{10}-C_{13} gave moderate hepatocyte hypertrophy. However, in the absence of any pathological changes, the CONTAM Panel consider this to be an adaptive response and not an adverse effect.

MOSH with carbon number in the range C_{16}-C_{40} are able to accumulate in tissues and cause microgranulomas. Accumulation occurs both in humans and in rats. In humans, there are in early publications (Boitnott and Margolis, 1970) some indications that there is a positive association between the hepatic concentration of MOH and microgranulomas in the liver. These granulomas were not associated with inflammatory responses or clinical abnormalities. Moreover, there are no quantitative human data describing MOSH exposure and associated effects that are suitable for hazard characterisation. In the following considerations of dose-response relationships data from animal experiments are used.

In rats, the available data for high molecular weight mixtures showed effects related to MOSH accumulation in the liver and in MLN. In comparison to the Sprague Dawley rat, possibly due to a stronger tendency for accumulation, Fischer 344 rats showed a higher sensitivity to the development of liver and MLN granulomas and microgranulomas following the subchronic exposure to mineral oils and waxes. The CONTAM Panel considered that the presence of microgranulomas/histocytosis in MLN is a non specific, adaptative change of low toxicological concern (see Section 7.3.1). In the

liver, however, the microgranulomas were surrounded by inflammatory cells. Evidence from a two year carcinogenicity study on two high viscosity mixtures (P70(H) and P100(H)) suggest that the microgranulomas in liver and MLN do not cause a prolonged inflammatory response or other severe pathological changes following chronic exposure in Fischer rats (Trimmer et al., 2004). However, the CONTAM Panel concluded that it would be prudent to assume that liver microgranulomas, observed in the Fischer 344 rats, could be potentially relevant to humans and therefore the critical effect for the risk assessment of MOSH.

Because the development of liver granulomas appears to be dependent on accumulation new information on toxicokinetics provided by CONCAWE (Cnubben and van Stee, 2010 and Bakker, 2011) was assessed. This clearly indicated that Fischer 344 rats are more prone to accumulate MOH than Sprague Dawley rats. The CONTAM Panel noted that in the study on human volunteers a single oral dose of 1 mg/kg b.w. P15(H) was administered, which did not result in measurable concentrations in blood. It was therefore not possible to determine the extent to which P15(H) would accumulate in humans from this study.

Liver microgranulomas in Fischer 344 rats have been observed for various MOSH products intended for food use (see Tables 16 and 17). Except for two studies on P70(H) and P100(H) of two year duration, the more potent mixtures were only tested in 90-day studies, which were therefore used to identify the respective NOAELs. The published data did not allow modelling of the dose-response data of the different studies, because there was insufficient information on the relevant endpoint. As it can be observed from Table 18 the lowest NOAEL for white mineral oils was observed for two poorly characterised products (OTWO and HTWO, NOAELs of 46 and 45 mg/kg b.w. per day, respectively). One product, P15(H), showed no statistically significant increase in liver microgranulomas up to 190 mg/kg b.w. per day in one study (Smith et al., 1996), but in another study with a different batch a similar dose (161 mg/kg b.w. per day) was associated with a clear increase in liver microgranulomas (Firriolo et al., 1995). The NOAEL for the other products was always ≥ 190 mg/kg b.w. per day. For the waxes (Table 17) a considerable difference is observed between LMPW and IMPW (NOAEL = 19 mg/kg b.w. per day), and the HMPW (NOAEL = 1 900 mg/kg b.w. per day, the highest dose tested).

The CONTAM panel concluded that these NOAELs could be used to select Reference Points (RPs) for establishing health based guidance values.

7.5.2. Health-based guidance values for mineral hydrocarbons

7.5.2.1. Products intended for food use - applicability of the existing ADIs

The existing ADIs have been established for specific products intended for food use. The current classifications of food grade-MOH set by SCF (1995), FAO/WHO (2002) and ANS Panel (EFSA, 2009) are compared in Table 23 and discussed in Section 3 of this Opinion. Based on the new information assessed in the opinion, the following observations can be made on the previously established ADIs:

- The established ADIs are based on toxicological studies with poorly characterised products with regard to chemical composition. Ideally MOSH mixtures should be assessed by considering the molecular mass range and subclass composition (e.g. n-, branched- or cyclo-alkanes), rather than on physico-chemical properties such as viscosity, as has been done in the past for all MOSH mixtures tested.

- SCF (1995) and FAO/WHO (2002) established the ADIs for a number of MOSH mixtures on the basis of the occurrence of MLN hystiocytosis in Fischer 344 rats. Doses required to produce microgranulomas in the liver, considered the critical effect by the CONTAM Panel, are about 100 times higher than those producing effects in MLN, for most of the tested grades of oils and waxes (Tables 16 and 17).

- New information was provided by CONCAWE on toxicokinetics in Fischer 344, and Sprague Dawley rats and humans (Cnubben and van Stee, 2010 and Bakker, 2011). However, the CONTAM Panel noted that these studies were carried out by single administration and this limitation should be taken into account if these data are used for deriving an ADI.

On the grounds of the considerations listed above, the CONTAM Panel concluded that a revision of the temporary group ADI established by FAO/WHO for medium- and low-viscosity mineral oils class II and III is warranted.

With respect to microcrystalline waxes, high-viscosity mineral oils and medium- and low-viscosity class I mineral oils, the ADIs established by SCF, FAO/WHO and EFSA were based on toxicological studies in which no effects were observed at any tested dose. For those grades, the CONTAM Panel concluded that the existing ADIs are of low priority for revision, although they are based on products with a poor chemical characterisation.

Table 23: Comparison of (temporary-)ADIs established by SCF (1995), FAO/WHO (2002) and EFSA (2009).

	SCF (1995)				FAO/WHO (2002)				EFSA (2009)			
	ADI (mg/kg b.w. per day)	NOAEL (mg/kg b.w. per day)	Uncertainty factor	Comments	ADI (mg/kg b.w. per day)	NOAEL (mg/kg b.w. per day)	Uncertainty factor	Comments	ADI (mg/kg b.w. per day)	NOAEL (mg/kg b.w. per day)	Uncertainty factor	Comments
High viscosity P100(H)	0-4[a]	1 951	500	90-day NOAEL	0-20	1 951	100	90-day NOAEL	12	1 200	100	2-year NOAEL
Medium and low viscosity, class I P70(H)	0-4[a]	1 951	500	90-day NOAEL	0-10	1 200	100	2-year NOAEL	(12)[b]	(1 200)	(100)	2-year NOAEL
Medium and low viscosity, class II N70(H)	No ADIs established				0-0.01[a]	2	200	90-day NOAEL	–	–	–	
Medium and low viscosity, class III P15(H), N15(H)	No ADIs established				0-0.01[a]	2	200	90-day NOAEL	–	–	–	
Microcrystalline wax high melting point wax	0-20	1 951	100	90-day NOAEL	0-20	1 951	100	90-day NOAEL	–	–	–	
Low melting point wax	No ADI established				Withdrawn				–	–	–	

a: Temporary group ADI.
b: EFSA concluded that the ADI established for high viscosity mineral oil could have been potentially applicable also to medium- and low-viscosity mineral oil class I.

7.5.2.2. General MOSH exposure in humans - derivation of a health based guidance value

The carbon-numbers of the MOSH to which humans are exposed via food range from C_{12} to C_{40} with centres ranging from C_{18} to C_{34} (Table 3). Evidence from analyses of the MOSH content of human tissues show an accumulation of MOSH with carbon numbers between n-C_{16} to n-C_{35} with centres around n-C_{23} to n-C_{24}. The accumulating constituents appear to be unresolved MOSH mainly branched- and alkyl-substituted cycloalkanes. None of the existing ADIs was considered adequate for the risk characterisation of the range of MOH present in the background exposure of humans.

In order to be prudent and protect humans from toxicity to the whole range of MOSH exposure, the whole MOSH fraction should be considered in total. There are no studies in experimental animals with MOSH mixtures typical to those humans are exposed to. The existing data are from studies on specific fractions of MOSH characterised by physico-chemical parameters (i.e. viscosity and melting point) with unknown chemical composition. Hence, the CONTAM Panel did not find the existing data appropriate for establishment of a health based guidance value for MOSH, such as a TDI.

Given the deficiencies in the toxicity data base, the CONTAM Panel therefore decided to use an MOE approach and considered the results from toxicity testing of a range of different MOSH mixtures (see Tables 16 and 17) to select an RP for this purpose. The CONTAM Panel selected the NOAEL for microgranulomas in the liver of 19 mg/kg b.w. per day for the most potent mixtures (LMPW and IMPW) from the 90-day study of Smith et al. (1996) for use as an RP in the calculation of MOEs for the human background MOSH exposure (see Table 16).

The range of MOSH mixtures involved in the high exposure scenarios (MOSH used as release agents for bread and for spraying of grains) is more restricted than that for the background exposure. The CONTAM Panel therefore concluded that it was not appropriate to use the RP from LMPW and IMPW in the risk characterisation for these scenarios. Hence, the CONTAM Panel used the highest NOAEL of 45 mg/kg b.w. per day from the study of Baldwin et al. (1992) below the lowest LOAEL of 161 mg/kg b.w per day (Firriolo et al., 1995) for these grades of MOSH (see Table 17).

8. Risk characterisation

MOAH

Because no RP could be derived and no appropriate exposure data for MOAH exist, it is not possible to characterise the risk associated with human exposure to MOAH via food. The MOAH content of MOH present in food are mostly around 20 %, but may in vegetable oil and oil seeds be up to 30 - 35 % of the MOH levels. The MOAH fraction could represent a carcinogenic risk. Therefore the CONTAM Panel considers the exposure to MOAH through food to be of potential concern.

MOSH

Background exposure from all sources

The summary of chronic dietary exposure scenarios for MOSH across Europe is given in Table 9. MOEs for the different age categories were calculated using the Reference Point of 19 mg/kg b.w. per day (see Table 24).

Table 24: Margins of exposure (MOEs) calculated for the background exposure scenario (average and high consumption, minimum LB and maximum UB across European dietary surveys)

Background exposure scenario (19 mg/kg b.w. per day as Reference Point)		
	Average (min LB - max UB)	**P95 (min LB - max UB)**
Infants [(a)]	500 – 110	160 – 150
Toddlers	230 – 100	110 – 73
Other children	290 – 110	140 – 59
Adolescents	680 – 200	300 – 95
Adults	610 – 280	320 – 160
Elderly	610 – 320	330 – 200
Very elderly	590 – 350	280 – 230

b.w.: body weight; LB: lower-bound; UB: upper-bound;
(a): estimates available only from two dietary surveys for the mean and only one for the 95th percentile.

For average consumption the MOEs (based on maximum UB and minimum LB exposures across European dietary surveys, respectively) for toddlers and children and for adolescents and adults were from 100 to 290 and from 200 to 680, respectively. For high consumption the MOEs for toddlers and children and for adolescents and adults were from 59 to 140 and from 95 to 330, respectively.

High exposure scenarios – regular consumption of bread or grains with high levels of MOSH

Chronic high exposure scenarios were calculated for regular consumption of bread and rolls high in MOSH due to their use as release agents or for grains (mainly rice) sprayed with MOSH. Summary statistics of these scenarios following chronic intake of such products were given in Tables 25 and 26. In calculation of MOEs for these scenarios an RP of 45 mg/kg b.w. per day was used because the white oils and not waxes were used in these cases. Except for infants the MOEs (based on maximum and minimum exposure across European dietary surveys, respectively) obtained for the different age classes ranged from 16 to 55 for average consumption, and in some cases were below 10 for high consumption (P95) of bread and rolls with high levels of MOSH.

For the regular mean consumers of grains high in MOSH the MOEs varied greatly between different age classes and ranged between 35 (toddlers, maximum exposure across European dietary surveys) and 1 900 (other children, minimum exposure across European dietary surveys). For high (P95) consumption the MOEs were between 12 (other children) and 200 (elderly).

Table 25: Margin of exposure to MOSH across European dietary surveys, related to continued consumption of 'Bread and rolls' with high MOSH levels (from white oils) over a long period (average and high consumption, minimum and maximum exposure across European dietary surveys)**.**

High exposure scenario - bread (45 mg/kg b.w. per day as Reference Point)		
	Average (min - max)	**P95 (min - max)**
Infants[(a)]	1300-49	12
Toddlers	41-15	12-7.8
Other children	41-16	18-7.0
Adolescents	54-30	24-13
Adults	65-38	32-18
Elderly	51-38	25-21
Very elderly	51-41	25-18

b.w.: body weight;
(a): estimates available only from two dietary surveys for the mean and only one for the 95th percentile;

Table 26: Margin of exposure to MOSH across European dietary surveys, related to continued consumption of 'Grains for human consumption' with high MOSH levels (from white oils) over a long period (average and high consumption, minimum and maximum exposure across European dietary surveys)**.**

High exposure scenario - grains (45 mg/kg b.w. per day as Reference Point)		
	Average (min - max)	**P95 (min - max)**
Infants	150-120	30
Toddlers	190-35	24-15
Other children	1900-51	66-12
Adolescents	410-69	100-18
Adults	560-130	125-35
Elderly	830-160	200-41
Very elderly	940-170	85-38

b.w.: body weight;
(a): estimates available only from two dietary surveys for the mean and only one for the 95th percentile;

Comments on the MOEs

The CONTAM Panel took into account that the basis for the RPs was 90-day studies and that some of these compounds might have very long elimination half lives in humans, when interpreting the obtained MOEs. In the view of this the CONTAM Panel considers the MOEs obtained for the chronic background exposure to MOSH via food to be low and therefore this exposure is of potential concern.

The MOEs obtained in the two high exposure scenarios, particularly the regular intake of bread high in release agents were low, in most cases below 100, and some cases below 10 for high consumption of bread. This exposure scenario was considered to be of particular concern. In the case of spraying of grains, mainly high consumption, but also some age groups among average consumers had low MOEs that might be of concern

Hence, the CONTAM Panel concluded that there is potential concern associated with the current background exposure to MOSH in Europe and in particular with the use of white oils as release agents for bread and to some extent for spraying of grains.

9. Uncertainty

The evaluation of the inherent uncertainties in the assessment of exposure to mineral oil hydrocarbons has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006) the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model, and model input (parameters). In addition uncertainties related to hazard characterisation and risk characterisation were included.

9.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference. The CONTAM Panel assessed the new occurrence data that were collected by EFSA between 6 August and 15 October 2010. In its risk assessment the CONTAM Panel focused on the exposure to MOSH.

9.2. Exposure scenarios/Exposure model

In response to EFSA's request to submit occurrence data on mineral oil hydrocarbons in food, results for 1 455 samples were provided for several food groups. Most of the occurrence data were provided

by a single enforcement laboratory and a large part of them results from targeted sampling. This fact, together with the uncertainty related to the possibility that other food commodities could also contain appreciable amounts of MOH, are the main limitations of the dataset. In the case of two food groups, 'Bread and rolls' and 'Grains for human consumption', the CONTAM Panel noted that the distribution of analytical values is bi-modal and the high occurrence values are related to specific production practices using food grade white oils. The mean values for both the background and the high values were determined using a log-normal maximum likelihood fitting. Although the CONTAM Panel recognised that the use of a statistical model introduced some uncertainty, a better presentation of these high occurrence data in bread and grains was achieved.

The estimates of exposure to MOH from dry foods packaged in recycled paper and board are based on occurrence data from one Member State with a total of 120 samples of dry foods analysed, which were assumed as representative of the European occurrence data. The samples tested were from 12 different food categories. Therefore only a small number of samples was analysed from each food category. For the four food categories considered for the exposure assessment the number of samples tested ranged from 8 (semolina) to 24 (breakfast cereals).

Only occurrence data on foods packaged without a barrier to migration between the food and the recycled paper and board were considered, which provides a conservative exposure estimate.

9.3. Model input (parameters)

There are no prescribed fixed official methods or defined performance criteria for the analysis of MOH and laboratories can use any method of analysis, provided it can be demonstrated in a traceable manner that they fulfil the requirements according to ISO 17025. This may have added to the uncertainty in the analytical results. The absence of certified reference standards and certified reference materials is a limitation when the method performance for the analytical procedures for analysis of MOH in food is assessed.

9.4. Toxicological data

The CONTAM Panel considered the toxicological data retrieved on single MOSH and MOAH components of the relevant mixtures as inappropriate for the risk assessment for MOH mixtures.

The MOSH mixtures tested were defined on physico-chemical properties, with little relationship to their chemical compositions, which can vary from batch to batch. The tested MOSH mixtures cannot be related to the MOH mixtures to which humans are exposed.

The incidence of hepatic microgranulomas in rats following exposure to MOSH was considered the critical effect for the selection of the NOAELs. However, several uncertainties regarding the extrapolation from data on experimental animals to humans exist, in particular the relevance of these lesions for humans and the sensitivity of humans in comparison with the most sensitive species tested, Fischer 344 rats.

It is assumed that the accumulation of MOSH plays an important role in microgranuloma formation both in rats and humans. The low and intermediate melting point waxes, which consist mainly of n-alkanes that do accumulate to a much lesser extent than branched- and cyclic-alkanes, are the most potent mixtures tested. This fact would indicate that unknown mechanisms other than accumulation of MOSH *per se* are involved in the pathogenesis of the microgranuloma formation.

The CONTAM Panel based the risk characterisation on the NOAEL for the most potent mixtures for the calculation of MOE. However there is uncertainty regarding how well this NOAEL applies to the MOSH to which humans are currently exposed. The CONTAM Panel concluded that it is likely that the NOAEL used will be sufficiently protective for the range of MOSH to which humans are exposed.

In view of the paucity of data available on the compositional characterisation of the MOAH fraction, of the toxicological effects, and on the human exposure to MOAH, the CONTAM Panel was not able to perform a meaningful risk assessment for this class of MOH.

9.5. Summary of uncertainties

In Table 27, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

Table 27: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the human dietary exposure of MOH.

Sources of uncertainty	Direction
Lack of certified reference standards, certified reference materials and regular proficiency tests	+/-[(a)]
Occurrence data not representative of all food commodities in which MOH could be present	-
Occurrence data not equally distributed across Europe	+/-
Use of a mathematical model to calculate the average occurrence values in bread and grains	+/-
Occurrence data in foods packaged in recycled paper limited in number	+/-
Occurrence data mainly related to targeted investigations	+
The MOSH mixtures tested in toxicological studies were characterised by physico-chemical properties. Chemical composition may vary from batch to batch.	+/-
The composition of the mixtures tested in toxicological assays may differ from the compositions of the mixtures of MOH to which humans are exposed via food	+/-
Relevance of liver microgranulomas for humans and the sensitivity of humans in comparison with the most sensitive species tested, Fischer 344 rats.	+
Absence of exposure and toxicological data for MOAH.	-
The selection of the NOAEL of the most potent mixtures as the Reference Point for the MOSH to which humans are currently exposed.	+

LB: lower bound; UB upper bound;
(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk.

The CONTAM Panel considered the impact of the uncertainties on the risk assessment of human exposure to MOH and concluded that overall uncertainty is substantial.

10. Advice on future monitoring

The last point in the Terms of Reference specifies “Advise on classes to be included if monitoring would be set up for the presence of mineral oil in food”. “Classes” are understood as classes of MOH as well as classes of food products.

10.1. Classes of mineral oil hydrocarbons

As the MOH in the mineral oil products encountered in food vary widely and differ in toxicological relevance, monitoring should distinguish between them as far as technically possible. It is necessary to distinguish between MOSH and MOAH because of their different modes of action and toxicological and carcinogenic effects.

Classification of MOSH:

- Distinction by molecular mass; cuts in the gas chromatograms after the given n-alkane:

- MOSH up to n-C_{16}, which are used as solvent in printing inks;
- MOSH n-C_{16} (cut after elution of n-C_{16}) up to n-C_{35}; fraction corresponding to the MOSH accumulated by the human body;

- MOSH above n-C_{35} are considered to be less relevant for monitoring because of the low gastrointestinal absorption.

- Distinction of n-alkanes eluted on top of the patterns of unresolved peaks (relative to MOSH), which are mainly composed of branched and cyclic MOSH.

- Distinction between paraffins (open chain MOSH) and naphthenes (cyclic MOSH). This separation probably requires analysis by GCxGC.

- Distinction of hydrocarbons, such as PAO and POSH, from the MOSH, if possible.

Classification of MOAH:

Total MOAH should be monitored. Presently there is no suitable analytical method to separate different structural subclasses of MOAH. Analytical methods should be developed for this purpose.

10.2. Classes of food to be included in future monitoring

With respect to the food classes to include in an eventual monitoring, the food groups with a relevant contribution to the background exposure, including the special cases related to use of white oils should be taken into account. In particular, 'Animal fat', 'Bread and rolls', 'Confectionery (non chocolate)', 'Fine bakery wares', 'Fish meat', 'Fish products (canned fish)', 'Grains for human consumption', 'Ices and desserts', 'Pasta', 'Sausages', 'Vegetable oil' should be included.

It is recommended to investigate whether other food groups not included in the present evaluation also provide a relevant contribution to the total chronic exposure.

10.3. Performance of monitoring

Monitoring needs to be planned in a structured manner, e.g. starting from the sources, as otherwise the complexity of the occurrence requires an excessive number of samples to be analysed.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Mineral oil hydrocarbons (MOH) consist of three major classes of compounds: paraffins (constituted of linear and branched alkanes), naphthenes (constituted of alkyl-substituted cyclo-alkanes), and aromatics (including polyaromatic hydrocarbons, which are generally alkyl-substituted). MOH may also include minor amounts of heteroatom-containing compounds. Within these classes there are enormous numbers of individual components.

- In this opinion MOH have been divided in two main types, mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH).

- MOH are derived by physical separations (such as distillation or extraction) and chemical conversion processes (cracking, hydrogenation, alkylation, isomerisation, etc.) from crude oils and/or synthetic products derived from liquefaction of coal, natural gas or biomass. Of the many products, little is known about the composition, as specifications generally are expressed in terms of physico-chemical properties (such as viscosity) connected with the

applications of the products. Even products with the same specification may considerably vary in their composition, depending on the source of the oil and the processes used.

- The food grade MOH products are treated in such a way that the MOAH content is minimised. Technical grades MOH typically contain 15 - 35 % MOAH.

Methods of analysis

- Because of the complexity of the MOH mixtures it is not possible to separate these into individual components. However, within the boundaries of the associated analytical uncertainties, it is possible to measure the concentration of MOSH and MOAH fractions, as well as certain sub-classes, using methods based on gas chromatography (GC).

- Currently the most efficient method to analyse MOSH and MOAH in food and feed comprises extraction followed by pre-separation by high performance liquid chromatography (HPLC) on-line coupled to GC with flame ionisation detection (FID). Detection limits depend on the mass distribution, the sample matrix and any prior enrichment, and can be as low as 0.1 mg/kg.

- Comprehensive GCxGC-FID enables a rough separation and quantification of paraffins and naphthenes in the MOSH fraction, but it is of limited practicality for routine analysis.

- Contamination with polyolefin oligomeric saturated hydrocarbons (POSH), e.g. from plastic bags, heat sealable layers or adhesives, may interfere with MOSH analysis.

- Analytical capacity to distinguish the different MOAH subclasses in food is limited. GCxGC appears to be the most effective method.

- Due to the complexity and the variable compositions of the MOH mixtures, there are limitations in defining certified standards of general applicability.

Occurrence

- The Panel on Contaminants in the Food Chain (CONTAM Panel) identified the following sources for the presence of mineral oils in food:

  - Food contact materials:

    - Food packaging materials made from recycled paper and board;
    - Off-set printing inks applied to paper and board for food packaging;
    - Mineral oils used as additives in the manufacture of plastics for food contact (e.g. internal lubricants in polystyrene, polyolefins);
    - Wax paper and board;
    - Jute or sisal bags with mineral batching oil;
    - Lubricants for can manufacture;
    - Wax coating directly applied to food.
    - In addition, some types of adhesives may contain mineral oil components. Systematic studies on the migration from adhesives have not been carried out.

  - Contaminants:

    - Environmental contaminants: lubricating oil from engines without catalyst (mainly diesel), unburned fuel oil, debris from tyres and road bitumen.
    - Harvesting machinery: diesel oil, lubricating oil.

  - Lubricating oils in pumps, syringe type dosing machinery and other industrial installations used in food processing.
  - Cleaning agents, solvents consisting of pure MOH or C_{10}-C_{14} mixtures.

- Food additives, processing aids and other uses:

  - Release agents for bakery ware and sugar products.
  - Oils for surface treatment of foods, such as rice, confectionery.
  - Mineral oils in feeds, e.g. binders for minor additives added as powder.
  - Defoamers.
  - Authorised paraffinic waxes (e.g. for chewing gum or coating of certain fruits)
  - Pesticide formulations.
  - Anti-dusting agents for cereals.

- Occurrence data were available only for a limited number of food groups and only from a few countries. Part of the occurrence data is from targeted sampling.

- Nearly all data refer to total MOSH and little information is available on the sub-classes such as open chain linear or branched, and cyclic alkanes.

- For MOSH detected in the different food categories the number of carbon atoms typically ranges from 12 to 40.

- MOAH measurements were not available for the majority of the samples, but MOAH concentrations can be estimated from the typical composition of the mineral oil product detected.

- In the available dataset, not considering the high values for 'Bread and rolls' and 'Grains for human consumption' (rice), the highest mean occurrence values for MOSH (lower bound – upper bound (LB-UB), when different) are in 'Confectionery (non-chocolate)' (46 mg/kg), 'Vegetable oil' (41-45 mg/kg), 'Fish products' (canned fish) (40 mg MOSH/kg) and 'Oilseeds' (38 mg MOSH/kg), followed by 'Animal fat' (22-24 mg MOSH/kg), 'Fish meat' (21 mg MOSH/kg), 'Tree nuts' (20-21 mg MOSH/kg) and 'Ices and desserts' (14 mg MOSH/kg).

- In the groups 'Bread and rolls' and 'Grains for human consumption' (mainly represented by rice) some high values were reported that can be due to specific production practices with use of food grade MOH. In these cases, the distribution of all values was modelled using a maximum likelihood log-normal fitting in order to identify a mean value for both the 'background' occurrence and the high levels of occurrence The mean background occurrence for 'Bread and rolls' and 'Grains for human consumption' was 1.8 and 4.1 mg/kg, respectively. The mean occurrence values for the same food groups with high levels of MOSH were 532 mg /kg and 977 mg /kg.

- Occurrence data in foods that could be attributed to the use of recycled paperboard were available from two different surveys conducted in two Member States. Mean concentrations of MOH were up to 32 mg/kg for MOSH found in creme/pudding mix and 4.5 mg/kg MOAH found in noodles. Maximum values of occurrence were 100 mg/kg in semolina and 17 mg/kg in noodles, for MOSH and MOAH, respectively.

Human exposure

- Chronic exposure scenarios were calculated for different age classes of the population based on mean occurrence values. These values were considered to represent the contamination normally expected in the respective food groups.

- In adults and elderly the dietary exposure to MOSH across European dietary surveys was between 0.03 and 0.07 mg/kg body weight (b.w.) per day for average consumers and between 0.06 and 0.1 mg/kg b.w. per day for high consumers (P95).

- Considering all age classes, the highest dietary exposure estimate per kg b.w. was found for 'other children' (3 to 10 years old). In this age class, the UB exposure to MOSH ranges across dietary surveys from 0.068 to 0.17 mg/kg b.w. per day for average consumers, and from 0.14 to 0.32 mg/kg b.w. per day for high consumers (P95).

- The percentage contribution of the different food groups to the exposure distributions was calculated for each available survey/age class combination. In 'Infants', the major contributors were 'Breast milk', 'Vegetable fat' and 'Animal fat'. In 'Toddlers', 'Ices and desserts', 'Vegetable oil' and 'Bread and rolls'. In 'Other children', 'Ices and desserts', then 'Confectionery (Non-Chocolate)' and 'Fine bakery wares'. In 'Adolescents', 'Vegetable oil', 'Ices and desserts' and 'Fish meat'. In 'Adults' the most important contributors were 'Fish meat', 'Fine bakery wares', 'Vegetable oil' and 'Bread and rolls'. In 'Elderly', 'Fish meat', 'Animal fat', 'Bread and rolls' and 'Vegetable oil'. In 'Very elderly', 'Fish meat', 'Vegetable oil', 'Animal fat' and 'Bread and rolls'.

- Additional exposure on top of the background was calculated for specific consumers of 'Bread and rolls' and 'Grains for human consumption' with high levels of MOSH deriving from their use as release agents or spraying agents. Though these high values cannot be included in the background contamination, it cannot be excluded that some groups of consumers (buying always from the same source or having brand loyalty) are exposed on a regular basis to these high concentrations of MOSH. Excluding infants, the additional exposure across European dietary surveys and age classes is up to 6.4 mg/kg b.w. per day for the 'Bread and rolls' scenario and up to 3.8 mg/kg b.w. per day in the 'Grains for human consumption' scenario.

- For the subgroup of exclusively breast-fed infants an exposure to MOSH of about 0.3 - 0.5 mg/kg b.w. per day was calculated.

- Based on the occurrence estimated proportion of MOAH in other MOH, a background exposure to MOAH ranging from 15 to 35% of total MOH can be expected. Exposure to MOAH from use of food grade white mineral oils, such as in bread treated with release agents or sprayed grains, can be considered as negligible

- Exposure to MOSH and MOAH attributed to migration from recycled paper and board packaging was estimated based on limited occurrence data from two Member States. Toddlers and other children were the age classes of consumers potentially more exposed to MOH. Exposure to MOSH, for high consumers in these age classes was up to 0.04 mg/kg b.w. per day from bakery wares, 0.07 mg/kg b.w. per day from breakfast cereals and 0.11 mg/kg b.w. per day from rice. Background exposure to MOSH from all sources was estimated to be up to 0.3 mg kg b.w. per day. These data therefore indicate that exposure to MOSH from migration into dry foods packaged in recycled paper and board without a barrier to migration may contribute significantly to the total dietary exposure. MOAH consistently represents approximately 15% of MOSH migrating from recycled paper.

Hazard identification

Toxicokinetics

- Absorption of alkanes may occur through the portal and/or the lymphatic system. For n-alkanes the absorption varies from 90 % for carbon numbers in the range C_{14}-C_{18} to 25 % for C_{26}-C_{29}. The absorption further decreases with increasing carbon number.

- Limited data suggest that cyclo-alkanes are absorbed to a similar extent as n-alkanes of comparable molecular weight, whereas absorption of branched alkanes is slightly less.

- Alkanes are oxidised to the corresponding fatty alcohols through cytochrome P450s and are then generally biotransformed to fatty acids. This reaction is more rapid for n-alkanes than for branched- and cyclo-alkanes.

- MOSH having carbon numbers between C_{16} and C_{35} may accumulate in different tissues including adipose tissue, lymph nodes, spleen and liver.

- In rats, the terminal half-life in blood of MOSH in P15(H) white oils was between 23 and 59 hours, depending on the strain.

- MOSH concentrations observed in human tissues (mainly lymph nodes, liver, spleen and adipose tissue) demonstrate that accumulation of these compounds, mostly branched- and cyclo-alkanes, occurs in humans.

- Although limited information exists on toxicokinetics of MOAH, the available data indicate that these compounds are well absorbed and are rapidly distributed amongst all organs. The data also indicate that MOAH are extensively metabolised and do not bioaccumulate.

Toxicity

- MOSH and MOAH have low acute oral toxicity and given the current exposure via food the CONTAM Panel did not consider this relevant.

- Low molecular weight alkanes can cause α_{2u}-globulin related nephrotoxicity in male rats. This effect is not relevant for humans.

- MOSH mixtures with carbon number in the range C_{10}-C_{13} gave moderate hepatocyte hypertrophy. However, in the absence of pathological effects the CONTAM Panel did not consider this to be an adverse effect.

- In rats, MOSH with carbon number higher than C_{16} can bioaccumulate and may lead to formation of microgranulomas in the liver and mesenteric lymph nodes (MLN). Microgranulomas in MLN are considered of low toxicological concern because they are not associated with an inflammatory response or necrosis, do not progress to adverse lesions and available studies did not show an effect on immune functions. In the liver, microgranulomas were associated with inflammatory reactions.

- Lipogranulomas have been observed in humans in liver, spleen, lymph nodes and other organs, together with MOSH, but these changes are pathologically different from other granulomas and have not been associated with adverse consequences. There is no information on exposure levels at which these effects occur in humans.

- In arthritis-prone rodent models, intradermal and intraperitoneal injections of high doses of certain MOSH can induce autoimmune responses. Weaker effects were observed following short term percutaneous exposure. Whether long term oral exposure would have similar consequences is unknown although one short term study suggests this might not be the case.

- All MOH mixtures are mutagenic unless they are treated specifically to remove MOAH. The mutagenicity of MOH is caused mainly by 3-7 ring MOAH, including alkylated polycyclic aromatic hydrocarbons (PAHs) and non-alkylated PAHs. The latter group is mainly formed by the heating of the oil, is a minor fraction and some of these are covered by monitoring programmes in food. Many MOAH with three or more aromatic rings and little or no alkylation, and heterocyclic-containing analogues, can be activated by P450 enzymes into chemically reactive genotoxic carcinogens.

- MOSH are not carcinogenic, though long chain MOSH can act as tumour promoters at high doses.

- Some highly alkylated MOAH can also act as tumour promoters.

- Some simple MOAH, such as naphthalene, are carcinogenic most likely by a non-genotoxic mode of action, involving cytotoxicity and proliferative regeneration.

Hazard characterisation

Critical effects and Reference Points

- In view of the complexity and the limited information on the chemical composition of the MOAH and MOSH mixtures, and lack of possibility to separate the mixtures into individual compounds, it is not meaningful to base the hazard characterisation on single compounds or indicator substances. Hence, if possible, chemically-defined whole-mixture studies should be used for this purpose.

- For MOAH mixtures there are no dose-response data on the carcinogenicity and hence it is not possible to establish a Reference Point (RP) upon which to base a margin of exposure (MOE) calculation, which would normally be the approach for the risk characterisation of MOAH mixtures.

- The CONTAM Panel considered the formation of liver microgranulomas in the Fischer 344 rats as the critical effect of MOSH with carbon number between C_{16} and C_{35}. These have been observed for various MOSH products intended for food use. From the available information on the different white oils and waxes tested in toxicological studies it is not possible to differentiate between subclasses (e.g. n-, branched- or cyclo-alkanes) of MOSH. Studies used to identify the respective NOAELs were 90-day studies. The published data did not allow modelling of the dose-response data of the different studies. The CONTAM panel concluded that these NOAELs could potentially be used to select RPs for establishing health based guidance values.

Health based guidance values – products intended for food use

- The existing ADIs have been established for specific products intended for food use. The current classifications of food grade-MOH were set by SCF (1995), FAO/WHO (2002) and EFSA (2009), and are all based on toxicological studies with poorly characterised products with regard to chemical composition. Ideally, MOSH mixtures should be assessed by considering the molecular mass range and subclass composition (e.g. n-, branched- or cyclo-alkanes), rather than on physico-chemical properties such as viscosity.

- Based on new information about the lack toxicological relevance of the effects in MLN observed in the (sub)chronic studies in Fischer 344 rats and on newly available toxicokinetics studies, the CONTAM Panel concluded that:

  - a revision of the temporary group ADI established by JECFA for medium- and low-viscosity mineral oils class II and III is warranted;

  - with respect to microcrystalline waxes, high-viscosity mineral oils and medium- and low-viscosity class I mineral oils, the existing ADIs are of low priority for revision, although they are based on products with a poor chemical characterisation.

Reference Points for background and high level exposure scenarios

- The distribution of carbon numbers of the MOSH mixtures to which humans are exposed via food ranges from C_{12} to C_{40} with centres ranging from C_{18} to C_{34} in different foods. None of the existing ADIs was considered adequate for the risk characterisation of the range of MOH present in the background exposure of humans.

- In the absence of toxicological studies on MOSH mixtures typical to those humans are exposed to, the CONTAM Panel considered it inappropriate to establish a health based guidance value for MOSH.

- Given the deficiencies in the toxicity data base, the CONTAM Panel decided to use an MOE approach and for the background exposure selected as an RP the NOAEL for the most potent MOSH grades for microgranulomas of the liver, which was 19 mg/kg b.w. per day for low and intermediate melting point waxes.

- The range of MOSH involved in the high exposure scenarios (MOSH used as release agents for bread and rolls and for spraying of grains) is more restricted than that for the background exposure and therefore the CONTAM Panel used the highest NOAEL below the lowest LOAEL (lowest-observed-adverse-effect level) for these grades of MOSH, 45 mg/kg b.w. per day, as an RP.

Risk characterisation

MOAH

- The MOAH content of MOH present in food are mostly around 20 %, but may be up to 30 - 35 % of the MOH levels in vegetable oil and oil seeds. The MOAH fraction may be both mutagenic and carcinogenic, but no MOE for MOAH exposure via food could be derived. Because of its potential carcinogenic risk, the CONTAM Panel considers the exposure to MOAH through food to be of potential concern.

MOSH

- For background exposure from all sources for average consumption, the MOEs (based on maximum UB and minimum LB exposure across European dietary surveys, respectively) for toddlers and children and for adolescents and adults were from 100 to 290 and from 200 to 680, respectively. For high consumers of the same groups MOEs varied from 59 to 140 and from 95 to 330, respectively.

- In the high exposure scenarios with regular consumption of bread with high contents of MOSH, the MOEs (based on maximum and minimum exposure across European dietary surveys, respectively) were from 16 to 55 for average consumption, and in some cases below

10 for high consumption of bread and rolls. For the regular mean consumers of grains the MOEs varied greatly between different age classes and were between 35 (toddlers, maximum exposure across European dietary surveys) and 1 900 (other children, minimum exposure across European dietary surveys), and between 12 (toddlers, maximum exposure across European dietary surveys) and 200 (elderly, minimum exposure across European dietary surveys) for high consumers.

- The CONTAM Panel took into account that the basis for the RPs was 90-day studies and that some of these compounds might have very long elimination half lives in humans when interpreting the obtained MOEs.

- The CONTAM Panel considers that there is potential concern associated with the current background exposure to MOSH in Europe and in particular with the use of white oils as release agents for bread and to some extent for spraying of grains.

RECOMMENDATIONS

- There is a need for certified reference standards and reference materials for MOH to allow method development and (inter-laboratory) validation.

- Future monitoring should distinguish between MOAH and MOSH, and between subclasses of MOSH based on carbon numbers and chemical structures.

- The food classes to include for eventual monitoring should be based on the food groups making a relevant contribution to the background exposure and the special cases related to use of white oils.

- Sources of the contamination at various stages of food production should be identified in order to design an appropriate monitoring programme.

- MOH contamination of food by the use of recycled paperboard as packaging material may be a significant source of dietary exposure. It can be effectively prevented by the inclusion of functional barriers into the packaging assembly. Other measures may include segregation of recovery fibre sources intended for recycling and the increasing of the recyclability of food packages by avoiding the use of materials and substances with MOH in the production of food packages.

- Disposition data for multi-branched and cyclic MOSH are needed.

- The relevance of liver microgranulomas found in rat strains (Fischer 344 and Sprague Dawley) for the risk assessment in humans in respect of metabolic fate, sensitivity and potency of the different structural sub-classes of MOSH should be further investigated.

- It should be investigated whether MOSH exposure also via the oral route is associated with systemic autoimmune diseases or altered immune function as observed after some parenteral exposure.

- The toxicological evaluation of MOH should be focus on the molecular mass range and structural sub-classes, rather than chemico-physical properties such as viscosity.

DOCUMENTATION PROVIDED TO EFSA

1. Documentation Submitted to EFSA by CONCAWE

Bakker GCM, 2011. Absorption and kinetics of P15H oil after single oral administration of P15H oil in human volunteers. TNO Quality of Life Report V9501 (unpublished study report). Submitted in May 2011.

CONCAWE, 2011a. Executive summary – Single dose pharmacokinetic study of P15 white oil in two rat strains (TNO report V8503). Submitted in May 2011

CONCAWE, 2011b. Executive summary - Absorption and kinetics of P15H oil after single oral administration of p15h oil in human volunteers (TNO report V9051). Submitted in May 2011

CONCAWE, 2011c. Proposal for an internal exposure-based approach to derive an Acceptable Daily Intake (ADI) for low and medium viscosity mineral oils. Submitted in May 2011

Cnubben NHP, van Stee LLP, 2010. Single dose pharmacokinetic study of P15 white oil in two rat strains. TNO Quality of Life Report V8503 (unpublished study report). Submitted in May 2011

Van Stee LLP, 2011. Validation of an analytical method for the quantitative determination of P15 white oil in rat and human whole blood. TNO Triskelion report V8477 (unpublished study report). Submitted in May 2011

2. Documentation submitted to EFSA through BfR by ExxonMobil Biomedical

Exxon Biomedical Sciences, 1991a. 90-day subchronic oral toxicity study in rats with MRD-89-582. Project 158270 (unpublished study report). Submitted in August 2011.

Exxon Biomedical Sciences, 1991b. Test material MRD-90-868. 90-day oral toxicity in the rat. Project 186870 (unpublished study report). Submitted in August 2011.

Exxon Biomedical Sciences, 1991c. In vivo mammalian bone marrow micronucleus assay: oral gavage dosing method: MRD-89-582. Project 158830 (unpublished study report). Submitted in August 2011.

3. Documentation submitted to EFSA through BfR by Chevron Phillips Chemicals

Hazleton Laboratories, 1983. In vitro sister chromatide exchange in Chinese hamster ovary cells – Sotrol 130. Project No. 652-129 (unpublished study report). Submitted in August 2011.

4. Documentation submitted to EFSA by Shell Chemicals

Shell, 2009. Shell Process Oils. Shell Process Oil Range. January 2009, 48 pp. Submitted in August 2010.

Shell, 1998a. Shellsol D60: bacterial mutagenicity assay (Ames) in *Salmonella typhimurium*. Toxicology Report No. 98.1315 (unpublished study report). Submitted in May 2012.

Shell, 1998b. Shellsol D70: chromosome aberration study in cultured human lymphocytes. Toxicology Report No. 98.1317 (unpublished study report). Submitted in May 2012.

Shell, 1999. Shellsol TD: bacterial mutagenicity assay (Ames) in *Salmonella typhimurium*. Toxicology Report No. 99.1407 (unpublished study report). Submitted in May 2012.

REFERENCES

Abdo KM, Eustis SL, McDonald M, Jokinen MP, Adkins BJ and Haseman JK, 1992. Naphthalene: a respiratory tract toxicant and carcinogen for mice. Inhalation Toxicology, 4, 393-409.

Abdo KM, Grumbein S, Chou BJ and Herbert R, 2001. Toxicity and carcinogenicity study in F344 rats following 2 years of whole-body exposure to naphthalene vapors. Inhalation Toxicology, 13, 931-950.

Agarwal R, Kumar S and Mehrotra NK, 1986. Polyaromatic hydrocarbon profile of a mineral oil (JBO-P) by gas chromatography. Journal of Chromatographic Science, 24, 289-292.

Agarwal R, Shukla Y, Kumar S and Mehrotra NK, 1988. Evaluation of carcinogenic effect of jute batching oil (JBO-P) fractions following topical application to mouse skin. Archives of Toxicology, 62, 406-410.

Albro PW, 1976. Bacterial waxes. In: Chemistry and Biochemistry of Natural Waxes. Ed Kolattukudy PE. Elsevier, 419-450.

Albro PW and Fishbein L, 1970. Absorption of aliphatic hydrocarbons by rats. Biochimica et Biophysica Acta, 219, 437-446.

Albro PW and Thomas R, 1974. Intestinal absorption of hexachlorobenzene and hexachlorocyclohex and isomers in rats. Bulletin of Environmental Contamination and Toxicology, 12, 289-294.

Alnafouri AJ and Franz R, 1999. A study on the equivalence of olive oil and the EU official substitute test media for migration testing at high temperatures. Food Additives and Contaminants, 16, 419-431.

Ambaye RY, Panse TB and Tilak BD, 1961. Metabolism of Thiosters of Carcinogenic Hydrocarbons. Part I. Metabolism of Dibenzothiophene Proceedings of the Indian Academy of Sciences, Section B, 53, 149-156.

Amoruso MA, Gamble JF, McKee RH, Rohde AM and Jaques A, 2008. Review of the toxicology of mineral spirits. International Journal of Toxicology, 27, 97-165.

Anderson PN and Potter M, 1969. Induction of plasma cell tumours in BALB-c mice with 2,6,10,14-tetramethylpentadecane (pristane). Nature, 222, 994-995.

ANSES (French Food Safety Agency), 2009. Summary of the Report of the 2006/2007 Individual and National Study on Food Consumption 2 (INCA 2). Available from www.afssa.fr/Documents/PASER-Sy-INCA2.pdf.

API (American Petroleum Institute), 1961. Selected values of properties of hydrocarbons and related compounds. Research Project 44, Department of Chemistry, Agricultural and Mechanical College of Texas, TX, USA.

API (American Petroleum Institute), 2003. Robust Summary Information on aromatic extracts. Available from http://www.petroleumhpv.org/docs/aromatic_extracts/121503_aromext_robsumm_final.pdf.

ASTM (American Standardization for Testing and Materials), D86-10a. Standard test method for distillation of petroleum products at atmospheric pressure, American Standardization for Testing and Materials, West Conshohocken, PA, USA.

ASTM (American Standardization for Testing and Materials), D2887-08. Standard test method for boiling range distribution of petroleum fractions by gas chromatography. American Standardization for Testing and Materials, West Conshohocken, PA, USA.

ASTM (American Standardization for Testing and Materials), D7500-10. Standard test method for determination of boiling range distribution of distillates and lubricating base oils—in boiling range from 100 to 735 °C by gas chromatography. American Standardization for Testing and Materials, West Conshohocken, PA, USA.

ASTM (American Standardization for Testing and Materials), E 1687-95. Standard Test Method for Determining Carcinogenic Potential of Virgin Base Oils in Metalworking Fluids-Designation. American Standardization for Testing and Materials, West Conshohocken, PA, USA.

ATSDR (Agency for Toxic Substances and Disease Registry), 2005. Toxicological profile for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. U.S. Department of Health and Human Services – Public Health Service, August 2005, Atlanta, GA. Available from http://www.atsdr.cdc.gov/toxprofiles/tp67.pdf.

Avigan J and Blumer M, 1968. On the origin of pristane in marine organisms. Journal of Lipid Research, 9, 350-352.

Baird WM, Hooven LA and Mahadevan B, 2005. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environmental and Molecular Mutagenesis, 45, 106-114.

Baldwin MK, Berry PH, Esdaile DJ, Linnett SL, Martin JG, Peristianis GC, Priston RA, Simpson BJ and Smith JD, 1992. Feeding studies in rats with mineral hydrocarbon food grade white oils. Toxicologic Pathology, 20, 426-435.

Barrowman JA, Rahman A, Lindstrom MB and Borgstrom B, 1989. Intestinal absorption and metabolism of hydrocarbons. Progress in Lipid Research, 28, 189-203.

Baxter CS and Miller ML, 1987. Mechanism of mouse skin tumor promotion by n-dodecane. Carcinogenesis, 8, 1787-1790.

Baxter JH, Steinberg D, Mize CE and Avigan J, 1967. Absorption and metabolism of uniformly 14C-labeled phytol and phytanic acid by the intestine of the rat studied with thoracic duct cannulation. Biochimica et Biophysica Acta, 137, 277-290.

Becker W and Pearson M, 2002. Riksmaten 1997-98, Kostvanor och näringsintag I Sverige. Metod- och resultatanalysis.(Dietary habits and nutrient intake in Sweden 1997-98) Livsmedelsverket (National Food Administration) 1-201. Available from http://www.slv.se/upload/dokument/rapporter/kostundersokningar/riksmat.pdf

Beens J, 1998. Chromatographic couplings for unraveling oil fractions, PhD dissertation. University of Amsterdam. ISBN 90-901233-1.

Beens J, Blomberg J and Schoenmakers PJ, 2000. Proper tuning of comprehensive two-dimensional gas chromatography (GC x GC) to optimize the separation of complex oil fractions. Journal of High Resolution Chromatography, 23, 182-188.

BfR (Bundeninstitut für Risikobewertung), 2009. Übergänge von Mineralöl aus Verpackungsmaterialien auf Lebensmittel. Stellungnahme Nr. 008/2010 des BfR vom 09 Dezember 2009. Available from http://www.bfr.bund.de/cm/343/uebergaenge_von_mineraloel_aus_verpackungsmaterialien_auf_lebensmittel.pdf

BfR (Bundesinstitut für Risikobewertung), 2011. 7. Sitzung der BfR-Kommission für Bedarfsgegenstände (BeKo) – Protokoll der Sitzung vom 14 April 2011. Available from http://www.bfr.bund.de/cm/343/7_sitzung_der_bfr_kommission_fuer_bedarfsgegenstaende.pdf.

BfR (Bundeninstitut für Risikobewertung), 2012. Bestimmung von Kohlenwasserstoffen aus Mineralöl (MOSH und MOAH) oder Kunststoffen (POSH, PAO) in Verpackungsmaterialien und trockenen Lebensmitteln mittels Festphasenextraktion und GC-FID. Available at: http://www.bfr.bund.de/cm/343/bestimmung-von-kohlenwasserstoffen-aus-mineraloel-oder-kunststoffen.pdf

Biedermann-Brem S and Grob K, 2011. Removal of mineral oil migrated from paperboard packing during cooking of foods in boiling water. European Food Research and Technology 232, 1035-1041

Biedermann-Brem S, Kasprick N, Simat T and Grob K, 2012. Migration of polyolefin oligomeric saturated hydrocarbons (POSH) into food. Food Additives Contaminants. Part A, 29, 449-460.

Biedermann M, Fiselier K and Grob K, 2009. Aromatic hydrocarbons of mineral oil origin in foods: method for determining the total concentration and first results. Journal of Agricultural and Food Chemistry, 57, 8711-8721.

Biedermann M and Grob K, 2009. How "white" was the mineral oil in the contaminated Ukrainian sunflower oils? European Journal of Lipid Science and Technology, 111, 313-319.

Biedermann M and Grob K, 2010. Is recycled newspaper suitable for food contact materials? Technical grade mineral oils from printing inks. European Food Research and Technology, 230, 785-796.

Biedermann M and Grob K, 2012a. On-line coupled high performance liquid chromatography – gas chromatography (HPLC-GC) for the analysis of mineral oil. Part 1: method of analysis in foods, environmental samples and other matrices. A review. Journal of Chromatography A, in press.

Biedermann M and Grob K, 2012b. On-line coupled high performance liquid chromatography – gas chromatography (HPLC-GC) for the analysis of mineral oil; Part 1: Part 2: migrated from paperboard into dry foods: interpretation of chromatograms. A review. Journal of Chromatography A, in press.

Biedermann M, Uematsu Y and Grob K, 2011. Mineral oil contents in paper and board recycled to paperboard for food packaging. Packaging Technology and Science, 24, 61-73.

Bingham E, 1988. Carcinogenicity of mineral oils. Annals of the New York Academy of Sciences, 534, 452-458.

Bingham E and Barkley W, 1979. Bioassay of complex mixtures derived from fossil fuels. Environ Health Perspect, 30, 157-163.

Bird MG, Lewis SC, Freeman JJ, Smith JH, Hogan GK, and Scala RA, 1990. Subchronic feeding study of white mineral oils in rats and dogs. Toxicologist 10: 143.

Blackburn GR, Deitch RA, Schreiner CA and Mackerer CR, 1986. Predicting carcinogenicity of petroleum distillation fractions using a modified Salmonella mutagenicity assay. Cell Biology and Toxicology, 2, 63-84.

Blackburn GR, Deitch RA, Schreiner CA, Mehlman MA and Mackerer CR, 1984. Estimation of the dermal carcinogenic activity of petroleum fractions using a modified Ames assay. Cell Biology and Toxicology, 1, 67-80.

Blewitt RW, Bradbury K, Greenall MJ and Burrow H, 1977. Hepatic damage associated with mineral oil deposits. Gut, 18, 476-479.

BLIC (European Association of the Rubber Industry), 2005. Literature study on substances leached from shredded and whole used tyres. Brussels. July 2005.

Bock KW and Kohle C, 2006. Ah receptor: dioxin-mediated toxic responses as hints to deregulated physiologic functions. Biochemical Pharmacology, 72, 393-404.

Boitnott JK and Margolis S, 1970. Saturated hydrocarbons in human tissues. 3. Oil droplets in the liver and spleen. Johns Hopkins Medical Journal, 127, 65-78.

Booth ED, Brandt HC, Loose RW and Watson WP, 1998. Correlation of 32P-postlabelling-detection of DNA adducts in mouse skin *in vivo* with the polycyclic aromatic compound content and mutagenicity in *Salmonella typhimurium* of a range of oil products. Archives of Toxicology, 72, 505-513.

Bradley EL and Castle L, 2007. Food packaging adhesives and chemical migration into foods. In: Chemical migration and food contact materials. Eds Barnes KA, Sinclair R and Watson D. Woodhead Publishing, Cambridge, U.K., 320-332.

Brandenberger S, Mohr M, Grob K and Neukomb HP, 2005. Contribution of unburned lubricating oil and diesel fuel to particulate emission from passenger cars. Atmospheric Environment, 39, 6985-6994.

Brandt HC, Booth ED, de Groot PC and Watson WP, 1999. Development of a carcinogenic potency index for dermal exposure to viscous oil products. Archives of Toxicology, 73, 180-188.

Bray HG, Carpanini FM and Waters BD, 1971. The metabolism of thiophen in the rabbit and the rat. Xenobiotica, 1, 157-168.

Brooks TM, Priston RA, Wright AS and Watson WP, 1995. Evaluation of modified bacterial mutagenicity assays for the genotoxicity testing of mineral oils. Mutagenesis, 10, 409-415.

Burleson GR, 2001. Unpublished study conducted by Burleson Research Technologies, Raleigh, North Carolina, USA for American Petroleum Institute, Washington DC, USA, as described in WHO/IPCS, 2003.

Calvani N, Caricchio R, Tucci M, Sobel ES, Silvestris F, Tartaglia P and Richards HB, 2005. Induction of apoptosis by the hydrocarbon oil pristane: implications for pristane-induced lupus. Journal of Immunology, 175, 4777-4782.

Cannon GW, Woods ML, Clayton F and Griffiths MM, 1993. Induction of arthritis in DA rats by incomplete Freund's adjuvant. Journal of Rheumatology, 20, 7-11.

Carlson BC, Jansson AM, Larsson A, Bucht A and Lorentzen JC, 2000. The endogenous adjuvant squalene can induce a chronic T-cell-mediated arthritis in rats. American Journal of Pathology, 156, 2057-2065.

Carlton WW, Boitnott JK, Dungworth DL, Ernst H, Hayashi Y, Mohr U, Parodi AL, Pattengale PK, Rittinghausen S and Ward JM, 2001. Assessment of the morphology and significance of the lymph nodal and hepatic lesions produced in rats by the feeding of certain mineral oils and waxes. Proceedings of a pathology workshop held at the Fraunhofer Institute of Toxicology and Aerosol Research Hannover, Germany, May 7-9, 2001. Experimental and Toxicologic Pathology, 53, 247-255.

Castle L, Kelly M and Gilbert J, 1991. Migration of mineral hydrocarbons into foods. 1. Polystyrene containers for hot and cold beverages. Food Additives and Contaminants, 8, 693-699.

Castle L, Kelly M and Gilbert J, 1993a. Migration of mineral hydrocarbons into foods. 3. Cheese coatings and temporary casings for skinless sausages. Food Additives and Contaminants, 10, 175-184.

Castle L, Kelly M and Gilbert J, 1993b. Migration of mineral hydrocarbons into foods. 2. Polystyrene, ABS, and waxed paperboard containers for dairy products. Food Additives and Contaminants, 10, 167-174.

Cerbulis J, Flanagan VP and Farrell HM, Jr., 1985. Composition of the hydrocarbon fraction of goats' milk. Journal of Lipid Research, 26, 1438-1443.

Chevron Phillips, 2010. Soltron 130 Isoparaffin – Issued sales specification. Available from http://www.cpchem.com/bl/specchem/en-us/tdslibrary/Soltrol_130_Isoparaffin.pdf

Chou MW, Yang SK, Sydor W and Yang CS, 1981. Metabolism of 7,12-dimethylbenz(a)anthracene by rat liver nuclei and microsomes. Cancer Research, 41, 1559-1564.

CONCAWE (Conservation of Clean Air and Water in Europe), 1984. Assessment and comparison of the composition of food-grade white oils and waxes manufactured from petroleum by catalytic hydrogenation versus conventional treatment. CONCAWE report N°84/60. CONCAWE, Brussels, Belgium.

CONCAWE (Conservation of Clean Air and Water in Europe), 1993. White oils and waxes-summary of 90-day studies. CONCAWE Report N°93/56. CONCAWE, Brussels, Belgium.

CONCAWE (Conservation of Clean Air and Water in Europe), 2006. Application dossier for the use of of high viscosity white mineral oils (CAS # 8042-47-5) as a food additive and as a processing aid in foods. Submitted by the 'European Oil Company Organisation for Environment, Health and Safety' (CONCAWE), Belgium.

Concin N, Hofstetter G, Plattner B, Tomovski C, Fiselier K, Gerritzen K, Fessler S, Windbichler G, Zeimet A, Ulmer H, Siegl H, Rieger K, Concin H and Grob K, 2008. Mineral oil paraffins in human body fat and milk. Food and Chemical Toxicology, 46, 544-552.

Concin N, Hofstetter G, Plattner B, Tomovski C, Fiselier K, Gerritzen K, Semsroth S, Zeimet A, Marth C, Siegl H, Rieger K, Ulmer H, Concin H and Grob K, 2011. Evidence for cosmetics as source of mineral oil contamination in women. Journal of Women's Health, 20, 1713-1719.

Coppock RW, Khan AA, Geleta L, Dziwenka MM, Nation PN and Hiltz MN, 2002. Translocation of biomarker chemicals into sheep tissues after oral exposure to crude oil. Toxicological Sciences, 66, 64 (Abs. 311).

Coppock RW, Khan AA, Geleta LM and Hiltz MN, 2001. Biomarker chemicals in fat after oral exposure of cattle to petroleum. Toxicological Sciences, 60, 232 (Abs. 1105).

Cox JC and Coulter AR, 1997. Adjuvants - a classification and review of their modes of action. Vaccine, 15, 248-256.

Cravedi J, Thibaut R, Tulliez J and Perdu E, 2011. Comparative in vitro study of the biotransformation of n-alkanes by liver and small intestine microsomes from different rat strains. Toxicology Letters, 205, S188-S188.

Cravedi JP and Tulliez J, 1981. Distribution and elimination routes of a naphthenic hydrocarbon (dodecylcyclohexane) in rainbow trout (*Salmo gairdneri*). Bulletin of Environment Contamination and Toxicology, 26, 337-344.

Cravedi JP and Tulliez J, 1982. Accumulation, distribution and depuration in trout of naphthenic and isoprenoid hydrocarbons (dodecylcyclohexane and pristane). Bulletin of Environment Contamination and Toxicology, 28, 154-161.

Cravedi JP and Tulliez J, 1986. Metabolism of n-alkanes and their incorporation into lipids in the rainbow trout. Environmental Research, 39, 180-187.

Cravedi JP and Perdu E, 2012. In vitro metabolic study on alkanes in hepatic microsomes from humans and rats. EFSA External Scientific Report. Supporting Publication, 263, 64 pp.

Croisy A, Mispelter J, Lhoste JM, Zajdela F and Jacquignon P, 1984. Thiophene analogues of carcinogenic polycyclic hydrocarbons. Elbs pyrolysis of various aroylmethylbenzo[b]thiophenes. Journal of Heterocyclic Chemistry, 21, 353-359.

Cruickshank B, 1984a. Follicular (mineral oil) lipidosis: I. Epidemiologic studies of involvement of the spleen. Human Pathology, 15, 724-730.

Cruickshank B and Thomas MJ, 1984b. Mineral oil (follicular) lipidosis: II. Histologic studies of spleen, liver, lymph nodes, and bone marrow. Human Pathology, 15, 731-737.

Dahlgren J, Takhar H, Anderson-Mahoney P, Kotlerman J, Tarr J and Warshaw R, 2007. Cluster of systemic lupus erythematosus (SLE) associated with an oil field waste site: a cross sectional study. Environmental Health, 6, 8.

Dalbey WE and Biles RW, 2003. Respiratory toxicology of mineral oils in laboratory animals. Applied Occupational & Environmental Hygiene, 18, 921-929.

Danish Food Composition Databank - ed. 7.01 – Available from http://www.foodcomp.dk/v7/fcdb_default.asp (retrieved 13.12.2011).

Dansette PM, Thang DC, el Amri H and Mansuy D, 1992. Evidence for thiophene-S-oxide as a primary reactive metabolite of thiophene in vivo: formation of a dihydrothiophene sulfoxide mercapturic acid. Biochem Biophys Res Commun, 186, 1624-1630.

Davidsohn I and Stern K, 1960. Letter to J.I. Weeks. As reported from FAO/WHO, 1993.

De Vriese S, Huybrecht I, Moreau M, De Henauw S, De Backer G, Kornlitzer M, Leveque A and Van Oyen H, 2005. The Belgian food consumption survey: aim, design and methods. Archives of Public Health, 63, 1-16.

Delladetsima JK, Horn T and Poulsen H, 1987. Portal tract lipogranulomas in liver biopsies. Liver, 7, 9-17.

Diehl JW and Di Sanzo FP, 2005. Determination of aromatic hydrocarbons in gasolines by flow modulated comprehensive two-dimensional gas chromatography. Journal of Chromatography A, 1080, 157-165.

Dill JA, Fuciarelli AF, Lee KM, Mellinger KM, Chan PC, Burka LT and Roycroft JH, 2003a. Single administration toxicokinetic studies of decalin (decahydronaphthalene) in rats and mice. Toxicological Sciences, 72, 210-222.

Dill JA, Lee KM, Renne RA, Miller RA, Fuciarelli AF, Gideon KM, Chan PC, Burka LT and Roycroft JH, 2003b. Alpha 2u-globulin nephropathy and carcinogenicity following exposure to decalin (decahydronaphthalene) in F344/N rats. Toxicological Sciences, 72, 223-234.

Dincsoy HP, Weesner RE and MacGee J, 1982. Lipogranulomas in non-fatty human livers. A mineral oil induced environmental disease. American Journal of Clinical Pathology, 78, 35-41.

Doak SM, Brown VK, Hunt PF, Smith JD and Roe FJ, 1983. The carcinogenic potential of twelve refined mineral oils following long-term topical application. British Journal of Cancer, 48, 429-436.

Droz C and Grob K, 1997. Determination of food contamination by mineral oil material from printed cardboard using on-line coupled LC-GC-FID. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-Food Research and Technology, 205, 239-241.

Du J, 2002. Combined algorithms for constrained estimation of finite mixture distributions with grouped data and conditional data. Master of Science, McMaster University (Statistics), Hamilton, Ontario.124 pp.

Dubuisson C, Lioret S, Touvier M, Dufour A, Calamassi-Tran G, Volatier JL and Lafay L, 2010. Trends in food and nutritional intakes of French adults from 1999 to 2007: results from the INCA surveys. British Journal of Nutrition, 103, 1035-1048.

Dukan P, 2010. The Dukan Diet. Ed. Hodder & Stoughton Ltd.

ECB (European Chemical Bureau), 2003. European Union Risk Assessment Report – Naphthalene. Institute for Health and Consumer Protection – ECB, Existing Substances 1st Priority List, Vol. 33. Available from http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/naphthalenereport020.pdf.

ECHA (European Chemicals Agency), 2012a. Published information on the REACH Registration Dossier on White mineral oil (petroleum) (CAS Number 8042-47-5). Available from http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances.

ECHA (European Chemicals Agency), 2012b. Published information on the REACH Registration Dossier on Distillates (petroleum), light paraffinic (CAS Number 64741-50-0). Available from http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances.

ECHA (European Chemicals Agency), 2012c. Published information on the REACH Registration Dossier on Distillates (petroleum), solvent-refined heavy paraffinic (CAS Number 64741-88-4). Available from http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances.

Edam R, Blomberg J, Janssen HG and Schoenmakers PJ, 2005. Comprehensive multi-dimensional chromatographic studies on the separation of saturated hydrocarbon ring structures in petrochemical samples. Journal of Chromatography. A, 1086, 12-20.

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related the use of mineral oils in jute and sisal bags. The EFSA Journal, 162, 1-6.

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment. The EFSA Journal, 438, 1-57.

EFSA (European Food Safety Authority), 2008a. Conclusion on pesticide peer review regarding the risk assessment of the active substance paraffin oil (CAS 8042-47-5, chain lengths C18-C30, reliable boiling point range not available). EFSA Scientific Report The EFSA Journal, 219, 1-61.

EFSA (European Food Safety Authority), 2008b. Conclusion on pesticide peer review regarding the risk assessment of the active substance paraffin oil (CAS 8042-47-5, chain lengths C17-C31, boiling point 280-460 °C). EFSA Scientific Report The EFSA Journal, 220, 1-42.

EFSA (European Food Safety Authority), 2008c. Conclusion on pesticide peer review regarding the risk assessment of the active substance paraffin oil CAS 64742-46-7 chain lengths C11-C25, CAS 72623-86-0 chain lengths C15-C30, CAS 97862-82-3 chain lengths C11-C30. EFSA Scientific Report. The EFSA Journal, 216, 1-59.

EFSA (European Food Safety Authority), 2008d. Polycyclic Aromatic Hydrocarbons in Food - Scientific Opinion of the Panel on Contaminants in the Food Chain. The EFSA Journal, 724, 1-114.

EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp.

EFSA (European Food Safety Authority), 2011a. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 27 pp.

EFSA (European Food Safety Authority), 2011b. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp.

EFSA (European Food Safety Authority), 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2009. Scientific Opinion on the use of high viscosity white mineral oils as a food additive. EFSA Journal 2009;7(11):1387, 1-39.

Elliott TH, Robertson JS and Williams RT, 1966. The metabolism of cis- and trans-decalin. Biochemical Journal, 100, 403-406.

Elmadfa I, Freisling H, Nowak V and Hofstädter D, 2009. Austrian Nutrition Report 2008 (Österreichischer Ernährungsbericht 2008). 1st edition, Vienna.

EMEA (The European Agency for the Evaluation of Medicinal Products), 1995. The European Agency for the Evaluation of Medicinal Products. Committee for Veterinary medicinal Products. Mineral hydrocarbons – Summary Report. EMEA/CVMP/069/95-FINAL. Available from http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015093.pdf.

Epler JL, Rao TK and Guerin MR, 1979. Evaluation of feasibility of mutagenic testing of shale oil products and effluents. Environmental Health Perspectives, 30, 179-184.

FAO/WHO (Food and Agriculture Organization/World Health Organization), 1993. Evaluation of certain food additives: thirty-ninth meeting of the Joint FAO/WHO Expert Committee on food

Additives. WHO Food Additive Series, No. 30. Available from http://www.inchem.org/documents/jecfa/jecmono/v30je14.htm.

FAO/WHO (Food and Agriculture Organization/World Health Organization), 1995. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives. Mineral oil. Available from http://www.inchem.org/documents/jecfa/jeceval/jec_1655.htm.

FAO/WHO (Food and Agriculture Organization/World Health Organization), 2002. Evaluation of certain food additives: fifty-ninth report of the Joint FAO/WHO Expert Committee on food Additives. WHO Technical Report Series 913. Available from http://whqlibdoc.who.int/trs/WHO_TRS_913.pdf.

FAO/WHO (Food and Agriculture Organization/World Health Organization), 2006. Safety Evaluation of Certain Contaminants in Food, WHO Food Additives Series: 55, WHO, Geneva, 2006. Polycyclic aromatic hydrocarbons, pp 563-743. Available from http://whqlibdoc.who.int/publications/2006/9241660554_PAH_eng.pdf.

FAO/WHO (Food and Agriculture Organization/World Health Organization), 2009. Evaluation of certain food additives: sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives: Evaluation of Calcium lignosulfonate (40-65). WHO Technical Report Series 952. Available from http://whqlibdoc.who.int/trs/WHO_TRS_952.pdf.

Fehler SW and Light RJ, 1972. Biosynthesis of methylheptadecanes in Anabaena variabilis. In vitro incorporation of S-(methyl- 14 C)adenosylmethionine. Biochemistry, 11, 2411-2416.

Felix K, Kelliher K, Bornkamm GW and Janz S, 1998. Association of elevated mutagenesis in the spleen with genetic susceptibility to induced plasmacytoma development in mice. Cancer Research, 58, 1616-1619.

Felix K, Kelliher KA, Bornkamm GW and Janz S, 1999. Elevated mutant frequencies in lymphoid tissues persist throughout plasmacytoma development in BALB/c.lambdaLIZ mice. Cancer Research, 59, 3621-3626.

Felix K, Potter M, Bornkamm GW and Janz S, 1997. In vitro mutagenicity of the plasmacytomagenic agent pristane (2,6,10,14-tetramethylpentadecane). Cancer Letters, 113, 71-76.

Fineli ® - Finnish Food Composition Database - http://www.fineli.fi/index.php?lang=en (retrieved 13.12.2011).

Fiorini D, Fiselier K, Biedermann M, Ballini R, Coni E and Grob K, 2008. Contamination of grape seed oil with mineral oil paraffins. Journal of Agricultural and Food Chemistry, 56, 11245-11250.

Fiorini D, Paciaroni A, Gigli F and Ballini R, 2010. A versatile splitless injection GC-FID method for the determination of mineral oil paraffins in vegetable oils and dried fruit. Food Control, 21, 1155-1160.

Firriolo JM, Morris CF, Trimmer GW, Twitty LD, Smith JH and Freeman JJ, 1995. Comparative 90-day feeding study with low-viscosity white mineral oil in Fischer-344 and Sprague-Dawley-derived CRL:CD rats. Toxicologic Pathology, 23, 26-33.

Fiselier K, Fiorini D and Grob K, 2009a. Activated aluminum oxide selectively retaining long chain n-alkanes. Part I, description of the retention properties. Analytica Chimica Acta, 634, 96-101.

Fiselier K, Fiorini D and Grob K, 2009b. Activated aluminum oxide selectively retaining long chain n-alkanes: Part II. Integration into an on-line high performance liquid chromatography-liquid chromatography-gas chromatography-flame ionization detection method to remove plant paraffins for the determination of mineral paraffins in foods and environmental samples. Analytica Chimica Acta, 634, 102-109.

Fiselier K and Grob K, 2008. Method demonstrated at the workshop of the European Commission and the Official Food Control Authority of the Canton of Zurich "Mineral oil material in foods: analytical methods, occurrence, evaluation", 17/18 Sept. 2008.

Fiselier K and Grob K, 2009. Determination of mineral oil paraffins in foods by on-line HPLC-GC-FID: lowered detection limit; contamination of sunflower seeds and oils. European Food Research and Technology, 229, 679-688.

Fisher HG, Priestley W, Jr., Eby LT, Wanless GG and Rehner J, Jr., 1951. Properties of high-boiling petroleum products; physical and chemical properties as related to carcinogenic activity. Archives of Industrial Hygiene and Occupational Medicine, 4, 315-324.

Fleming KA, Zimmerman H and Shubik P, 1998. Granulomas in the livers of humans and Fischer rats associated with the ingestion of mineral hydrocarbons: a comparison. Regulatory Toxicology and Pharmacology, 27, 75-81.

Flesher JW, 1967. Distribution of radioactivity in the tissues of rats after oral administration of 7,12-dimethylbenz(A)anthracene-3H. Biochemical Pharmacology, 16, 1821-1831.

Food Chemical Risk Analysis, 2001. The usage, occurrence and dietary intake of white mineral oils and waxes and naturally occurring hydrocarbons in Europe. Prepared for CONCAWE and EWF.

FSA (Food Standards Agency), 2003. Mineral hydrocarbons in food contact materials,. Food Survey Information Sheet Number 34/03. 29th January 2003. http://www.food.gov.uk/science/surveillance/fsis2003/34fcm.

FSA (Food Standards Agency), 2011. Migration of selected ink components from printed packaging materials into foodstuffs and screening of printed packaging for the presence of mineral oils. Food Survey Information Sheet Number 03/11 December 2011, http://www.food.gov.uk/multimedia/pdfs/fsis0311.pdf.

Gabrijelčič Blenkuš M, Gregorič M, Tivadar B, Koch V, Kostanjevec S, Turk Fajdiga V, Žalar A, Lavtar D, Kuhar D and Rozman U, 2009. Prehrambene navade odraslih prebivalcev Slovenije z vidika varovanja zdravja. Ljubljana, Pedagoška fakulteta, Inštitut za varovanje zdravja RS.

Gaines TB, 1969. Acute toxicity of pesticides. Toxicology and Applied Pharmacology, 14, 515-534.

Gary JH and Handwerk GE, 1984. Petroleum Refining Technology and Economics, 2nd Edition, Marcel Dekker, Inc., 1984.

Granella M and Clonfero E, 1991. The mutagenic activity and polycyclic aromatic hydrocarbon content of mineral oils. International Archives of Occupational and Environmental Health, 63, 149-153.

Griffis LC, Twerdok LE, Francke-Carroll S, Biles RW, Schroeder RE, Bolte H, Faust H, Hall WC and Rojko J, 2010. Comparative 90-day dietary study of paraffin wax in Fischer-344 and Sprague-Dawley rats. Food and Chemical Toxicology, 48, 363-372.

Grob K, 1991. On-Line Coupled LC-GC. Hüthig, Heidelberg. ISBN 3-7785-1872-0.

Grob K, 2007. Verification of results to improve the quality of analytical data: A proposal. Journal of Chromatography A, 1150, 93-99.

Grob K, Artho A, Biedermann M, Caramaschi A and Mikle H, 1992b. Batching oils on sisal bags used for packaging foods: analysis by coupled LC-GC. Journal of AOAC International, 75, 283-287.

Grob K, Artho A, Biedermann M and Egli J, 1991a. Food contamination by hydrocarbons from lubricating oils and release agents - determination by coupled LC-GC. Food Additives and Contaminants, 8, 437-446.

Grob K, Artho A, Biedermann M and Mikle H, 1993. Contamination of hazel nuts and chocolate by mineral oil from jute and sisal bags. Zeitschrift fur Lebensmittel-Untersuchung und -Forschung, 197, 370-374.

Grob K, Biedermann M, Artho A and Egli J, 1991b. Food contamination by hydrocarbons from packaging materials determined by coupled LC-GC. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung, 193, 213-219.

Grob K, Biedermann M and Bronz M, 1994. Results of a control of edible oils: Frauds by admixtures, contaminations. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene, 85, 351-365.

Grob K, Biedermann M, Caramaschi A and Pacciarelli B, 1991d. LC-GC analysis of the aromatics in a mineral-oil fraction - batching oil for jute bags. Journal of High Resolution Chromatography, 14, 33-39.

Grob K, Huber M, Boderius U and Bronz M, 1997. Mineral oil material in canned foods. Food Additives and Contaminants, 14, 83-88.

Grob K, Lanfranchi M, Egli J and Artho A, 1991c. Determination of food contamination by mineral-oil from jute sacks using coupled LC-GC. Journal of the Association of Official Analytical Chemists, 74, 506-512.

Grob K, Vass M, Biedermann M and Neukom HP, 2001. Contamination of animal feed and food from animal origin with mineral oil hydrocarbons. Food Additives and Contaminants, 18, 1-10.

Grova N, Feidt C, Laurent C and Rychen G, 2002. C-14 Milk, urine and faeces excretion kinetics in lactating goats after an oral administration of C-14 polycyclic aromatic hydrocarbons. International Dairy Journal, 12, 1025-1031.

Grundböck F, Fiselier K and Grob K, 2010a. Method demonstrated at the workshop of the Bundesinstitut für Risikobewertung (BfR, Berlin) and the Official Food Control Authority of the Canton of Zurich "Mineralölanalytik im Lebensmittelbereich. Methoden für MOSH und MOAH, Vorkommen und Risikobewertung", 10/11 June 2010.

Grundböck F, Fiselier K, Schmid F and Grob K, 2010b. Mineral oil in sunflower seeds: the sources. European Food Research and Technology, 231, 209-213.

Guinda A, Lanzón A, Albi T, 1996. Differences in hydrocarbons of virgin olive oils obtained from several olive varieties. Journal of Agricultural and Food Chemistry 44, 1723-1726.

Halladay JS, Mackerer CR, Twerdok LE and Sipes IG, 2002. Comparative pharmacokinetic and disposition studies of [1-14C]1-eicosanylcyclohexane, a surrogate mineral hydrocarbon, in female Fischer-344 and Sprague-Dawley rats. Drug Metabolism and Disposition: The Biological Fate of Chemicals, 30, 1470-1477.

Han J and Calvin M, 1969. Hydrocarbon distribution of algae and bacteria, and microbiological activity and sediments Proceedings of the National Academy of Science USA 64, 436–443.

Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE and Valcovic LR, 1993. Hazard evaluation of chemicals that cause accumulation of alpha 2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environmental Health Perspectives, 99, 313-349.

Harrington KE, Robson PJ, Kiely M, Livingstone MB, Lambe J and Gibney MJ, 2001. The North/South Ireland Food Consumption Survey: survey design and methodology. Public Health Nutrition, 4, 1037-1042.

Heimbach JT, Bodor AR, Douglass JS, Barraj LM, Cohen SC, Biles RW and Faust HR, 2002. Dietary exposures to mineral hydrocarbons from food-use applications in the United States. Food and Chemical Toxicology, 40, 555-571.

Henderson L, Gregory J and Swan G, 2002. NDNS (National Diet and Nutrition Survey), National Diet and Nutrition Survey: Adults Aged 19 to 64 Years: volume 1: types and quantities of foods consumed, London: TSO.

Hill P, 2007. "Can-Making". Presentation made at the UK-Defra LINK FSQ45 dissemination seminar 'New technologies and chemistries for food can coatings'. Leeds University, Leeds (UK), 2nd May 2007.

Höke H and Zellerhoff R, 1998. Metabolism and toxicity of diisopropylnaphthalene as compared to naphthalene and monoalkyl naphthalenes: a minireview. Toxicology, 126, 1-7.

Holm BC, Svelander L, Bucht A and Lorentzen JC, 2002. The arthritogenic adjuvant squalene does not accumulate in joints, but gives rise to pathogenic cells in both draining and non-draining lymph nodes. Clinical and Experimental Immunology, 127, 430-435.

Holmdahl R, Lorentzen JC, Lu S, Olofsson P, Wester L, Holmberg J and Pettersson U, 2001. Arthritis induced in rats with nonimmunogenic adjuvants as models for rheumatoid arthritis. Immunological Reviews, 184, 184-202.

Horton AW, Bolewicz LC, Barstad AW and Butts CK, 1981. Comparison of the promoting activity of pristane and n-alkanes in skin carcinogenesis with their physical effects on micellar models of biological membranes. Biochimica et Biophysica Acta: Protein Structure and Molecular Enzymology, 648, 107-112.

Horton AW, Denman DT and Trosset RP, 1957. Carcinogenesis of the skin. II. The accelerating properties of aliphatic and related hydrocarbons. Cancer Research, 17, 758-766.

Horton AW, Eshleman DN, Schuff AR and Perman WH, 1976. Correlation of cocarcinogenic activity among n-alkanes with their physical effects on phospholipid micelles. Journal of the National Cancer Institute, 56, 387-391.

Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, Neve Md, Arcella D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T, Oltarzewski M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-Majem L, Volatier JL, Trolle E and de Neve M, 2011. Dietary exposure assessments for children in Europe (the EXPOCHI project): rationale, methods and design. Archives of Public Health, 69, 1-7.

IARC (International Agency for Research on Cancer), 1984. Polynuclear Aromatic Compounds, Part 2. Carbon blacks, mineral oils and some nitroarenes. IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans, vol. 33. Lyon, France: International Agency for Research on Cancer. 245 pp.

IARC (International Agency for Research on Cancer), 1987. Genetic and related effects: An updating of selected IARC monographs from Volumes 1 to 42. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, Supplement 6 IARC Press, Lyon, France. 729 pp.

IARC (International Agency for Research on Cancer), 1993. Scientific Publications No. 147 Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis Edited by C.C. Capen, E. Dybing, J.M. Rice and J.D. Wilbourn.

IARC (International Agency for Research on Cancer), 2002. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans, vol. 82. IARC Press, Lyon, France. 601 pp.

IARC (International Agency for Research on Cancer), 2010. Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans, Vol. 92. IARC Press, Lyon, France. 853 pp.

Ichihara K, Ishihara K, Kusunose E and Kusunose M, 1981. Some properties of a hexadecane hydroxylation system in rabbit intestinal mucosa microsomes. Journal of Biochemistry, 89, 1821-1827.

ImmunoTox 2001. Effects of dietary mineral hydrocarbons on T-cell dependent antibody production in two strains of rats. Unpublished study ITI 1298 prepared for American Petroleum Institute, Washington DC, as described by WHO/IPCS, 2003.

Ingram AJ and Phillips JC, 1993. The dermal bioavailability of radiolabelled benzo[a]pyrene from acetone or from oils of differing viscosity, assessed by DNA and protein binding. Journal of Applied Toxicology, 13, 25-32.

Ingram AJ, Phillips JC and Davies S, 2000. DNA adducts produced by oils, oil fractions and polycyclic aromatic hydrocarbons in relation to repair processes and skin carcinogenesis. Journal of Applied Toxicology, 20, 165-174.

IOM (Institute of Medicine of the U.S. National Academies of Sciences), 1991. Nutrition During Lactation. National Academy Press. Washington, D.C.

IPCS (International Programme on Chemical Safety), 1998. Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria 202. International Programme on Chemical Safety, World Health Organization, Geneva.

Iversen OH, 1989. Studies of the carcinogenesis and tumorigenesis of skin applications of dodecylbenzene on hairless mice. British Journal of Industrial Medicine, 46, 608-616.

Iwahara S, 1974. Studies on metabolism of di-isopropylnaphthalene. National Defense Medical Journal, 21, 273-290.

Jackson LL and Blomquist GL, 1976. Cuticular lipids of insects: VIII. Alkanes of the Mormon cricket *Anabrus simplex*. Lipids 11, 77-79.

Jacob S, 1990. Sulfur analogues of polycyclic aromatic hydrocarbons (thiarenes). Cambridge University Press, Cambridge, 295 pp.

Jakobsen BM, Hass U, Jull F, Kjaergaard S, 1986. Prenatal toxicity of white spirit inhalation in the rat. Teratology 354, 415.

Janz S and Shacter E, 1995. Disposition of the plasmacytomagenic alkane pristane (2,6,10,14-tetramethylpentadecane) in mice. Cancer Biochemistry Biophysics, 15, 25-34.

Jickells SM, Nichol J and Castle L, 1994. Migration of mineral hydrocarbons into foods .6. Press lubricants used in food and beverage cans. Food Additives and Contaminants, 11, 595-604.

JRC (Joint Research Centre), 2008. Scientific and Technical Report, EUR 23811EN (2009). Final report on "Proficiency test on the determination of mineral oil in sunflower oil. Available from http://irmm.jrc.ec.europa.eu/html/publications/technical_reports/food,_biotechnology_and_health/EUR_23811_sunflower_oil.pdf.

Kaneda T, 1968. Biosynthesis of long-chain hydrocarbons. II. Studies on the biosynthetic pathway in tobacco. Biochemistry 7, 1194-1202

Kasprick N, Grob K, Biedermann M and Simat TJ 2010. Migration resorbierbarer Kohlenwasserstoffe aus PE und PP in fettreiche Lebensmittel. Poster at Deutscher Lebensmittelchemikertag, Stuttgart, Sept. 20-22, 2010.

Kent JC, Mitoulas L, Cox DB, Owens RA and Hartmann PE, 1999. Breast volume and milk production during extended lactation in women. Experimental Physiology, 84, 435-447.

Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schoch G, 1998. Measured consumption of commercial infant food products in German infants: results from the DONALD study. Dortmund Nutritional and Anthropometrical Longitudinally Designed. Journal of Pediatric Gastroenterology and Nutrition, 27, 547-552.

Khan AA and Kolattukudy PE, 1974. Decarboxylation of long chain fatty acids to alkanes by cell free preparations of pea leaves (*Pisum sativum*). Biochemical and Biophysical Research Communication 61, 1379-1386.

Khanduja KL, Dogra SC and Sharma RR, 1981. Glucuronic acid conjugates of metabolites of 7, 12-dimethylbenz(a)anthracene excreted through bile ducts in rat. Indian journal of experimental biology, 19, 375-378.

Kiely M, Flynn A, Harrington KE, Robson PJ and Cran G, 2001. Sampling description and procedures used to conduct the North/South Ireland Food Consumption Survey. Public Health Nutrition, 4, 1029-1035.

Kilanowicz A, Sapota A and Czerski B, 2002. Disposition and metabolism of 1,6-dimethylnaphthalene in rats. Toxicology Letters, 134, 227-235.

Kojima S, Honda T, Nakagawa M, Kiyozumi M and Takadate A, 1982. Urinary metabolites of 2,6-diisopropylnaphthalene in rats. Drug Metabolism and Disposition, 10, 429-433.

Kolattukudy PE, Croteau R and Buckner JS, 1976. Biochemistry of plant waxes. In: Chemistry and Biochemistry of Naturals Waxes. Ed Kolattukudy PE. Elsevier, Amsterdam, pp. 290–334.

Kolattukudy PE and Hankin L, 1966. Metabolism of a plant wax paraffin (N-nonacosane) in rat. Journal of Nutrition, 90, 167-174.

Koprivniak O, Procida G and Favretto L, 1997. Determination of aliphatic hydrocarbons of virgin olive oils of four autochthonous cultivars from Krk Island (Croatia). Food Technology and Biotechnology, 35, 125-131.

Krems C, Bauch A, Gotez A, Heuer T, Hild A, Möseneder J and Borombach C, 2006. Methoden der nationalesn Verzehrstudie II. Ernahrungs umschau 53. Heft, 2, 44-50.

Kreutz TG, Larson ED, Liu G and Williams RH, 2008. Fischer-Tropsch fuels from coal and biomass. Prepared for 25th Annual International Pittsburgh Coal Conference, 29 September – 2 October, 2008 Pittsburgh, Pennsylvania, USA.

Kropp KG and Fedorak PM, 1998. A review of the occurrence, toxicity, and biodegradation of condensed thiophenes found in petroleum. Canadian Journal of Microbiology, 44, 605-622.

Kuksis A, 1964. Hydrocarbon composition of some crude and refined edible seed oils. Biochemistry, 3, 1086-1093.

Kumar S, Lin JM, Whysner J, Sikka HC and Amin S, 2004. Mutagenicity of benzo[b]phenanthro[2,3-d]thiophene (BPT) and its metabolites in TA100 and base-specific tester strains (TA7001-TA7006) of *Salmonella typhimurium*: evidence of multiple pathways for the bioactivation of BPT. Mutation Research, 545, 11-21.

Kuroda Y, Akaogi J, Nacionales DC, Wasdo SC, Szabo NJ, Reeves WH and Satoh M, 2004. Distinctive patterns of autoimmune response induced by different types of mineral oil. Toxicological Sciences, 78, 222-228.

Kusunose M, Ichihara K and Kusunose E, 1969. Oxidation of n-hexadecane by mouse liver microsomal fraction. Biochimica et Biophysica Acta, 176, 679-681.

Lagana SM, Moreira RK and Lefkowitch JH, 2010. Hepatic granulomas: pathogenesis and differential diagnosis. Clinical Liver Disease, 14, 605-617.

Laher JM and Barrowman JA, 1987. Role of the lymphatic system in the transport of absorbed 7,12-dimethylbenzanthracene in the rat. Lipids, 22, 152-155.

Laher JM and Barrowman JA, 1988. Intestinal absorption of carcinogenic hydrocarbons. Annals of the New York Academy of Sciences, 534, 565-574.

Laher JM, Chernenko GA and Barrowman JA, 1983. Studies of the absorption and enterohepatic circulation of 7,12-dimethylbenz[a]anthracene in the rat. Canadian Journal of Physiology and Pharmacology, 61, 1368-1373.

Laher JM, Rigler MW, Vetter RD, Barrowman JA and Patton JS, 1984. Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat. Journal of Lipid Research, 25, 1337-1342.

LaKind JS, Berlin CM and Naiman DQ, 2001. Infant exposure to chemicals in breast milk in the United States: what we need to learn from a breast milk monitoring program. Environmental Health Perspectives, 109, 75-88.

Laragione T, Brenner M, Sherry B and Gulko PS, 2011. CXCL10 and its receptor CXCR3 regulate synovial fibroblast invasion in rheumatoid arthritis. Arthritis & Rheumatism, 63, 3274-3283.

Larrañaga N, Amiano Etxezarreta P, Gorostiza Garai E, Pérez Díez Y, Bidaurrazaga Van-Dierdonck J, Sarasqueta Eizaguirre C, Arrizabalaga Abasolo JJ, Espada Sáez-Torres M and Méndez Navas I

2006. Encuesta de nutrición 2005: Hábitos alimentarios y estado de salud de la población vasca de 4 a 18 años. Primeros resultados. Vitoria-Gasteiz Servicio Central de Publicaciones del Gobierno Vasco, DL. Available from www.osanet.euskadi.net/r85-13714/es/contenidos/informacion/sanidad_alimentaria/es_1247/adjuntos/DietaSana_c.pdf.

LaVoie EJ, Bedenko V, Tulley-Freiler L and Hoffmann D, 1982. Tumor-initiating activity and metabolism of polymethylated phenanthrenes. Cancer Research, 42, 4045-4049.

LaVoie EJ, Coleman DT, Rice JE, Geddie NG and Hoffmann D, 1985. Tumor initiating activity, mutagenicity, and metabolism of methylated anthracenes. Carcinogenesis, 6, 1483-1488.

LaVoie EJ, Tulley-Freiler L, Bedenko V and Hoffman D, 1981. Mutagenicity, tumor-initiating activity, and metabolism of methylphenanthrenes. Cancer Research, 41, 3441-3447.

Le Bon AM, Cravedi JP and Tulliez JE, 1988. Disposition and metabolism of pristane in rat. Lipids, 23, 424-429.

Leclercq C, Arcella D, Piccinelli R, Sette S, Le Donne C and Turrini A, 2009. The Italian National Food Consumption Survey INRAN-SCAI 2005-06: main results in terms of food consumption. Public Health Nutrition, 12, 2504-2532.

Leffler WL, 1985, Petroleum refining for the nontechnical person, 2nd Edition, PennWell Books, ISBN 0-87814-280-0

Leighton FA, 1989. Acute oral toxicity of dibenzothiophene for male CD-1 mice: LD50, lesions, and the effect of preinduction of mixed-function oxidases. Fundamental and Applied Toxicology, 12, 787-792.

Lester DE, 1979. Normal paraffins in living matter--occurrence, metabolism and pathology. Progress in Food and Nutrition Science, 3, 1-66.

Levine WG, 1974. Hepatic uptake, metabolism, and biliary excretion of 7,12-dimethylbenzanthracene in the rat. Drug Metabolism and Disposition: The Biological Fate of Chemicals, 2, 169-177.

Lin CY, Wheelock AM, Morin D, Baldwin RM, Lee MG, Taff A, Plopper C, Buckpitt A and Rohde A, 2009. Toxicity and metabolism of methylnaphthalenes: comparison with naphthalene and 1-nitronaphthalene. Toxicology, 260, 16-27.

Lioret S, Dubuisson C, Dufour A, Touvier M, Calamassi-Tran G, Maire B, Volatier JL and Lafay L, 2010. Trends in food intake in French children from 1999 to 2007: results from the INCA (Etude Individuelle Nationale des Consommations Alimentaires) dietary surveys. Br J Nutr, 103, 585-601.

Lorentzen JC, Flornes L, Eklow C, Backdahl L, Ribbhammar U, Guo JP, Smolnikova M, Dissen E, Seddighzadeh M, Brookes AJ, Alfredsson L, Klareskog L, Padyukov L and Fossum S, 2007. Association of arthritis with a gene complex encoding C-type lectin-like receptors. Arthritis & Rheumatism, 56, 2620-2632.

Lorenzini R, Fiselier K, Biedermann M, Barbanera M, Braschi I and Grob K, 2010. Saturated and aromatic mineral oil hydrocarbons from paperboard food packaging: Prediction of long-term migration from contents in the paperboard; data from the market. Food Additives and Contaminants. Part A, 27, 1765-1774.

Lushbaugh CC, Green JW Jr. and Redemann CE, 1950. Effects of prolonged inhalation of oil fogs on experimental animals. Archives of Industrial Hygiene and Occupational Medicine, 1, 237-247.

Lyhne N, Christensen T, Groth MV, Fagt S, Biltoft-Jensen A, Hartkopp H, Hinsch H-J, Matthiessen J, Møller A, Saxholt E and Trolle E, 2005. Dietary habits in Denmark 2000-2002. Main results. Copenhagen: Danish Institute for Food and Veterinary Research, Department of Nutrition.

Machinery Lubrication, 2010. The evolution of base oil technology industry focus. July 2010.

Mackerer CR, Griffis LC, Grabowski Jr JS and Reitman FA, 2003. Petroleum mineral oil refining and evaluation of cancer hazard. Applied Occupational & Environmental Hygiene, 18, 890-901.

Madison RM, Rabstein LS and Bryan WR, 1968. Mortality rate and spontaneous lesions found in 2,928 untreated BALB/cCr mice. Journal of the National Cancer Institute, 40, 683-685.

Mattie DR, Marit GB, Flemming CD and Cooper JR, 1995. The effects of JP-8 jet fuel on male Sprague-Dawley rats after a 90-day exposure by oral gavage. Toxicology and Industrial Health, 11, 423-435.

McCarthy RD, 1964. Mammalian Metabolism of Straight-Chain Saturated Hydrocarbons. Biochimica et Biophysica Acta, 84, 74-79.

McFall T, Booth GM, Lee ML, Tominaga Y, Pratap R, Tedjamulia M and Castle RN, 1984. Mutagenic activity of methyl-substituted tri- and tetracyclic aromatic sulfur heterocycles. Mutation Research, 135, 97-103.

McKee RH, Plutnick RT and Rzygoda RT, 1989. The carcinogenic initiating and promoting properties of a lightly refined paraffinic oil. Fundamental and Applied Toxicology, 12, 748-756.

Mehrotra NK and Saxena AK, 1979. Evaluation of carcinogenic effect of mineral oil used in the processing of jute fibres. British Journal of Experimental Pathology, 60, 518-525.

MHW (Japanese Ministry of Health and Welfare), 1996. n-Undecane – Repeat Dose and Reproductive/Developmental Toxicity. Available from http://dra4.nihs.go.jp/mhlw_data/home/file/file1120-21-4.html

Miller MJ, Lonardo EC, Greer RD, Bevan C, Edwards DA, Smith JH and Freeman JJ, 1996. Variable responses of species and strains to white mineral oils and paraffin waxes. Regulatory Toxicology and Pharmacology, 23, 55-68.

Mitchell MP and Hubscher G, 1968. Oxidation of n-hexadecane by subcellular preparations of guinea pig small intestine. European Journal of Biochemistry, 7, 90-95.

Mold JD, Means RE and Ruth JM, 1966. The higher fatty acids of flue-cured tobacco: Methyl and cyclohexyl branched acids. Phytochemistry, 5, 59-66.

Moret S, Grob K and Conte LS, 1996. On-line high-performance liquid chromatography-solvent evaporation-high-performance liquid chromatography-capillary gas chromatography-flame ionisation detection for the analysis of mineral oil polyaromatic hydrocarbons in fatty foods. Journal of chromatography. A, 750, 361-368.

Moret S, Grob K and Conte LS, 1997. Mineral oil polyaromatic hydrocarbons in foods, eg from jute bags, by on-line LC-solvent evaporation (SE)-LC-GC-FID. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-Food Research and Technology, 204, 241-246.

Moret S, Populin T, Conte LS, Grob K and Neukom HP, 2003. Occurrence of C-15-C-45 mineral paraffins in olives and olive oils. Food Additives and Contaminants, 20, 417-426.

Morrison DS, Schmidt J and Paulli R, 1996. The scope of mineral oil in personal care products and its role in cosmetic formulation. Journal of Applied Cosmetology, 14, 111-118.

Motiuk K, 1980. Wool wax hydrocarbons: a review. Journal of the American Oil Chemistry Society, 57, 145-146.

MRI (Max Rubner-Institut), 2008. Ergebnisbericht der Nationalen Verzehrsstudie II - Teil 1 (in German: Report of the National Nutrition Survey II - Part 1). Karlsruhe (Germany), 2008, p. 144. Available from http://www.was-esse-ich.de/uploads/media/NVS_II_Abschlussbericht_Teil_1.pdf.

Murata Y, Denda A, Maruyama H and Konishi Y, 1993. Chronic toxicity and carcinogenicity studies of 1-methylnaphthalene in B6C3F1 mice. Fundamental and Applied Toxicology, 21, 44-51.

Murata Y, Denda A, Maruyama H, Nakae D, Tsutsumi M, Tsujiuchi T and Konishi Y, 1997. Chronic toxicity and carcinogenicity studies of 2-methylnaphthalene in B6C3F1 mice. Fundamental and Applied Toxicology, 36, 90-93.

Murphy SE, Amin S, Coletta K and Hoffmann D, 1992. Rat liver metabolism of benzo[b]naphtho[2,1-d]thiophene. Chemical Research in Toxicology, 5, 491-495.

Nacionales DC, Kelly KM, Lee PY, Zhuang H, Li Y, Weinstein JS, Sobel E, Kuroda Y, Akaogi J, Satoh M and Reeves WH, 2006. Type I interferon production by tertiary lymphoid tissue developing in response to 2,6,10,14-tetramethyl-pentadecane (pristane). American Journal of Pathology, 168, 1227-1240.

Nash JF, Gettings SD, Diembeck W, Chudowski M and Kraus AL, 1996. A toxicological review of topical exposure to white mineral oils. Food and Chemical Toxicology, 34, 213-225.

Neukom HP, Grob K, Biedermann M and Noti A, 2002. Food contamination by C-20-C-50 mineral paraffins from the atmosphere. Atmospheric Environment, 36, 4839-4847.

Nochomovitz LE, Uys CJ and Epstein S, 1975. Massive deposition of mineral oil after prolonged ingestion. South African Medical Journal, 49, 2187-2190.

Noti A, Grob K, Biedermann M, Deiss U and Bruschweiler BJ, 2003. Exposure of babies to C-15-C-45 mineral paraffins from human milk and breast salves. Regulatory Toxicology and Pharmacology, 38, 317-325.

NTP (National Toxicology Program), 2005. Toxicology and Carcinogenesis of Decalin (CAS No. 91-17-8) in F344/N Rats and B6C3F1 Mice (Inhalation Study). Technical Rerport Series No. 213. National Toxicology Program, Research Triangle Park, N.C.

National Toxicology Program (NTP), 2009.Toxicology and Carcinogenesis Studies of Tetralin (CAS NO. 119-64-2) in F344/N Rats and B6C3F1 mice (Inhalation Study). Technical Rerport Series No. 561. National Toxicology Program, Research Triangle Park, NC.

NIEHS (National Institute of Environmental Health Sciences), 2002. Tumor incidence in control animals in 2-year NTP-diet studies, F344/N rats. National Institute of Environmental Health Sciences. Analytical Sciences, Durham, NC. Historical Control Information for Fischer 344 rats. Available from html://htp.db.niehs.nih.gov/htdocs/HCRS/Hist_2002 Tumor_Rats.html

Ocké MC, Hulshof KFAM and van Rossum CTM, 2005. The Dutch national food consumption survey 2003. Methodological issues. Archives of Public Health 63, 227-241.

OECD (Organisation for Economic Co-Operation and Development), 2001. Guideline for the testing of chemicals. Proposal for updating guideline 414. Available from http://www.oecd.org/dataoecd/18/15/1948482.pdf.

OECD (Organisation for Economic Co-Operation and Development), 2004. High Production Volume Screening Information Dataset (OECD SIDS) on 1,2,3,4-tetrahydronaphthalene. Available from http://www.chem.unep.ch/irptc/sids/oecdsids/119642.pdf.

Olson CT, Yu KO and Serve MP, 1986. Metabolism of nephrotoxic cis- and trans-decalin in Fischer-344 rats. Journal of Toxicology and Environment Health, 18, 285-292.

Onyango AW, Receveur O and Esrey SA, 2002. The contribution of breast milk to toddler diets in western Kenya. Bulletin of the World Health Organization, 80, 292-299.

Ortega RM, López-Sobaler AM, Ballesteros JM, Pérez N, Rodríguez- Rodríguez E, Aparicio A, Perea JM and Andrés P, 2010. Estimation of salt intake by 24-hour urinary sodium excretion in a representative sample of Spanish adults. British Journal of Nutrition, 105, 787-794.

Paturi M, Tapanainen H and Reinivuo H, 2008. The National FINDIET 2007 Survey. (In Finnish, summary, figures and tables in English) No. B23. Helsinki: National Public Health Institute. Available from http://www.ktl.fi/attachments/suomi/julkaisut/julkaisusarja_b/2008/2008b23.pdf.

Pelroy RA, Stewart DL, Tominaga Y, Iwao M, Castle RN and Lee ML, 1983. Microbial mutagenicity of 3- and 4-ring polycyclic aromatic sulfur heterocycles. Mutation Research, 117, 31-40.

Perdu-Durand EF and Tulliez JE, 1985. Hydrocarbon hydroxylation system in liver microsomes from four animal species. Food and Chemical Toxicology, 23, 363-366.

Petrova S and Angelova K, 2006. Food-Based Dietary Guidelines for Bulgarian adults – scientific background for development and formulation. Advances in Bulgarian Science, 4, 19-33.

Petrova S, Ovcharova D, Rangelova L, Duleva V, Angelova K, Kalinov K, Dimitrov P, Bojilova D, Baikova D, Vatralova K, Popivanova A, Marinova M, Antonova Z and Duneva Z, 2009. National survey on nutrition of infants and children under 5 years and family child rearing. A Report for UNICEF Bulgaria. NCPHP, 1 – 361 (In Bulgarian). Available from www.ncphp.government.bg.

Pokrovskiĭ AA, Markelova VF, Tatsievskiĭ VA and Levachev MM, 1969. Transformation of hydrocarbons in animals. Biokhimiia, 34, 791-794.

Pomerleau J, McKee M, Robertson A, Vaask S, Pudule I, Grinberga D, Kadziauskiene K, Abaravicius A and Bartkeviciute R, 1999. Nutrition and lifestyle in the Baltic Republics. European centre on health of societies in transition and world health organization regional office for Europe, 1-61.

Poon R, Davis H, Lecavalier P, Liteplo R, Yagminas A, Chu I and Bihun C, 1997. Effects of benzothiophene on male rats following short-term oral exposure. Journal of Toxicology and Environment Health, 50, 53-65.

Poon R, Davis H, Park G, Lecavalier P, Yagminas A, Elwin J, Chu I and Valli VE, 1998. Subchronic toxicity of benzothiophene on rats following dietary exposure. Journal of Toxicology and Environmental Health, Part A, 55, 133-150.

Popovic M, Gernecevic N and Dimovski J, 1973. Absorption of n-paraffins in the rat. Acta Pharm. Jugoslav., 23, 195-201.

Populin T, Biedermann M, Grob K, Moret S and Conte L, 2004. Relative hopane content confirming the mineral origin of hydrocarbons contaminating foods and human milk. Food Additives and Contaminants, 21, 893-904.

Potter M, 2003. Neoplastic development in plasma cells. Immunological Reviews, 194, 177-195.

Potter M and Wax JS, 1981. Genetics of susceptibility to pristane-induced plasmacytomas in BALB/cAn: reduced susceptibility in BALB/cJ with a brief description of pristane-induced arthritis. Journal of Immunology, 127, 1591-1595.

Rahman A, Barrowman JA and Rahimtula A, 1986. The influence of bile on the bioavailability of polynuclear aromatic hydrocarbons from the rat intestine. Canadian Journal of Physiology and Pharmacology, 64, 1214-1218.

Reddy MV, Blackburn GR, Schreiner CA and Mackerer CR, 1997. Correlation of mutagenic potencies of various petroleum oils and coal tar mixtures with DNA adduct levels *in vitro*. Mutation Research, 378, 89.

Reeves WH, Lee PY, Weinstein JS, Satoh M and Lu L, 2009. Induction of autoimmunity by pristane and other naturally occurring hydrocarbons. Trends in Immunology, 30, 455-464.

Reich A, G., Douglass JS, van der Reit B, Eickhoff JC and Heimbach JT, 1998. Dietary exposure assessment for direct and indirect food use applications for mineral hydrocarbons. Prepared for the American Petroleum Institute by TAS-ENVIRON.

Requejo AM, Ortega AM, Lopez Sobaler AM, Navia B, Andres P, Jodral M, Quintas E, Redondo R, Menendez L, Perea JM, Aparicio A, Lozano MC, Bermejo L, Mena C, Faci M, Lolo JM, Rodriguez N, Cocho M, Diez C and Alvarez C, 2002. Estudio sobre dietas y habitos alimentarios en la poblacion Espanola. Consejo de seguridad nuclear, 1-303.

Ridder GM, Von Bargen EC, Alden CL and Parker RD, 1990. Increased hyaline droplet formation in male rats exposed to decalin is dependent on the presence of alpha 2u-globulin. Fundamental and Applied Toxicology, 15, 732-743.

RIKILT, 2008. Bulder AS, Hoogenboom LAP, Kan CA (ASG), van Raamsdonk LVD, Traag WA, Bouwmeester H. Risk assessment of Nickel, Mineral Oils, Polycyclic Aromatic Hydrocarbons and Volatile Organic Compounds in animal feed materials. Report 2007.020, November 2008, 1-102.

Rivedal E, Mikalsen SO, Roseng LE, Sanner T and Eide I, 1992. Effects of hydrocarbons on transformation and intercellular communication in Syrian hamster embryo cells. Pharmacology & Toxicology, 71, 57-61.

Rodler I, Biró L, Greiner E, Zajkás G, Szórád I, Varga A, Domonkos A, Agoston H, Balazs A, Mozsary E, Vitrai J, Hermann D, Boros J, Nemeth R and Zsuzsanna K, 2005. Taplalkozasi vizsgalat Magyarorszagon, 2003-2004. (Dietary survey in Hungary, 2003-2004) Orvosi Hetilap, 146. Evfolyam, 34, 1781-1789.

Roy TA, Johnson SW, Blackburn GR and Mackerer CR, 1988. Correlation of mutagenic and dermal carcinogenic activities of mineral oils with polycyclic aromatic compound content. Fundamental and Applied Toxicology, 10, 466-476.

Ruiz-Bravo A, Perez M and Jimenez-Valera M, 1995. The enhancement of growth of a syngeneic plasmacytoma in BALB/c mice by pristane priming is not due to immunosuppressive effects on antibody-forming cell or mitogen-responsive splenocytes. Immunology Letters, 44, 41-44.

Rundhaug JE and Fischer M 2010. Molecular mechanisms of mouse skin tumor promotion. Cancers 2:436-482. Safety Evaluation of Certain Contaminants in Food, WHO Food Additives Series 55, WHO, Geneva, 2006. Polycyclic aromatic hydrocarbons. 563-743.

Ruprich J, Dofkova M, Rehurkova I, Slamnenikova E and Resova D, 2006. Individual food consumption - the national study SISP04. Prague: CHFCH NIPH. Available from www.chpr.szu.cz/spotrebapotravin.htm.

Salvayre R, Negre A, Rocchiccioli F, Duboucher C, Maret A, Vieu C, Lageron A, Polonovski J and Douste-Blazy L, 1988. A new human pathology with visceral accumulation of long-chain n-alkanes; tissue distribution of the stored compounds and pathophysiological hypotheses. Biochimica et Biophysica Acta: Protein Structure and Molecular Enzymology, 958, 477-483.

Šantare D, Ozoliņš G and Joffe R, 2008. Latvijas iedzīvotāju pārtikas patēriņa pētījums: mērķi, norise, metodes. LLU Raksti, Jelgava, iesniegta publicēšanai.

Sargent JR, 1976. The structure, metabolism and function of lipids in marine organisms. In: Biochemical and Biophysical Perspectives in Marine Biology, Volume 3. Eds Malins DC and Sargent JR. Academic Press, New York. pp. 149-212.

Satoh M, Kuroda Y, Yoshida H, Behney KM, Mizutani A, Akaogi J, Nacionales DC, Lorenson TD, Rosenbauer RJ and Reeves WH, 2003. Induction of lupus autoantibodies by adjuvants. Journal of Autoimmunity, 21, 1-9.

Savary P and Constantin MJ, 1967. On the micellar solubilization of hexadecane and its passage into thoracic duct lymph of the rat. Biochimica et Biophysica Acta, 137, 264-276.

SCF (Scientific Committee for Food), 1989. Minutes of the 67th meeting. III/3681/89-EN.

SCF (Scientific Committee for Food), 1995. Opinion on mineral and synthetic hydrocarbons, SCF reports: 37th series. 25 Sept 1995. Available from http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_37.pdf.

Scheuer PJ and Lefkowitch JH, 2006. Liver biopsy interpretation. 7th ed. Elsevier Science, 448 pp.

Schoenmakers PJ, Oomen J, Blomberg J, Genuit W and van Velzen G, 2000. Comparison of comprehensive two-dimensional gas chromatography and gas chromatography - mass spectrometry for the characterization of complex hydrocarbon mixtures. Journal of Chromatography A, 892, 29-46.

Scotter MJ, Castle L, Massey RC, Brantom PG and Cunninghame ME, 2003. A study of the toxicity of five mineral hydrocarbon waxes and oils in the F344 rat, with histological examination and

tissue-specific chemical characterisation of accumulated hydrocarbon material. Food and Chemical Toxicology, 41, 489-521.

Sekula W, Nelson M, Figurska K, Oltarzewski M, Weisell R and Szponar L, 2004. Comparison between household budget survey and 24 hour recall data in a nationally representative sample of Polish households. Public Health Nutrition 8, 430-439.

Servé MP, Llewelyn BM, Yu KO, McDonald GM, Olson CT and Hobson DW, 1989. Metabolism and nephrotoxicity of tetralin in male Fischer 344 rats. Journal of Toxicology and Environment Health, 26, 267-275.

Shaheen VM, Satoh M, Richards HB, Yoshida H, Shaw M, Jennette JC and Reeves WH, 1999. Immunopathogenesis of environmentally induced lupus in mice. Environmental Health Perspectives, 107 Suppl 5, 723-727.

Shell, 2007a. Shellsol TD – Data sheet. Available from http://www.scdynamiccontent.shell.com/Files/isoparaffins_shellsoltd_europe.pdf.

Shell, 2007b. Shellsol D60 – Data sheet. Available from http://www.granddor.ru/pdf/shellsol_d60.pdf.

Shell, 2007c. Shellsol D70 – Data sheet. Available from http://www.coralenergy.gr/media/ximika/TDS_SSOL_D70.pdf.

Shoda T, Toyoda K, Uneyama C, Takada K and Takahashi M, 1997. Lack of carcinogenicity of medium-viscosity liquid paraffin given in the diet to F344 rats. Food and Chemical Toxicology, 35, 1181-1190.

Shopp GM, White KL, Jr., Holsapple MP, Barnes DW, Duke SS, Anderson AC, Condie LW, Jr., Hayes JR and Borzelleca JF, 1984. Naphthalene toxicity in CD-1 mice: general toxicology and immunotoxicology. Fundamental and Applied Toxicology, 4, 406-419.

Shoshkes M, Banfield WG, Jr. and Rosenbaum SJ, 1950. Distribution, effect and fate of oil aerosol particles retained in the lungs of mice. Archives of Industrial Hygiene and Occupational Medicine, 1, 20-35.

Shubik P and Saffiotti U, 1955. The carcinogenic and promoting action of low boiling catalytically cracked oils. Acta Unio Internationalis Contra Cancrum, 11, 707-711.

Shubik P, Saffiotti U, Lijinsky W, Pietra G, Rappaport H, Toth B, Raha CR, Tomatis L, Feldman R and Ramahi H, 1962. Studies on the toxicity of petroleum waxes. Toxicology and Applied Pharmacology, 4(Suppl), 1-62.

Sicé J, 1966. Tumor-promoting activity of n-alkanes and 1-alkanols. Toxicology and Applied Pharmacology, 9, 70-74.

Silin DS, Lyubomska OV, Jirathitikal V and Bourinbaiar AS, 2007. Oral vaccination: where we are? Expert Opinion on Drug Delivery, 4, 323-340.

Sinclair C, 2005. Dictionary of Food. International Food and Cooking Terms from A to Z. A&C Black Publishers Ltd, London, UK, 640 pp.

Smith JH, Mallett AK, Priston RA, Brantom PG, Worrell NR, Sexsmith C and Simpson BJ, 1996. Ninety-day feeding study in Fischer-344 rats of highly refined petroleum-derived food-grade white oils and waxes. Toxicologic Pathology, 24, 214-230.

Smith PB, Veley KE, Yarrington JT and Slauter RW 1999. 90-day oral gavage toxicity study of C9-C16 aromatic fraction JET-A in female Sprague-Dawley CD rats and male C57BL/6 mice. United States Air Force Research Laboratory, Report Number AFRL-HE-WP-TR-1999-0229.

Smith WE, Sunderland DA and Sugiura K, 1951. Experimental analysis of the carcinogenic activity of certain petroleum products. Archives of Industrial Hygiene and Occupational Medicine, 4, 299-314.

Speight JG, 2006. The chemistry and technology of petroleum, 3rd edition. CRC Press. Boca Raton, USA. ISBN 9780849390678.

Stoker J, 2007. "Food canning". Presentation made at the UK-Defra LINK FSQ45 dissemination seminar 'New technologies and chemistries for food can coatings'. Leeds University, Leeds (UK), 2nd May 2007.

Stula EF and Kwon BK, 1978. Pulmonary pathology from inhalation of a complex mineral oil mist in dogs, rats, mice and gerbils. American Industrial Hygiene Association Journal, 39, 393-399.

Summers SA, Hoi A, Steinmetz OM, O'Sullivan KM, Ooi JD, Odobasic D, Akira S, Kitching AR and Holdsworth SR, 2010. TLR9 and TLR4 are required for the development of autoimmunity and lupus nephritis in pristane nephropathy. Journal of Autoimmunity, 35, 291-298.

Sverdrup B, Kallberg H, Bengtsson C, Lundberg I, Padyukov L, Alfredsson L, Klareskog L and Epidemiological Investigation of Rheumatoid Arthritis Study G, 2005. Association between occupational exposure to mineral oil and rheumatoid arthritis: results from the Swedish EIRA case-control study. Arthritis Research & Therapy, 7, R1296-1303.

Sverdrup B, Klareskog L and Kleinau S, 1998. Common commercial cosmetic products induce arthritis in the DA rat. Environmental Health Perspectives, 106, 27-32.

Swartz CD, King LC, Nesnow S, Umbach DM, Kumar S, Sikka H and Demarini DM, 2009. Mutagenicity, stable DNA adducts, and abasic sites induced in Salmonella by phenanthro[3,4-b]- and phenanthro[4,3-b]thiophenes, sulfur analogs of benzo[c]phenanthrene. Mutation Research, 661, 47-56.

Szponar L, Sekuła W, Nelson M and Weisell RC, 2001. The Household Food Consumption and Anthropometric Survey in Poland. Public Health Nutrition, 4, 1183-1186.

Szponar L, Sekuła W, Rychlik E, Ołtarzewski M and Figurska K, 2003. Badania indywidualnego spożycia żywności i stanu odżywienia w gospodarstwach domowych (Household Food Consumption and Anthropometric Survey). NFNI Studies 101, Warszawa (text in Polish, summary in English).

Tennant DR, 2004. The usage, occurrence and dietary intakes of white mineral oils and waxes in Europe. Food and chemical Toxicology, 42, 481-492.

Tolls J and van Dijk J, 2002. Bioconcentration of n-dodecane and its highly branched isomer 2,2,4,6,6-pentamethylheptane in fathead minnows. Chemosphere, 47, 1049-1057.

Trimmer GW, Freeman JJ, Priston RA and Urbanus J, 2004. Results of chronic dietary toxicity studies of high viscosity (P70H and P100H) white mineral oils in Fischer 344 rats. Toxicologic Pathology, 32, 439-447.

Trivalle C, Profit P, Bonnet B, Manchon ND, Ducastelle T, Hemet J, Bercoff E and Bourreille J, 1991. Lipogranulomes hépatiques à l'huile de paraffine responsables d'une fièvre au long cours. Gastroenterologie Clinique et Biologique, 15, 551-553.

Tulliez J, 1986. A new approach to the fate and effects of dietary and medicinal mineral oils. Médecine et Nutrition, 22, 302-309.

Tulliez J and Bories G, 1975a. Metabolism of paraffinic and naphthalenic hydrocarbons in higher animals. I. Retention of paraffins (normal, cyclo and branched) in rats. Annales de la nutrition et de l'alimentation, 29, 201-211.

Tulliez J, Bories G, Boudene C, Fevrier C., 1975. Hydrocarbons of the spiruline algae: nature, metabolism of heptadecane by rats and swine. Annales de la nutrition et de l'alimentation, 29, 563-572.

Tulliez J and Peleran JC, 1977. Oxidation of a naphthenic hydrocarbon, dodecylcyclohexane, in the rat. FEBS Letters, 75, 120-122.

Tulliez JE and Bories GF, 1975b. Metabolism of paraffinic and naphthalenic hydrocarbons in higher animals. II. Accumulation and mobilization in rats. Annales de la nutrition et de l'alimentation, 29, 213-221.

Tulliez JE and Bories GF, 1978. Metabolism of a n-paraffin, heptadecane, in rats. Lipids, 13, 110-115.

Tulliez JE and Bories GF, 1979. Metabolism of naphthenic hydrocarbons. Utilization of a monocyclic paraffin, dodecylcyclohexane, by rat. Lipids, 14, 292-297.

Tulloch AP, 1976. Chemistry of waxes'of higher plants. In: Kolattukudy PE (Ed.), Chemistry and Biochemistry of Naturals Waxes. Elsevier, Amsterdam, pp. 235-287.

US EPA (US Environmental Protection Agency), 1990. Fluorene (CAS 86-73-7) Integrated Risk Information System. Available from http://www.epa.gov/IRIS/subst/0435.htm.

US EPA (US Environmental Protection Agency), 1993. Pyrene (CAS 129-00-0) Integrated Risk Information System. Available from http://www.epa.gov/iris/subst/0445.htm.

US EPA (US Environmental Protection Agency), 1994. Acenaphthene (CAS 83-32-9) Integrated Risk Information System. Available from http://www.epa.gov/iris/subst/0442.htm.

US EPA (US Environmental Protection Agency), 1998. Naphthalene (CAS 91-20-3) Integrated Risk Information System. Available from http://www.epa.gov/iris/subst/0436.htm.

US EPA (US Environmental Protection Agency), 2003. Biopesticides registration action document on 2,6-diisopropylnaphthalene. Available from http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_055803.pdf.

van der Westhuizen R, Ajam M, De Coning P, Beens J, de Villiers A and Sandra P, 2011. Comprehensive two-dimensional gas chromatography for the analysis of synthetic and crude-derived jet fuels. Journal of Chromatography. A, 1218, 4478-4486.

Van Heyningen R and Pirie A, 1976. Naphthalene cataract in pigmented and albino rabbits. Experimental Eye Research, 22, 393-394.

Vater ST, Hollingsworth L, Baldwin DM and Warshawsky D, 1991. Comparative metabolism of 7,12-dimethylbenz[a]anthracene by the perfused liver and liver microsomal preparations from Sprague-Dawley rats. Carcinogenesis, 12, 2379-2382.

Vendeuvre C, Bertoncini F, Espinat D, Thiebaut D and Hennion MC, 2005. Multidimensional gas chromatography for the detailed PIONA analysis of heavy naphtha: hyphenation of an olefin trap to comprehensive two-dimensional gas chromatography. Journal of Chromatography A, 1090, 116-125.

Verordnung des EDI über Bedarfsgegenstände, 817.023.21 (2005).

Vollmer A, Biedermann M, Grundböck F, Ingenhoff J-E, Biedermann-Brem S, Altkofer W and Grob K, 2011. Migration of mineral oil from printed paperboard into dry foods: survey of the German market. European Food Research and Technology, 232, 175-182.

Wagner C, Neukom H-P, Galetti V and Grob K, 2001a. Determination of mineral paraffins in feeds and foodstuffs by bromination and preseparation on aluminium oxide: Method and results of a ring test. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 92, 231-249.

Wagner C, Neukom H-P, Grob K, Moret S, Populin T and Conte LS, 2001b. Mineral paraffins in vegetable oils and refinery by-products for animal feeds. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 92, 499-514.

Wagner WD, Wright PG and Stokinger HE, 1964. Inhalation Toxicology of Oil Mists. I. Chronic Effects of White Mineral Oil. American Industrial Hygiene Association Journal, 25, 158-168.

Walters DG, Sherrington KV, Worrell N and Riley RA, 1994. Formulation and analysis of food-grade mineral hydrocarbons in toxicology studies. Food and Chemical Toxicology, 32, 549-557.

Wanless IR and Geddie WR, 1985. Mineral oil lipogranulomata in liver and spleen. A study of 465 autopsies. Archives of Pathology and Laboratory Medicine, 109, 283-286.

Warnasuriya GD, Elcombe BM, Foster JR and Elcombe CR, 2010. A Mechanism for the induction of renal tumours in male Fischer 344 rats by short-chain chlorinated paraffins. Archives of Toxicology, 84, 233-243.

Watabe T, Ishizuka T, Isobe M and Ozawa N, 1982. A 7-hydroxymethyl sulfate ester as an active metabolite of 7,12-dimethylbenz[alpha]anthracene. Science, 215, 403-405.

Weete JD, 1976. Algal and fungal waxes. In: Chemistry and Biochemistry of Naturals Waxes. Ed Kolattukudy PE. Elsevier, Amsterdam, pp 349-418.

Whittle KJ, MacKie PR, Hardy R, McIntyre AD and Blackman RAA, 1977. The alkanes of marine organisms from the United Kingdom and surrounding waters. Rapport et Procès-verbaux des Réunions, Conseil international pour L'Exploration de la Mer 171, 72-78.

WHO/IPCS (World Health Organization, International Programme on Chemical Safety), 2003. Safety Evaluation of Certain Food Additives, WHO Food Additives Series 50, Mineral Oils (Medium- and Low-Viscosity) and Paraffin Waxes. Available from http://www.inchem.org/documents/jecfa/jecmono/v50je04.htm#3.2.

WHO/IPCS (World Health Organization, International Programme on Chemical Safety), 2008. Uncertainty and Data Quality in Exposure Assessment. Part 1: Guidance document on characterizing and communicating uncertainty in exposure assessment. Part 2: Hallmarks of data quality in chemical exposure assessment. Available from http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf.

WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food. International Programme on Chemical Safety (IPCS), Environmental Health Criteria 240.

Wislocki PG, Fiorentini KM, Fu PP, Yang SK and Lu AY, 1982. Tumor-initiating ability of the twelve monomethylbenz[a]anthracenes. Carcinogenesis, 3, 215-217.

Wislocki PG, Gadek KM, Chou MW, Yang SK and Lu AY, 1980. Carcinogenicity and mutagenicity of the 3,4-dihydrodiols and other metabolites of 7,12-dimethylbenz(a)anthracene and its hydroxymethyl derivatives. Cancer Research, 40, 3661-3664.

Wooley PH, Seibold JR, Whalen JD and Chapdelaine JM, 1989. Pristane-induced arthritis. The immunologic and genetic features of an experimental murine model of autoimmune disease. Arthritis & Rheumatism, 32, 1022-1030.

Wrigley & Co., 1959. Report on toxicity tests; ingredients used in the manufacture of the Wrigley brands of chewing gum; history and complete data. As reported in FAO/WHO, 1993.

Yamauchi T, Komura S and Yagi K, 1986. Serum lipid peroxide levels of albino rats administered naphthalene. Biochemistry International, 13, 1-6.

Yevich PP, 1965. Lung Structure--Relation to Response to Particulate. Archives of Environment Health, 10, 37-43.

Yuan ZX, Sikka HC, Munir S, Kumar A, Muruganandam AV and Kumar S, 2003. Metabolism of the polynuclear sulfur heterocycle benzo[b]phenanthro[2,3-d]thiophene by rodent liver microsomes: evidence for multiple pathways in the bioactivation of benzo[b]phenanthro[2,3-d]thiophene. Chemical Research in Toxicology, 16, 1581-1588.

Zhu H, Bodenheimer HC, Jr., Clain DJ, Min AD and Theise ND, 2010. Hepatic lipogranulomas in patients with chronic liver disease: association with hepatitis C and fatty liver disease. World Journal of Gastroenterology, 16, 5065-5069.

Zhu W, Meng L, Jiang C, Hou W, Xu J, Wang B and Lu S, 2011. Induction of toll-like receptor 2 positive antigen-presenting cells in spleen of pristane-induced arthritis in rats. Molecular Biology Reports, 39, 3667-3673.

Zuelzer WW and Apt L, 1949. Acute hemolytic anemia due to naphthalene poisoning; a clinical and experimental study. JAMA: Journal of the American Medical Association, 141, 185-190.

APPENDICES

A. CONCENTRATIONS OF MINERAL OILS MEASURED IN CONNECTION WITH FOOD CONTROL AND PUBLISHED

Food sample	n	MOH type	Mean (range) in mg/kg	References
Plums	6	[1]P	(< 5-4.2)	Fiorini et al., 2010
Abricots	4	P	(6-28.2)	
Dates	2	P	(1.8-2)	
Raisins	3	P	(2.3-2.4)	
Coconut	1	P	< 5	
Mango	1	P	3.3	
Pineapple	1	P	3.4	
Rice	1	[3]MOH	19	Biedermann and Grob, 2010
		[4]MOSH	15.4	
		[5]MOAH	4	
Sunflower seeds (industrial)	4	MOSH	(3.3-9.3)	Fiselier and Grob, 2009
Sunflower seeds (manually picked from field)	2	MOSH	(0.14-0.25)	
Sunflower from gardens	4	MOSH	(0.25-0.77)	
Sunflower oil, crude	4	P+[2]A	(435-3 000)	Biedermann and Grob, 2009
Sunflower oil, refined	14	P+A	(155-4 900)	
Argan oil	1	MOSH	220	Biedermann et al., 2009
		MOAH	< 5	
Poppy seed oil	1	MOSH	28	
		MOAH	< 3	
Cotton seed oil 1	1	MOSH	65	
		MOAH	10	
Cotton seed oil 2	1	MOSH	275	
		MOAH	60	
Refined olive oil	1	MOSH	21	
		MOAH	4	
Olive pomace oil	1	MOSH	320	
		MOAH	55	
Rice 1	1	MOSH	10	
		MOAH	4.3	
Rice 2	1	MOSH	1.7	
		MOAH	< 0.1	
Chocolate	1	MOSH	24	
		MOAH	6	
Mung beans	1	MOSH	14	
		MOAH	7.5	
Rusk	1	MOSH	910	
		MOAH	< 2	
Grape seed oil	11	P	(43-247)	Fiorini et al., 2008
Extra virgin olive oil (olive mill)	12	P	< 1	Moret et al., 2003
Extra virgin olive oil (market)	10	P	< 1 (< 1-11)	
Lampante virgin olive oil (olive mill)	6	P	< 1	
Olive pomace oil (re-extracted)	7	P	59 (16-145)	
Olive oil (market)	13	P	14 (6-30)	
Olive pomace oil (crude)	3	P	230 (100-300)	
Olive pomace oil (market)	7	P	145 (121-250)	
Sunflower (extracted)	10	P	(5-53)	Wagner et al., 2001b
Sunflower (pressed)	20	P	< 5	
Sunflower (imported)	5	P	(5-20)	
Rapeseed (pressed)	24	P	< 5	

Food sample	n	MOH type	Mean (range) in mg/kg	References
Rapeseed (imported)	5	P	(< 5-80)	
Soybean (pressed)	2	P	(12-20)	
Soybean (extracted)	2	P	< 3	
Soybean (extracted)	5	P	(5-15)	
Safflower	11	P	(< 5-20)	
Peanut oil (Argentinean)	52	P	35 (5-85)	
Peanut oil (African)	14	P	(< 5-50)	
Peanut oil (African)	6	P	11	
Corn	14	P	(< 3-35)	
Palm	7	P	(< 3-35)	
Palmkernel	5	P	(20-40)	
Cocos	6	P	(30-105)	
Rice bran oil	2	P	(14-260)	
Hazelnut	8	P	(< 5-120)	
Walnut	4	P	(< 5-60)	
Almond	4	P	(< 5-75)	
Wheat germ	6	P	(20-180)	
Sesame	7	P	(< 5-95)	
Linseed	3	P	(< 5-20)	
Pumpkin	5	P	(< 5-5)	
Egg yolk	?	P	30 (<2-80)	Grob et al., 2001
Pig fat	2	P	(<3-80)	
Bovine fat	3	P	(<2-10)	
Pig or cattle fat	36	P	25 (?-100)	
Chicken fat	20	P	(<5-150)	
Pig, calve, beef or cow fat	40	P	(<5-30)	
Sardines (in cans)	3	MO	[6](115-480) / (80-370)	Grob et al., 1997
Sardines (in cans, cleaned)	4	MO	(150-210) / (110-165)	
Anchovies (in cans)	2	MO	(95-150) / (40-95)	
Tuna (in cans)	2	MO	(50-120) / (30-35)	
Herring (in cans)	2	MO	(115-175) / (40-45)	
Tuna (in glass)	1	MO	(35) / (45)	
Herring (in glass)	3	MO	(30-115) / (50-155)	
Dry baby foods (packaged in aluminium-laminated bag)	26	MO	< 5	Droz and Grob, 1997
Dry baby foods (packaged in paper bag)	8	MO	(5-33)	
Rice	1	P	130	Moret et al., 1997
		A	30	
Chocolate	1	P	1300	
		A	30	
Safflower oil (unrefined)	1	MO	2100	
		P	1200	
Fish (sea-water and fresh-water)	20	P	(< 10-1 200)	
Bread (bottom crust)	1	P	330	Grob et al., 1991a
Bonbons or caramels	24	P	(50-1300)	
Milk chocolate	1	P	70	
Hazelnuts	50	P	(10-500)	Grob et al., 1991b
Walnuts	4	P	< 0.5	
Almonds	10	P	80 (10-200)	
Coffee	14	P	100 (150-230)	
Instant coffee	4	P	Up to 2	
Cocoa	5	P	(10-135)	
Chocolate	10	P	(5-270)	
Rice	6	P	100 (20-160)	

[1]p: paraffins; [2]a: aromatics; [3]MO: mineral oil; [4]MOSH: mineral oil saturated hydrocarbons; [5]MOAH: mineral oil aromatic hydrocarbons; [6]: in oil / in fish.

B. OCCURRENCE DATA COLLECTED ON FEED

The Official Food Control Authority of the Canton of Zürich – Kantonales Labor Zürich (KLZH) analysed the mineral oil saturated hydrocarbons (MOSH) concentration in feed produced and consumed in Switzerland. A total of 141 analytical results were provided, including 24 results below the limit of detection (LOD) (17 %). Descriptive statistics are summarised in Table B1. Mineral oil added as binders to admix the fines and contaminants of the added fats were reported as MOSH sources.

Table B1: Descriptive statistical analysis of MOSH results provided on feed (mg/kg).

Feed category	N		P5	Mean	Median	P95
feed fat	**76**	**LB**	0	254	20	1 800
		UB	5	259	25	1 800
feed for poultry	**48**	**LB**	20	109	80	400
		UB	20	109	80	400
feed unspecified	**17**	**LB**	3	33	25	80
		UB	3	33	25	80

LB: lower bound; UB: upper bound; N: number of samples; P5/P95: 5^{th}/95^{th} percentiles.

C. ESTIMATION OF WHITE MINERAL OILS, PARAFFIN WAX AND MICROCRYSTALLINE WAX IN VARIOUS CATEGORIES OF FOODS, BASED ON THE AMOUNTS ADDED TO FOODS AND MIGRATION STUDIES FROM COATINGS AND PACKAGING MATERIALS

Heimbach et al. (2002) and WHO/IPCS (2003), estimated the concentrations of 'white mineral oils, paraffin wax and microcrystalline wax' in various categories of foods, based on the amounts added to foods and migration studies from coatings and packaging materials. According to WHO/IPCS (2003), the data show that: (1) the principal dietary sources of mineral oil in the United Kingdom were bread divider oils (high- and low-viscosity oils) and grain dust control (low-viscosity oils) (Food Chemical Risk Analysis, 2001, cited by WHO/IPCS, 2003), while those in the USA were fruit and vegetable coatings (low-viscosity oils), confectionery (low-viscosity oils), grain dust control (low-viscosity oils), and bakery pan release oils (high-viscosity oils) (Reich et al., 1998, cited by WHO/IPCS, 2003); and (2) the sole dietary source of paraffin wax in Europe was wax paper packaging, and the principal source of microcrystalline wax was use as a confectionery glazing agent (Food Chemical Risk Analysis, 2001, cited by WHO/IPCS, 2003), while the main sources of both waxes in the USA were fruit and vegetable coatings and flexible packaging (Reich et al., 1998, cited by WHO, 2003).

In addition, WHO/IPCS (2003) reported data from other surveys on MOH concentrations in five major food groups:

Bakery products (bread):

Few data were available on mineral oil residues in commercially produced breads. The total concentration of mineral oils in loaves in the United Kingdom in one study ranged from 220 to 490 mg/kg. A similar study on mineral oil residues in bread in Germany showed 330 mg/kg in the bottom crust and < 1 mg/kg in the top crust and centre of the bread (Food Chemical Risk Analysis, 2001, cited by WHO/IPCS, 2003). A survey conducted by the American Bakers Association in 1998 (Reich et al., 1998, cited by WHO/IPCS, 2003) showed that the overall average total concentration of mineral hydrocarbons in bread from pan-release oils and trough grease was 533 mg/kg. Some bread may contain only mineral oil, some only petrolatum jelly, some both kinds, and some may contain no mineral oils from the baking process.

Fruits and vegetables:

In computing the concentrations of mineral hydrocarbons in foods resulting from use of mineral oil-based coatings, it was conservatively assumed that all the relevant fruits and vegetables are coated with a standard composite of white mineral oil, petrolatum jelly, paraffin wax and microcrystalline wax. The residual concentrations of these mineral oils in standard coatings would then be 160, 44, 44 and 44 mg/kg, respectively. This assumption therefore resulted in a greatly exaggerated estimate of intake from fruit and vegetable coatings.

Grains and oil seeds:

Reich et al. (1998, cited by WHO/IPCS, 2003) showed that the concentrations of mineral oil on wheat grain stored for 6 months after spraying with low-viscosity white mineral oil at 230 mg/kg was 180 - 250 mg/kg. As low-viscosity mineral oils are quite volatile, losses are expected at the high temperatures used in food processing. No detectable mineral oil residues were found in finished bread products from milled and baked mineral oil-treated wheat grains. When mineral oil was added at 200 or 500 mg/kg to wheat flour, which was then made into dough and baked into bread, the concentration of mineral oil was reduced by approximately 80 %. As a conservative measure, an 80 % reduction factor was recommended for use in estimating the residual content of mineral oil in baked products and breakfast cereals when mineral oil-treated grains were the raw material.

Except where other estimates exist, such as for maize, soya beans and wheat meals, 20 % of the maximum permitted level in the USA of 200 mg/kg (i.e. 40 mg/kg) was used as the mineral oil concentration in other grain products, namely rice, barley, oats and rye.

Confectionery:

White mineral oils and petrolatum jelly for use as release agents and as sealing and glazing agents in the manufacture of candy, chocolate and marshmallow-type items are approved in the USA, with a maximum permitted level of 200 mg/kg.

Foods in contact with wax packaging materials:

The overall concentration of paraffin wax in crackers, cereals, meats, cheeses, milk and beverages (alcoholic, acidic and aqueous) due to contact with wax packaging was estimated to be 17 mg/kg, and that of microcrystalline wax was 9.7 mg/kg (Reich et al., 1998, cited by WHO/IPCS, 2003).

D. MAXIMUM LIKELIHOOD MODELLING OF OCCURRENCE IN 'BREAD AND ROLLS' AND 'GRAINS FOR HUMAN CONSUMPTION'

The two food groups 'Bread and rolls' and 'Grains for human consumption' show a multi-modal distribution of MOSH occurrence values. In particular, the high values depending on use of mineral oils in the technological process appear reasonably distinct by the distribution contributed by other sources. It was therefore decided to use a maximum likelyhood approach to fit the data separating the high occurrence values from the rest of the data.

This method (Du, 2002) is implemented in the 'mixdist' package of the R statistical program. Before fitting the data, they were log transformed, under the assumption of log normal nature of the component distributions. The mean values calculated in the model were converted back to real values.

The fitting for 'Bread and rolls' is shown in Figure D1.

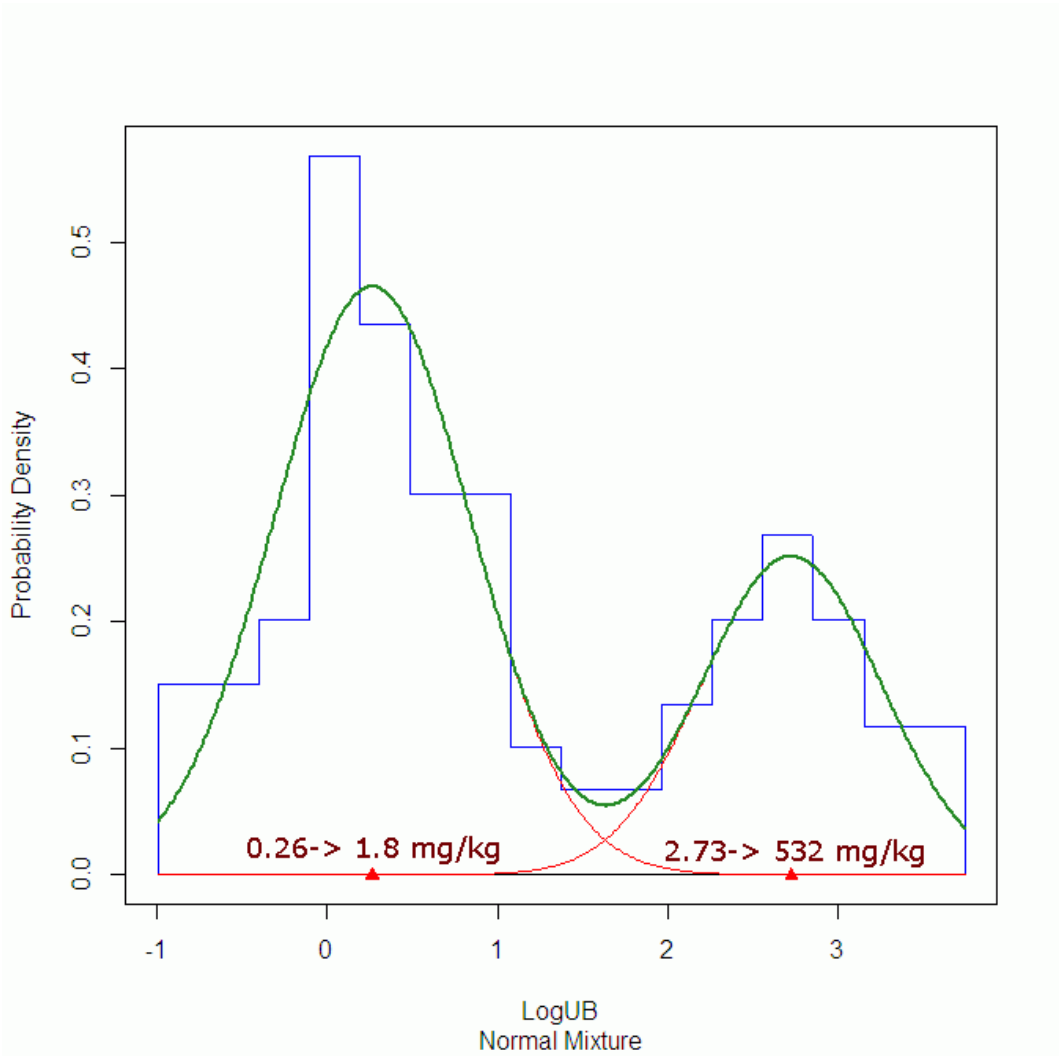


Figure D1: Bi-normal fitting of the log-transformed UB data on 'Bread and Rolls', calculated using the mixdist package of R.

The fitting for 'Grains for human consumption' is shown in Figure D2.

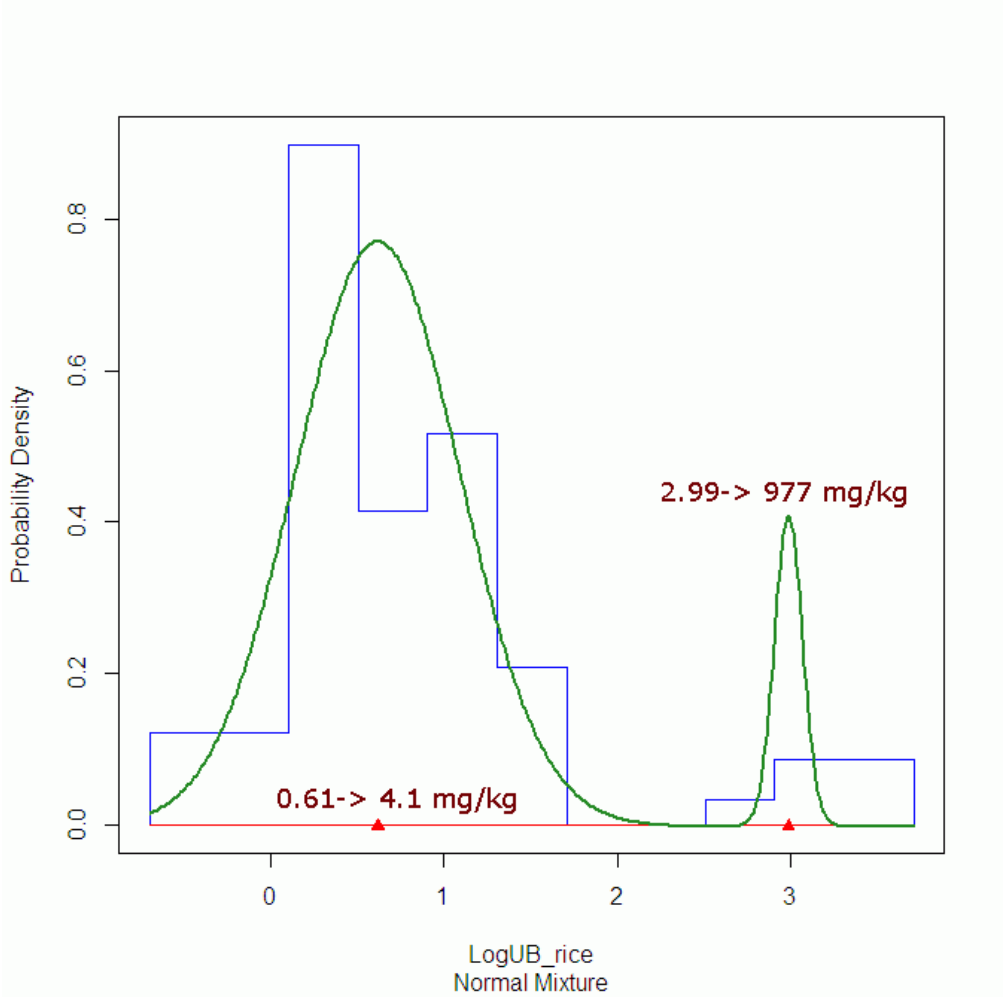


Figure D2: Bi-normal fitting of the log-transformed UB data on 'Grains for human consumption', calculated using the mixdist package of R.

E. INFORMATION ON THE EFSA "COMPREHENSIVE EUROPEAN FOOD CONSUMPTION DATABASE"

Table E1: Basic information on the dietary surveys included in the 'Comprehensive European Food Consumption Database'.

Country	Name of the dietary survey (Acronym)	Institution providing the data	Reference publication
Austria	Austrian Study On Nutritional Status (ASNS)	Institute of Nutritional Sciences - University of Vienna	Elmadfa et al., 2008
Belgium	Diet National 2004	Scientific Institute of Public Health	De Vriese et al., 2005
Bulgaria	National Survey Of Food Intake And Nutritional Status	National Centre of Public Health Protection	Petrova and Angelova, 2006
Bulgaria II	NUTRICHILD	National Centre of Public Health Protection	Petrova et al., 2009
Czech Republic	SISP04	National Institute of Public Health	Ruprich et al., 2006
Denmark	Danish National Survey of Dietary Habits and Physical Activity	National Food Institute, Technical University of Denmark	Lyhne et al., 2005
Estonia	NDS 1997	National Institute for Public Health Development	Pomerleau et al., 1999
Finland	FINDIET 2007	National Public Health Institute - Nutrition Unit[(a)]	Paturi et al., 2008.
France	INCA2	French Agency for food, environmental and occupation health safety (ANSES)	ANSES, 2009; Dubuisson et al., 2010; Lioret et al., 2010
Germany	German National Nutrition Survey II (NVS II)	Bundesforschungsinstitut für Ernährung und Lebensmittel (Max Rubner-Institut)	Krems et al., 2006; MRI, 2008
Hungary	National Repr Surv	Hungarian Food Safety Office	Rodler et al., 2005.
Ireland	NSIFCS	Food Safety Authority of Ireland	Harrington et al., 2001; Kiely et al., 2001
Italy	INRAN-SCAI 2005–06	National Research Institute for Food and Nutrition (INRAN)	Leclercq et al., 2009
Latvia	EFSA_TEST	Food Centre Food and Veterinary Service of Latvia	Šantare et al., 2008
Netherlands	VCP 2003	National Institute of Public Health and the Environment, TNO Quality of Life	Ocké et al., 2005
Poland	IZZ-FAO-2000	National Food and Nutrition Institute	Szponar et al., 2001; 2003; Sekula et al., 2004
Slovakia	SK MON 2008	Food Research Institute	Not available
Slovenia	CRP-2008	National Institute of Public Health of Slovenia	Gabrijelčič Blenkuš et al., 2009
Spain	AESAN –Fiab	Universidad Complutense de Madrid	Requejo et al., 2002
Spain II	AESAN	Universidad Complutense de Madrid	Ortega et al., 2010
Spain	Nutrition Survey of Basque population	Administración de la Comunidad Autónoma del País Vasco; Departamento de Sanidad	Larrañaga Larrañaga et al., 2006
Sweden	RIKSMATEN 1997-98	Swedish National Food Administration	Becker and Pearson, 2002
UK	National Diet and Nutrition Survey (NDNS)	Food Standards Agency (FSA)	Henderson et al., 2002

Table E2: Information on the dietary method used within the dietary surveys.

Country	Method	Number of replicates	Average distance between non consecutive replicates[(a)] (days)	Additional food frequency (FFQ) or propensity (FPQ) questionnaire[(b)]
Austria	24 h dietary recall	1	Not applicable	No
Belgium	24 h dietary recall	2	23	Yes
Bulgaria	24 h dietary recall	1	Not applicable	Yes
Bulgaria II	24 h dietary recall	2	3	Yes
Czech Republic	24 h dietary recall	2	79	Yes
Denmark	Food record	7	Consecutive days	No
Estonia	24 h dietary recall	1	Not applicable	Yes
Finland	48 h dietary recall	1	Not applicable	Yes
France	Food record	7	Consecutive days	No
Germany	24 h dietary recall	2	16	Dietary history
Hungary	Food record	3	2 consecutive days and 1 non consecutive day[(c)]	No
Ireland	Food record	7	Consecutive days	Yes, only focused on meat
Italy	Food record	3	Consecutive days	No
Latvia	24 h dietary recall	2	68	Yes
Netherlands	24 h dietary recall	2	11	Yes
Poland	24 h dietary recall	1	Not applicable	No
Slovakia	24 h dietary recall	1	Not applicable	No
Slovenia	24 h dietary recall	1	Not applicable	Yes
Spain	Food record	3	Consecutive days	Yes
Spain II	24 h dietary recall	2	3	Yes
Sweden	Food record	7	Consecutive days	No
United Kingdom	Food record	7	Consecutive days	No

Table E3: Dietary surveys included in the Comprehensive food consumption database, considered for the chronic dietary exposure assessment and number of subjects in the different age classes.

Country	Dietary survey[(a)]	Abbreviation[(b)]	Number of subjects							
			Total	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Belgium	Diet_National_2004	BE/1	3 118				584	1 304	518	712
	Regional Flanders	BE/2	661		36 [(c)]	625				
Bulgaria	NUTRICHILD	BG	1 721	860	428	433				
Cyprus	Childhealth	CY	303				303			
Czech Republic	SISP04	CZ	2 353			389	298	1 666		
Denmark	Danish_Dietary_Survey	DK	4 120			490	479	2 822	309	20[(c)]
Finland	DIPP	FI/1	1 430		497	933				
	FINDIET_2007	FI/2	2 038					1 575	463	
	STRIP	FI/3	250			250				
France	INCA2	FR	4 079			482	973	2 276	264	84
Germany	DONALD_2006	DE/1	303		92	211				
	DONALD_2007	DE/2	311		85	226				
	DONALD_2008	DE/3	307		84	223				
	National_Nutrition_Survey_II	DE/4	13 926				1011	10 419	2 006	490
Greece	Regional_Crete	GR	839			839				
Hungary	National_Repr_Surv	HU	1 360					1 074	206	80
Ireland	NSIFCS	IE	958					958		
Italy	INRAN_SCAI_2005_06	IT	3 323	16[(c)]	36[(c)]	193	247	2 313	290	228
Latvia	EFSA_TEST	LT	1 965			189	470	1 306		
Netherlands	DNFCS_2003	NL/1	750					750		
	VCP_kids	NL/2	1 279		322	957				
Spain	AESAN	ES/1	410					410		
	AESAN_FIAB	ES/2	1 067				86	981		
	NUT_INK05	ES/3	1 050			399	651			
	enKid	ES/4	382		17[(c)]	156	209			
Sweden	Riksmaten_1997_98	SE/1	1 210					1 210		
	NFAn	SE/2	2 491			1 473	1 018			
United Kingdom	NDNS	UK	1 724					1 724		

(a): More information on the dietary surveys is given in the Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b); (b) BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; (c): 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and therefore for these dietary surveys/age classes the 95th percentile estimates will not be presented in the exposure assessment.

ABBREVIATIONS

ABS	Acrylonitrile/butadiene/styrene
ADI	Acceptable daily intake
AESAN	Spanish Food and Drink Industry Federation
AESAN_FIAB	Spanish Food and Drink Industry Federation – Spanish dietary survey
AFC	EFSA former Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
AlOx	Aluminium oxide
ALT	Alanine aminotransferase
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
API	The American Petroleum Institute
AST	Aspartate aminotransferase
ASTM Intl.	American Society for Testing and Materials
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
BaA	Benz[*a*]anthracene
BaP	Benzo[*a*]pyrene
BCF	Bioconcentration factor
BE	Belgium
BfR	Bundesinstitut für Risikobewertung
BG	Bulgaria
BMELV	German Food Ministry of food, Agriculture and Consumer Protection
b.w.	Body weight
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
Childhealth	Childhealth (Cyprus, Dietary survey)
CHR	Chrysene
CI	Confidence interval
CONCAWE	Conservation of Clean Air and Water in Europe
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
CP	Citrulline containing peptides
CRM	Certified reference material
CY	Cyprus
CZ	Czech Republic
DA	Dark Agouti (rat)
Danish_Dietary_Survey	Danish dietary survey (Denmark, Dietary survey)
DCM	EFSA Dietary & Chemical Monitoring (former DATEX) Unit
DE	Germany
Diet_National_2004	Diet_National_2004 (Belgium, Dietary survey)
diMA	Dimethylbenzanthracene
DIPP	DIPP (Finland, Dietary survey)
DK	Denmark
DMBA	Dimethylbenz[*a*]anthracene

DMSO	Dimethyl sulfoxide
DNFCS_2003	Dutch National Food Consumption Survey
DONALD_2006	DONALD 2006 (Germany, Dietary survey)
DONALD_2007	DONALD_2007 (Germany, Dietary survey)
DONALD_2008	DONALD_2008 (Germany, Dietary survey)
EC	European Commission
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EFSA_TEST	EFSA_TEST (Latvia, Dietary survey)
EICO	eicosanylcyclohexane
EMEA	European Medicines Agency
enKid	Food preferences of Spanish children and young people
EPA	The US Environmental Protection Agency
ES	Spain
EXPOCHI	Article 36 project "Individual food consumption data and exposure assessment studies for children"
FA	Fluoranthene
FAO	Food and Agriculture Organization of the United Nations
FAT	Perirenal adipose tissue
FCC	Fluid catalytic cracker
FCM	Food contact material
FDA	The US Food and Drug Administration
FI	Finland
FINDIET_2007	FINDIET 2007 (Finland, Dietary survey)
FID	Flame ionisation detection
FLUO	Fluorene
FoodEx	Food classification system
FR	France
FSA	UK Food Standards Agency
FT	Fischer-Tropsch
GC	Gas chromatography
GC x GC	Two-dimensional gas chromatography
GC-/GC-MS	Two dimensional gas chromatography-mass spectrometry
GLC	Gas-liquid chromatography
GR	Greece
HC	Hydrocarbon
HF	Hydrofluoric acid
HMPW	High melting point wax
HPLC	High-performance liquid chromatography
HPV	High Production Volume
HTFT	High temperature Fischer-Tropsch
HTWO	Hydro-treated white oil

HU	Hungary
IARC	International Agency for Research on Cancer
IE	Ireland
IFN	Interferon
Ig	Immunoglobulin
IJO	International Jute Organisation
IL	Interleukin
IMPW	Intermediate melting point wax
INCA2	Enquête Individuelle et Nationale sur les Consommations Alimentaires
INRAN_SCAI_2005_06	– Italian National Food Consumption Survey
IOM	Institute of Medicine of the U.S. National Academies of Sciences
IPCS	International Programme on Chemical Safety
IP	Indeno[*1,2,3-cd*]pyrene
i.p.	Intraperitoneal
IR	Infra red
IT	Italy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	Joint Research Centre
KLZH	The Official Food Control Authority of the Canton of Zürich Kantonales Labor Zürich
LB	Lower bound
LC	Left censored
LMPW	Low melting point wax
LN	Lymph node
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
LOD	Limit of detection
LPG	Liquid propane gas
LOQ	Limit of quantification
LTFT	Low temperature Fischer-Tropsch
LV	Latvia
MA	Methylanthracene
MBA	Methylbenzanthracene
MCH	Methylchrysene
MFA	Methylfluoranthene
MHW	The Japanese Ministry of Health and Welfare
MI	Mutagenic index
MLN	Mesenteric lymph node
MOAH	Mineral Oil Aromatic Hydrocarbons
MOH	Mineral Oil Hydrocarbons
MOSH	Mineral Oil Saturated Hydrocarbons
MNAP	Methylnaphthalene
MRL	Maximum Residue Limit

MS	Mass spectrometry
MW	Molecular weight
NAP	Naphthalene
National_Nutrition_Survey_II	National_Nutrition_Survey_II (Germany, Dietary survey)
National_Repr_Surv	National_Repr_Surv (Hungary, Dietary survey)
NDNS	National Diet and Nutrition Survey (United Kingdom)
NFA	National Food Administration (Sweden, Dietary survey)
NL	The Netherlands
NOAEL	No-observed- adverse-effect-level
NOEL	No-observed-effect-level
NSF	National Science Foundation
NSIFCS	North/South Ireland Food Consumption Survey
NTP	US National Toxicology Program
NUT_INK05	NUT_INK05 (Spain, Dietary survey)
NUTRICHILD	NUTRICHILD (Bulgaria, Dietary survey)
NTP	The United States National Toxicology Programme
OECD	Organisation for Economic Co-operation and Development
OECD-SIDS	Organisation for Economic Co-operation and Development – Screening Information Dataset
OR	Odds ratio
OTWO	Oleum-treated white oil
PA	Polyamide
PAHs	Polycyclic aromatic hydrocarbons
PAO	Poly alpha olefins
PCB	Polychlorinated biphenyl
PE	Polyethylene
PE-HD	Polyethylene – high density
PET	Polyethylene terephthalate
PHEN	Phenanthrene
POSH	Polyolefin oligomeric saturated hydrocarbons
PP	Polypropylene
PPacr	Acrylate coated polypropylene
PRAPeR	EFSA Pesticide Risk Assessment and Peer Review Unit
PYR	Pyrene
QA	Quality assurance
RA	Rheumatoid arthritis
RASFF	Rapid Alert System for Food and Feed
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances
Regional_Crete	Regional_Crete (Greece, Dietary survey)
Regional Flanders	Regional Flanders (Belgium, Dietary survey)
RF	Rheumatoid factor
Riksmaten_1997_98	Swedish national food survey_1997_98

RP	Reference point
RR	Risk ratio
SAE	Society of Automotive Engineers
SCF	Scientific Committee for Food
SCoFCAH	Standing Committee on the Food Chain and Animal Health
SD	Standard deviation
SE	Sweden
SHCs	Synthesised hydrocarbons
SiOx	Silicium oxide
SISP04	Czech dietary Survey
SLE	Systemic lupus erythematosus
SML	Specific migration limit
SRBC	Sheep-red-blood-cell
STRIP	STRIP (Finland, Dietary Survey)
TDI	Tolerable daily intake
TLC	Thin layer chromatography
TLR	Toll-like Receptor
TPA	12-O-tetradecanoylphorbol-13-acetate
TPH	Total petroleum hydrocarbons
UB	Upper bound
UCM	Unresolved complex mixtures
UDP	Uridine diphosphate
UK	United Kingdom
USA	The United States of America
USDA	United States Department of Agriculture
USP	United States Pharmacopoeia
VCP_kids	VCP_kids (The Netherlands, Dietary survey)
VI	Viscosity index
WHO	World Health Organization