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Safety assessment of 'waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity' for use in food contact materials

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Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) assessed the safety of the 'waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity' (FCM No. 93), for which the uses were requested to be extended for articles in contact with fatty foods. Migration from low-density polyethylene samples containing 1% w/w of a representative wax was tested in food simulants. In fatty food simulants, the migration of mineral oil saturated hydrocarbons (MOSH) $\leq$ C35 was 142 mg/kg food, exceeding the overall migration limit for plastic FCM. Mineral oil aromatic hydrocarbons (MOAH) with at least two rings are largely removed during the manufacturing process. Based on various lines of evidence, the Panel concluded that any concern for the potential presence of MOAH with two or more conjugated aromatic rings can be ruled out. Based on the genotoxicity studies and on the content of polycyclic aromatic hydrocarbons (PAHs), the substance does not raise a concern for genotoxicity. Available toxicokinetic data showed a limited accumulation of MOSH. No adverse effects were observed up to the highest tested dose of 9 g/kg body weight per day in a 90-day repeated oral toxicity study in Sprague–Dawley rats. The available results showed that FCM No. 93 is devoid of endocrine activity. The provided information on chronic toxicity and carcinogenicity was limited and inadequate to reach conclusions on these endpoints. Therefore, the CEP Panel concluded that under the intended and tested conditions of uses, the substance does not raise safety concern for the consumer if used to a level ensuring that its migration into food is no more than 5 mg/kg.

Keywords: waxes, paraffinic, refined, derived from petroleum based or synthetic hydrocarbon feedstock, low viscosity, FCM No. 93, food contact materials, safety assessment, evaluation

Requestor: Ministry of Health, Welfare and Sport, the Netherlands

Question number: EFSA-Q-2018-00558

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Competing interests: R. Franz declared that Fraunhofer institute at which he is employed provides advisory services to private business operators active in the sector on food contact materials. In line with EFSA's Policy on Independence (https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf) and the Decision of the Executive Director on Competing Interest Management (https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf), a waiver was granted to R. Franz regarding his participation to the EFSA's Working Group on Food Contact Materials (FCM WG) in accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management. Pursuant to Article 21(6) of the above-mentioned Decision, the involvement of R. Franz is authorised as member in the FCM WG, allowing him to take part in the discussions and in the drafting phase of the scientific output, but he is not allowed to be, or act as, a chairman, a vice-chairman or rapporteur of the working group.

Note: The full opinion will be published in accordance with Article 10(6) of Regulation (EC) No 1935/2004 once the decision on confidentiality, in line with Article 20(3) of the Regulation, will be received from the European Commission. The following information has been provided under confidentiality and it is redacted awaiting the decision of the Commission: *Identity of the substance (Section 3.1.1)*.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Before a substance is authorised to be used in food contact materials (FCM) and is included in a positive list, EFSA's opinion on its safety is required. This procedure has been established in Articles 8, 9 and 10 of Regulation (EC) No 1935/2004[1] of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

According to this procedure, the industry submits applications to the Member States' competent authorities which transmit the applications to the European Food Safety Authority (EFSA) for their evaluation.

In this case, EFSA received an application from the Ministry of Health, Welfare and Sport, the Netherlands, requesting the evaluation of the substance waxes, paraffinic, refined, derived from petroleum based or synthetic hydrocarbon feedstock, low viscosity, with the FCM substance No. 93. The dossier was submitted on behalf of European Wax Federation aisbl. According to Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food, EFSA is asked to carry out an assessment of the risks related to the intended use of the substance and to deliver a scientific opinion.

2. Data and Methodologies

2.1. Data

The applicant has submitted a dossier in support of its application to extend the scope of the authorisation of 'waxes, paraffinic, refined, derived from petroleum based or synthetic hydrocarbon feedstock, low viscosity', to be used in plastic food contact materials.

Additional information was provided by the applicant during the assessment process in response to requests from EFSA sent on 17 September 2019 and 21 July 2021 (see 'Documentation provided to EFSA').

Data submitted and used for the evaluation are:

Non-toxicological data

- Data on identity and characterisation of the substance.
- Data on physical and chemical properties.
- Data on intended use and authorisation.
- Data on migration of the substance.

Toxicological data

- Gene mutation in bacteria.
- *In vitro* mammalian cell gene mutation test.
- *In vitro* chromosomal aberration test.
- A repeated dose 90-day oral toxicity study with Sprague–Dawley rats and published studies with Sprague–Dawley and Fisher 344 rats.
- Literature reviews on reproductive and developmental toxicity studies with similar substances.
- A systematic literature review and a published carcinogenicity study in Sprague–Dawley rats.
- Published studies on toxicokinetics in Sprague–Dawley rats, Fisher 344 rats and humans.

2.2. Methodologies

The assessment was conducted in line with the principles laid down in Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food. This Regulation underlines that applicants may consult the Guidelines of the Scientific Committee on Food (SCF) for the presentation of an application for safety assessment of a substance to be used in FCM prior to its authorisation (European Commission, 2001), including the corresponding data requirements. The dossier that the applicant submitted for evaluation was in line with the SCF guidelines (European Commission, 2001).

[1] Regulation (EC) No 1935/2004 of the European parliament and of the council 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. OJ L 338, 13.11.2004, pp. 4–17.

The methodology is based on the characterisation of the substance that is/are the subject of the request for safety assessment prior to authorisation, its impurities and reaction and degradation products, the evaluation of the exposure to those substances through migration and the definition of minimum sets of toxicity data required for safety assessment.

To establish the safety from ingestion of migrating substances, the toxicological data indicating the potential hazard and the likely human exposure data need to be combined. Exposure is estimated from studies on migration into food or food simulants and considering that a person may consume daily up to 1 kg of food in contact with the relevant FCM.

As a general rule, the greater the exposure through migration, the more toxicological data is required for the safety assessment of a substance. Currently there are three tiers with different thresholds triggering the need for more toxicological information as follows:

- In case of high migration (i.e. 5–60 mg/kg food), an extensive data set is needed.
- In case of migration between 0.05 and 5 mg/kg food, a reduced data set may suffice.
- In case of low migration (i.e. < 0.05 mg/kg food), only a limited data set is needed.

More detailed information on the required data is available in the SCF guidelines (European Commission, 2001).

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and considering the relevant guidance from the EFSA Scientific Committee.

3. Assessment

'Waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity' (FCM No. 93) are non-defined mixtures which are, according to the applicant, intended to be used as an additive in all kinds of polymers, including polyolefins and multi-layer multi-materials. Typical concentrations in the final articles are 1% to 3% w/w, depending on the intended applications, e.g. up to 1% w/w in polyolefins, and in all types of polymers in contact with fatty foods.

The substance was evaluated by the EFSA AFC Panel in 2006 (EFSA, 2006). It is currently listed in Regulation (EU) No 10/2011[2] under the FCM number 93 with a specific migration limit (SML) of 0.05 mg/kg food plus a specific restriction 'Not to be used for articles in contact with fatty foods for which simulant D is laid down. Average molecular weight not less than 350 Da. Viscosity at 100°C not less than 2.5 cSt (2.5×10^{-6} m^2/s). Content of hydrocarbons with carbon number less than 25, not more than 40% (w/w)'.

With this new application, the applicant requests to extend the use to all food contact types, i.e. to include fatty foods and more generally foods that have a lipophilic character for which simulants D1 and D2 are laid down in the regulation. Final articles containing the additive may be in contact with all types of food for long-term storage at room temperature or below.

According to the applicant, this group of paraffin waxes is also used as a component in paper production, in wax coatings on paper, in wax coatings on cheese and in the manufacture of construction products (polyvinylchloride (PVC)) which are intended to come into contact with drinking water. Paraffin waxes are also used in hot-melt adhesives and as ingredients for cosmetic preparations.

'Waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity', are not listed in the EU as a food additive.

3.1. Non-toxicological data

3.1.1. Identity of the substance[3]

FCM No. 93 waxes are non-defined mixtures, albeit within the restrictions imposed by the specifications (Section 3.1.2) and by the manufacturing process. They mostly consist of mineral oil saturated hydrocarbons (MOSH) and a small amount of mineral oil aromatic hydrocarbons (MOAH). According to the applicant, FCM No. 93 waxes are produced from petroleum-based feedstock or from polymerised synthetic feedstock of bio-mass or natural gas (Fischer–Tropsch).

[2] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1.
[3] Technical dossier/Consolidated version_Sept 22/Appendix B/Section 1 and Annexes 2, 3, 21, 22, 23.

When made from mineral oil (petroleum-based feedstock), the manufacturing process includes a number of isolation and purification steps:

1) The base oil fraction obtained by vacuum distillation is extracted with solvents. This step largely removes MOAH, especially those with two or more conjugated aromatic rings. According to the applicant, this renders the product free of carcinogenic MOAH [redacted]
2) Wax is obtained by crystallisation and de-oiling of the extracted base oil. This step is selective in precipitating MOSH with predominantly straight chains. This step is expected to reduce the MOAH content [redacted], again being selective for monocyclic MOAH with a straight alkyl group and preferentially removing those with two or more conjugated aromatic rings.
3) Waxes are finally hydrogenated. Hydrogenation is selective in saturating aromatic rings in conjugated polycyclic systems, while hydrogenation of isolated aromatic rings, such as alkylated benzenes, requires harsher conditions.

Waxes obtained from polymerised synthetic feedstock (Fischer–Tropsch) have a similar MOSH composition and are virtually free of MOAH.

As a result, FCM No. 93 waxes almost entirely consists of MOSH, namely *n*-alkanes (> 60%, mostly > 80%, Table 1), little branched isoalkanes with side-branches limited in number and length and located towards one end of the alkyl chain, as well as cyclohexanes and cyclopentanes with predominantly straight chain alkyl groups (naphthenes). The waxes also contain a small fraction of MOAH, almost exclusively alkylated and with a single aromatic ring.

The applicant submitted detailed hydrocarbon compositions of seven waxes that covered the range of FCM 93 specifications.[4] The analytical methods used were online high-performance liquid chromatography–gas chromatography-flame ionisation detection (HPLC-GC-FID) and comprehensive two-dimensional gas chromatography (GC×GC) with mass spectrometry (MS) or FID detection. This analysis provided a quantitative description of the seven waxes in terms of their content of hydrocarbon classes (normal-, branched-, cyclic- and aromatic hydrocarbons) at each point of the carbon number (Table 1).

Table 1: Physicochemical properties and composition of the different waxes analysed for this submission as provided by the applicant

		WAX 1 petroleum	WAX 2 petroleum	WAX 3 petroleum	WAX 4 petroleum	WAX 5 petroleum	WAX 6 petroleum	WAX 7 synthetic
Physicochemical properties								
Viscosity at 100°C (cSt)		3.5	3.3	3.4	3.81	6.0	9.9	9.5
Congealing point (°C)		54.0	53.5	53.0	58.0	62.0	73.0	80.0–85.0
Average molecular weight (g/mol)		377	375	371	393	481	609	598
Composition by hydrocarbon structural class (% w/w)								
MOSH	Normal alkanes	83.48	87.82	85.10	83.06	61.23	46.70	93.27
	Isoalkanes	12.66	9.63	10.76	13.92	18.16	33.61	6.66
	Monocyclic naphthenes	3.75	2.50	4.14	2.93	19.47	19.68	0.00
1-Alkenes		0.09	0.00	0.00	0.00	0.00	0.00	0.00
1-Alcohols		0.00	0.00	0.00	0.00	0.00	0.00	0.07
Aromatic hydrocarbons (MOAH)		0.01	0.04	0.00	0.09	1.14	0.00	0.00

[4] Wax 6 is at the lower end of FCM No. 94 waxes (specifications in Regulation (EU) no 10/2011: 'Average molecular weight not less than 500 Da. Viscosity at 100°C not less than 11 cSt (11×10^{-6} m^2/s). Content of mineral hydrocarbons with Carbon number less than 25, not more than 5% (w/w)') but very close to the higher end of FCM No. 93 waxes.

	WAX 1 petroleum	WAX 2 petroleum	WAX 3 petroleum	WAX 4 petroleum	WAX 5 petroleum	WAX 6 petroleum	WAX 7 synthetic
Carbon number range (%)							
C16–C20	0.78	0.48	0.93	0.04	0.06	0.01	0.31
C21–C25	34.36	40.76	42.21	21.54	2.68	0.46	0.93
C26–C30	53.37	48.02	48.28	59.12	21.53	2.51	2.08
C31–C35	10.80	8.85	7.36	18.34	39.42	5.42	9.76
C36–C40	0.56	1.53	0.99	0.57	23.54	14.36	24.62
C41–C45	0.08	0.26	0.18	0.23	8.85	41.44	27.81
C46–C50	0.00	0.05	0.04	0.06	2.19	29.13	21.98
C51–C55	0.00	0.01	0.01	0.01	0.58	6.27	12.18
C55–C60	0.00	0.00	0.00	0.00	0.00	0.40	0.28
Total	99.96	99.96	100.00	99.91	98.86	99.99	99.96
Fraction (%) in C21–C35 Range	99.31	98.10	98.78	99.05	63.69	8.40	13.09
Fraction (%) > C35	0.64	1.85	1.22	0.87	35.16	91.59	86.87

Additionally, the applicant provided analytical results and test certificates for MOAH in 22 other samples of waxes (covering the FCM No. 93 specifications), specified by the carbon-number ranges C10–C16, C17–C25, C26–C35 and C36–C50 (referring to retention times of *n*-alkanes in GC with a non-polar stationary phase). Summing these for C10–C35 and taking the middle-bound approach (assuming non-detects are at half the limit of detection (LoD) value), MOAH ≤ C35 was either not detected (< 0.15% in 15 samples) or was just detectable at 0.2%–0.3% w/w (in 6 samples), with one higher sample that contained 0.8% w/w MOAH ≤ C35. MOAH was also detected in the C36–C50 fraction. Extending the span to MOAH ≤ C50 (C10–C50), the results for 21 of the 22 waxes were in the range of 0.2% to 1.35% w/w with the sample that was highest in ≤ C35 content also being the highest in ≤ C50 MOAH content, at 3.9% w/w.

The applicant provided analytical results and test certificates for polycyclic aromatic hydrocarbons (PAHs) in five of the waxes reported in Table 1 (waxes 1, 2, 3, 4, 6), covering the range of waxes described by the EU specifications for FCM No. 93. Wax number 5 was not included, although, of the seven waxes in Table 1, it contained the highest content of MOAH (1.14% w/w, 12.7 times higher than 0.09% w/w for Wax 4). A total of 32 aromatic substances were tested for. Benzo[a]pyrene was not detected in any sample with a LoD of [redacted]. For the 16 genotoxic PAHs considered by the EFSA CONTAM Panel[5] (abbreviated as PAH16, EFSA, 2008a), the large majority of results were below this LoD ('non-detects') with only 12 of the 80 individual results (i.e. 5 samples times 16 analytes) being above the LoD. The sum of the PAH16 content (middle-bound, setting non-detects to half the LoD) for the five samples was in the range of 0.1 to 2.7 μg/kg wax.

The applicant provided limited data regarding the content of MOAH with two or more conjugated aromatic rings. GC×GC analysis of the wax EWF FCM 93 58[6] used in a sub-chronic toxicity study (Section 3.2.3.1) [redacted][7]

Among the five waxes analysed (Table 1), the non-genotoxic phenanthrene (not included in the 16 genotoxic PAHs considered by the EFSA CONTAM Panel, EFSA, 2008a) was by far the predominant

[5] The CONTAM Panel decided to cover the 15 PAHs identified by SCF in 2002 (European Commission, 2002) together with benzo[c]fluorene as suggested by JECFA in 2005. The 16 PAHs are: benzo[a]anthracene, benzo[c]fluorene benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, cyclopenta[cd]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene, 5-methylchrysene.

[6] Technical dossier/Consolidated version_Sept22/Annex 23.

[7] FCM No. 93 waxes are intended to be added up to 1% w/w in polyolefins and in all types of polymers in contact with fatty foods. Migration of wax components in fatty food simulants is much higher than in aqueous simulants (Section 3.1.3).

PAH with more than two aromatic rings. The highest concentration was around 21 μg/kg wax. This value can be used to estimate the potential content of alkylated 3-ring MOAH, some of which were shown to be positive in mutagenicity tests with S-9 mix (Wang et al., 2022a). For a batching oil, a cruder mineral oil fraction than FCM No. 93 waxes, it was shown that the non-alkylated 3-ring MOAH fraction (phenanthrene and anthracene) corresponded to 1.8% w/w of the total 3-ring MOAH content (Grob et al., 1991). From this, a total concentration of 3-ring MOAH of 1.17 mg/kg in the wax could be estimated.

3.1.2. Physical and chemical properties[8]

FCM No. 93 waxes are currently listed in Regulation (EU) No 10/2011 with the following specific restrictions:

- Average molecular weight not less than 350 Da.
- Viscosity at 100°C not less than 2.5 cSt (2.5×10^{-6} m^2/s).
- Content of hydrocarbons with carbon number less than 25, not more than 40% (w/w).

The applicant proposed to add the following specifications:

- Congealing point > 45°C.
- Purity requirements: the content of polycyclic aromatic compounds is verified by analytical purity tests as described in BfR recommendation XXV.1.1.2002 (similar methods and purity requirements are described in FDA § 172.886) and in the Dutch Food contact materials regulation (Annex B, Chapter II).

The melting points of the substance vary from 50 to 90°C. FCM No. 93 waxes are essentially insoluble in aqueous food simulants (water, 10% ethanol in water, 3% acetic acid in water). Solubility in olive oil at 22°C is 1%–2% w/w, at 40°C it is 10%–15% w/w and at 60°C the olive oil and the substance are completely miscible. In 95% ethanol, the solubility at 60°C is 0.5%–1% w/w. In isooctane, it is 10%–20% w/w at 22°C, 50%–70% w/w at 40°C and at 60°C the two are completely miscible. The substance is chemically inert and thermally stable up to ca. 250°C in air, which is approximately the onset of oxidation. Hence, the substance is stable under the maximum process temperature of plastics intended to contain the additive, which is indicated to be 240°C.

3.1.3. Specific migration[9]

Specific migration tests were conducted using Wax 1[redacted], also tested in the previous evaluation (EFSA, 2006). The distribution of hydrocarbons is centred (P50) at C26 and the P2.5 to P97.5 fraction, containing 95% by mass of hydrocarbons, is C21–C33. More than 99% of the wax is ≤ C35 (Table 1). Being one of the waxes with the lowest average carbon number, the Panel considered Wax 1 a suitable worse-case representative of FCM No. 93 waxes with regard to migration. The wax was added into low-density polyethylene (LDPE) at a nominal level of 1% w/w, which is the maximum use level indicated by the applicant for general use in plastics like polyolefins. LDPE was selected as the worse-case polymer for its high diffusivity. A variety of specific migration tests were conducted, following the time course of migration into the fatty food simulants Miglyol 812 (a medium-chain triglycerides mixture) and olive oil for up to 12 days at 60°C and up to 64 days at 40°C. Miglyol has the advantage in containing lower background levels of hydrocarbons than some samples of olive oil. Results in the two simulants were comparable. The Panel concluded that the test conditions of 64 days at 40°C were the most realistic, whilst still being severe compared to the intended contact conditions in the use of plastics with foods.

HPLC–GC-FID and GC×GC-MS/FID were used for the analysis of the content of the wax in the plastic and in the exposed simulants. The test plastic was found to contain less than the intended formulation level of wax (1% w/w) and so the migration results were corrected to correspond to a wax content of 1% w/w in the plastic. The migration of MOSH amounted to 23.6 mg/dm^2, this total being comprised of *n*-alkanes (16.2 mg/dm^2), isoalkanes (6.0 mg/dm^2) and naphthenes (1.4 mg/dm^2). Using the standard packaging surface to volume ratio of 6 dm^2/kg, these results would equate to a migration of total-hydrocarbons ≤ C35 of 142 mg/kg, i.e. 97 mg/kg of *n*-alkanes, 36 mg/kg of isoalkanes and 8.4 mg/kg of naphthenes. The Panel noted that the total migration is exceeding the overall migration limit of 10 mg/dm^2 or 60 mg/kg food.

[8] Technical dossier/Consolidated version_Sept22/Appendix B/Section 2 and Annexes 5, 6, 7, 13.
[9] Technical dossier/Consolidated version_Sept22/Appendix B/Section 5.1 and Annexes 8, 14, 19, 21.

The applicant did not provide results on the migration of the MOAH fraction. This can be estimated assuming that the MOAH migration is pro-rata with the migration of wax. As the MOAH content in the 22 wax samples was up to 3.9% w/w (Section 3.1.1), the resulting MOAH migration in fatty food simulants would be 0.19 and 2.3 mg/kg food for a migration of wax at 5 mg/kg food and 60 mg/kg food, respectively.

Specific migration into the food simulants water, 3% acetic acid and 10% ethanol were ≤ 5 μg/kg, as expected due to solubility limitations. The applicant also provided results for an in-mould-labelled polystyrene cup made of paper and 46% plastic with a hot-melt adhesive consisting of 54% paraffin wax. It is not further described here, since it is outside the scope of this evaluation.

3.1.4. Overall migration[10]

Overall migration was tested using Wax 1 added at 1% w/w to LDPE, with isooctane and olive oil at 10 days/20°C and 10 days/40°C, respectively, including intermediate timepoints. It was about 30 mg/dm^2 for both simulants, equating to ca. 180 mg/kg food or food simulant at the standard packaging surface to volume ratio of 6 dm^2/kg. Although the test conditions differed and a different methodology was used, these overall migration results are in broad agreement and supportive of the high specific migration results reported in the previous section.

Although LDPE is a worse-case plastic, the Panel expected high specific and overall migration into fatty foods also for other types of plastics, most likely exceeding 5 mg/kg food or simulant and perhaps under certain conditions also the OML of 10 mg/dm^2 or 60 mg/kg food.

3.2. Toxicological data

3.2.1. Genotoxic potential[11]

The genotoxic potential of the wax was assessed by the EFSA AFC Panel in the previous evaluation (EFSA, 2006) by using the three *in vitro* tests required[12] at that time. The wax selected [redacted] (Wax 1 in Section 3.1.1) as a test material for genotoxicity studies was considered representative for the low-molecular weight paraffin waxes used in food contact applications. As the test article is solid at room temperature, the wax was extracted with DMSO (compatible with the biological system) in order to obtain any soluble component that could potentially induce a mutagenic response. The actual concentrations of the test substance in the test solutions were not determined, however, the highest technically applicable volumes of extracts were tested. Under the test conditions used, [redacted] did not induce gene mutations in bacteria (four strains of *Salmonella* Typhimurium, TA1535, TA1537, TA98 and TA100 and one strain of *Escherichia coli*, WP2 *uvrA*) and mammalian cells (mouse lymphoma L5178Y cells), in the presence or absence of S9-mix, and was not clastogenic in Chinese Hamster Ovary cells. The studies were carried out in compliance with Good Laboratory Practice (GLP) principles and followed the appropriate OECD Test Guidelines.[13]

A micronucleus assay, required by the more recent Note for Guidance (EFSA, 2008b) in order to cover also aneuploidy, was not available. However, the results of the *in vitro* chromosomal aberration assay do not suggest an aneugenic potential, in view of the lack of induction of polyploidy and of any relevant inhibition of cell cycle progression, typically associated with chemically-induced chromosome malsegregation.

Based on the available data, the CEP Panel, in agreement with the previous opinion of the AFC Panel (EFSA, 2006), concluded that there is no evidence for a genotoxic potential of the tested DMSO extracts [redacted].

3.2.2. Toxicokinetics[14]

A limited number of studies were available on the toxicokinetic behaviour of low melting paraffin waxes or their individual components. As reported in EFSA's Scientific Opinion on mineral oil hydrocarbons in food (EFSA CONTAM Panel, 2012), oral absorption is recognised to decrease with

[10] Technical dossier/ Consolidated version_Sept22/Appendix B/Section 5.2 and Annex 14.
[11] Technical dossier/Consolidated version_Sept22/Appendix B/Section 8.1 and Annexes 9, 10, 11.
[12] Gene mutations in bacteria, *in vitro* mammalian cell gene mutation test, *in vitro* chromosomal aberration test.
[13] OECD TG 471 (1997) for the gene mutations test, TG 476 (1997) for the mouse lymphoma assay and TG 473 (1997) for the chromosomal aberrations test.
[14] Technical dossier/Consolidated version_Sept22/Appendix B/Section 8.3 and Annexes 15, 17, 19, 26, 28.

increasing carbon number. In the small intestine and liver, *n*-alkanes undergo oxidation to their corresponding fatty acids, which then enter the β-oxidation pathway or are incorporated into lipids. The oxidative metabolism of isoalkanes results in similar end products with additional terminal (ω) oxidation of the branched chain. The *n*-alkyl side chain of cycloalkanes can undergo ω-oxidation followed by β-oxidation and may undergo further transformations prior to urinary excretion.

Accumulation in Fischer 344 rats. The potential accumulation and toxic effects of hydrocarbon oils of various carbon number distributions were evaluated in a repeated-dose oral toxicity study with female Fischer 344 rats (Cravedi et al., 2017). Rats were exposed to three mixtures, including a mixture containing 1:1 of C25–C45 isoalkanes and alkylated cycloalkanes (no *n*-alkanes) and a wax of a similar carbon number range, for 120 days at 0, 400, 1,000 and 4,000 mg/kg feed, equivalent to 0, 22, 55 and 222 mg/kg body weight (bw) per day. MOSH, and in particular *n*-alkanes, accumulated predominantly in the liver (~50% of the total recovered dose), followed by adipose tissue, spleen and carcass. These experiments showed that the occurrence of hepatic granulomas (i.e. aggregates of macrophages) depended on *n*-alkanes and probably other wax components with carbon number > C25, assumed to be due to particularly slow metabolism and crystallisation in Fischer 344 rats.

Accumulation in humans and Sprague–Dawley rats. A different picture for bioaccumulation was reported for humans naturally exposed to MOSH. The composition, concentrations and molecular mass distributions of MOSH in various human tissues were measured in a study with a limited number of post-mortem patients (Barp et al., 2014; Biedermann et al., 2015). High amounts of MOSH (from C18 to C46, maximum at C25–C28) were found in liver and spleen. *n*-Alkanes were virtually absent, suggesting that they were efficiently metabolised and eliminated. A similar finding (hardly any *n*-alkanes detected) was reported in the liver of Sprague–Dawley rats in a recent 90-day repeated-dose oral toxicity study (Section 3.2.3.1). MOSH concentration (from C16 to C35, maximum at C23–C24) in the adipose tissue was approximately 15 times higher than that measured in the liver, which is probably due to a slower elimination from adipose tissue. The MOSH in adipose tissue and mesenteric lymph nodes (MLNs) included *n*-alkanes from natural sources, but hardly any of mineral or synthetic origin, which reflects the higher exposure to natural *n*-alkanes mainly from plant waxes.

Comparison among Fischer 344 rats, Sprague–Dawley rats and humans. *In vitro* metabolism studies with hepatic microsomes suggest that the hydroxylation rate of n-heptadecane is higher in humans than in rats (Cravedi and Perdu, 2012), with a less efficient metabolism in Fischer 344 females compared to Sprague–Dawley and Wistar rats (Cravedi et al., 2011). No metabolism was detected when pristane (branched-alkane, 19 carbon atoms) and dodecylcyclohexane were tested in human or rat hepatic microsomes (Cravedi and Perdu, 2012). Concerning alkyl cyclopentanes and cyclohexanes, with largely non-branched alkyl group, the plausible reported metabolic pathway (EFSA CONTAM Panel, 2012) was demonstrated by Halladay et al. (2002). The authors determined the toxicokinetic of 1-eicosanylcyclohexane in Fischer 344 and Sprague–Dawley rats. Higher bioavailability and higher levels of the test item in the liver as well as a slower clearance were observed in Fischer 344 compared to Sprague–Dawley rats. However, it was not demonstrated that the metabolism ended with complete degradation. Recently, Carrillo et al. (2021) proposed a mode of action analysis within an Adverse Outcome Pathway framework to better explain how the decreased metabolism, leading to an accumulation of *n*-alkanes in the Fischer 344 liver, were the key events triggering the production of wax crystals with the epithelioid granuloma formation (possibly triggering inflammation). The accumulation in humans, mainly in adipose tissue, does not reflect the accumulation in rats, especially the Fischer 344 strain, mainly due to the time course difference in hepatic clearance (Barp et al., 2017; Cravedi et al., 2017).

Overall, the available evidence suggests that the structural class distribution of MOSH in tissues and their bioavailability are different in Fischer 344 rats compared to Sprague–Dawley rats and humans. Based on this, the CEP Panel considers that the effects (i.e. *n*-alkane accumulation and granuloma formation) observed in Fischer 344 rats are not relevant for the evaluation of the toxicological properties of FCM No. 93 waxes in humans.

3.2.2.1. Data on accumulation from a 13-week oral toxicity study in Sprague–Dawley rats followed by a 6-week recovery period

A comprehensive analytical characterisation of hydrocarbons present in different tissues (i.e. liver, MLNs, adipose tissue, spleen and lungs), was carried out in the context of a 90-day repeated dose toxicity study with the test substance EWF FCM 93 58 (OECD TG 408, Section 3.2.3.1). The analysis was performed on samples from three animals (Sprague–Dawley rats) per sex in the control and high-

dose groups, during and after the entire period of exposure (30, 60 and 90 days) and after the recovery period (132 days). The hydrocarbon concentrations were higher in female liver (124 mg/kg at day 30) and remained constant until day 90, then decreased significantly after 42 days of recovery (9.4 mg/kg in females, consisting of 67% isoalkanes and 33% *n*-alkanes in the range C16–40 and returned to control values in males). The content in the MLNs (higher in females than in males) reached a steady-state at day 30, the first measurement point, with a strong decrease after the recovery period, excluding an outlier animal. Although some *n*-alkanes, monocyclic alkylated cycloalkanes and isoalkanes were still measured in MLNs after 60 days (489, 292 and 156 mg/kg, respectively), no effects considered adverse were observed in the parallel 90-day repeated toxicity study (OECD TG 408, Section 3.2.3.1).

Overall, the Panel noted that the accumulation of wax components in Sprague–Dawley rats reached a steady-state after 30 days (the first measurement point), which is much faster than what was observed for an oil virtually free of wax compounds administered to Fischer 344 rats (Barp et al., 2017), supporting that the large majority of the wax components are more rapidly eliminated than the oil components (which are richer in multibranched isoalkanes and naphthenes). *n*-Alkanes and other wax components were almost completely eliminated from the liver and spleen of Sprague–Dawley rats after a 6-week recovery period (5.7% residual MOSH, corrected for the controls after the recovery period).

3.2.3. Subchronic toxicity[15]

3.2.3.1. 13-Week oral toxicity study in Sprague–Dawley rats followed by a 6-week recovery period including toxicokinetics

Based on comprehensive analytical characterisation of the test material, the CEP Panel considered that EWF FCM 93 58 used in the repeated dose 90-day subchronic toxicity study in Sprague–Dawley rats (mentioned above in Section 3.2.2.1) is representative of the migrate of FCM No. 93 waxes. In the study (performed according to TG 408 and following GLP), three groups of animals (10 animals per sex per group) received the test material via the diet at doses of 2,000, 21,000 and 100,000 mg/kg (achieved dose level: 0.19, 0.24; 1.9, 2.0 and 9, 10 g/kg bw per day in males and females, respectively). A control group received the vehicle (powdered rodent diet). Control and high-dose groups included 10 additional animals per sex to be sacrificed after the 6-week recovery. Additional two satellite groups (control and high-dose; 10 animals per sex per group) were euthanised on day 30 and 60 for the evaluation of the accumulation of hydrocarbons in different organs and tissues (Section 3.2.2.1).

There were no unscheduled deaths throughout the study. No clinical signs of toxicological relevance were observed at any dose level during the study in either sexes. Functional performance tests (using grip strength and measurement of motor activity) and sensory reactivity assessments to different stimuli (auditory, visual and proprioceptive) did not indicate any treatment-related effects. No treatment-related changes were observed in body weight, body weight gain and food consumption during the whole duration of the study. Ophthalmic examination did not indicate any treatment-related effect. Clinical haematology evaluation revealed slight, but not dose-related, changes in mean cell haemoglobin concentration, erythrocytes during the dosing phase as well as lymphocytes and platelets in males, and eosinophils in females during the recovery phase, but not during the dosing phase. Sporadic, not dose-related. differences in alanine aminotransferase, creatinine, potassium, protein and albumin in males, and chloride, calcium and phosphorus in females, were observed. Thyroxine was statistically significantly lower than controls in males and higher in females from the high-dose group (−11% and +28%, respectively). The same change was also observed in females receiving 2 g/kg bw per day (+26%). As the observed differences are relatively minor and no other related changes (e.g. increase of thyroid stimulating hormones, decrease of triiodothyronine, thyroid weight) were recorded, these findings on T4 were not considered toxicologically relevant.

The evaluation of the oestrus cycle at least 2 weeks before the bleeding for hormones and at the end of the treatment period did not indicate any significant differences between groups. No treatment-related differences between the control and the treated groups were observed in semenology performed at the end of treatment and recovery periods regarding sperm motility, morphology and concentration (expressed as million sperm/g caudal epididymal tissue). No treatment-related differences in organ weight, including adrenal glands, pituitary gland, thyroid gland, testes, epididymides, epididymal cauda, seminal vesicles, uterus or ovaries were observed. MLN weights

[15] Technical dossier/Consolidated version_Sept22/Appendix B/Section 8.2.1 and Annexes 23, 25, 28.

increased at all dose levels and in both sexes. The increase was small in males (+11%, +20%, +18%) and more pronounced in females (+95%, +110%, +100%) for the low-, mid- and high-dose groups, respectively, reaching statistical significance. All the organ weights returned to the control values at the end of the recovery period.

No macroscopic findings were observed at necropsy examination that may be considered associated with treatment. Histopathological examination revealed no abnormal findings in the morphology of organs/tissues, including cervix, vagina, mammary glands and vaginal smear to determine stage of oestrus cycle. Granulomas were observed in the mesenteric lymph nodes. A dose-response relationship in the incidence and severity of this effect was observed in females, being minimal in 3, 2 and 5 animals (from the low-, mid- and high- dose groups, respectively); slight in 5 and 3 animals (from the mid- and high- dose groups, respectively); moderate in 2 animals (high-dose group). At the end of the 6-week recovery period in females, the incidence of this finding remained similar, although with a decrease in the severity (8 as minimal; 1 as slight; 1 as moderate at the high dose). The Panel noted that granulomas in MLNs are considered of low toxicological concern because they are not associated with an inflammatory response or necrosis, and do not progress to adverse lesions (Carlton et al., 2001; EFSA CONTAM Panel, 2012). No additional treatment-related effects were observed in rats after repeated dosing at dose levels of 0.2, 2 and 10 g/kg bw per day for 13 consecutive weeks and after a 6-week recovery period.

An increase in MLN weights correlating with granulomas in MLNs were noted mainly in females. These changes were expected to be non-adverse, based on the reversibility (weights) or on the evidence of ongoing recovery (microscopy) after a 6-week treatment-free period. As these effects were not related to any consequent necrosis and/or inflammatory processes, the CEP Panel considered this evidence as non-toxicologically relevant. This conclusion is also supported by the study of Dunster (2009), which reported that a 90-day repeated dose treatment of Sprague–Dawley rats with a gas-to-liquid base oil (a substance similar to FCM No. 93 waxes but with multibranched alkanes instead of *n*-alkanes) produced accumulation of alveolar macrophages and an increase in histiocytes in MLNs (only in females). The authors considered these effects as non-adverse, being a response to high-level exposure to inert materials, without any proliferative nor degenerative effects.

Based on the lack of toxicological relevant effects shown in the 90-day repeated dose oral toxicity study in Sprague–Dawley rats, the Panel identified the no observed adverse effect level (NOAEL) at the highest dose tested, 9 g/kg bw per day (highest achieved dose in males).

3.2.3.2. 13-Week oral toxicity study in Fischer 344 and Sprague–Dawley rats

The applicant provided a repeated dose 90-day oral toxicity study with a C19–C42 low melting paraffin wax (LMPW), whose results were included in Griffis et al. (2010).[16] The analysis of the tested wax confirmed that it can be considered representative of FCM No. 93 waxes. The study was performed in compliance with the GLP principles but was not designed according to any test guideline. The CEP Panel noted that raw data of histopathological observations are missing in the available original study report.

In this study, the test material was administered to female Fischer 344 and Sprague–Dawley rats up to 90 days via the diet, at 0.2% and 2.0% w/w, corresponding to 157, 160 and 1,609, 1,644 mg/kg bw per day, respectively, for the two strains. No treatment-related effect on mortality, clinical signs, body weight gain and food consumption were observed for either rat strain. Haematological evaluation and clinical chemistry measurements revealed increased of neutrophils, aspartate transaminase and gamma-glutamyl transferase only in Fischer 344 rats. In the same rat strain, increases in the absolute and relative weights of MLNs, liver and spleen were observed. Dose- and time-dependent histopathological changes (hepatic granuloma formation related to inflammatory changes in liver as well as granulomas and reticuloendothelial hyperplasia in MLNs) were observed in Fischer 344 rats. Only treatment-related effects with a lower incidence and severity were observed in the MLNs of Sprague–Dawley rats. These findings were correlated with a different accumulation of the test material in the two rat strains: a time and dose-related LMPW hydrocarbon accumulation (around 20 mg/kg) was observed in the Fischer 344 liver. In Sprague–Dawley rats, no LMPW alkane residues (below the limit of quantification) were found in the liver in either dose group or at any of the time points. Retention of alkanes in the MLNs was found in the Fischer 344 (at higher levels) and Sprague–Dawley rats, after 90 days at both doses.

[16] Technical dossier/Consolidated version_Sept22/ Annex 17.

Firriolo et al. (1995) showed that although both strains showed increases in the levels of hepatic mineral hydrocarbons after 90-day feeding with 2,000 and 20,000 mg/kg (~160 and 1,600 mg/kg bw per day, respectively), Fischer 344 rats had 2.2- and 2.0-fold (5.6 and 8.2 mg/g) greater amounts of mineral hydrocarbons in the liver than Sprague–Dawley rats (1.7 and 4.1 mg/g).

Overall, the results showed that Fischer 344 rats are more sensitive and have a higher potential for accumulation (mainly in the liver) than Sprague–Dawley rats, as also concluded in Section 3.2.2.

The CEP Panel identified the no observed adverse effect level (NOAEL) at 1,644 mg LMPW/kg bw per day (the highest dose tested), after 90-day repeated dietary administration in Sprague–Dawley rats. Considering the study's deviations from the current OECD TG 408, in terms of tested doses (only two) and sex (only females), the Panel considered the study a supporting study to identify a NOAEL in Sprague–Dawley rats.

3.2.3.3. Considerations on studies with Fischer 344 rats

Already in 1995, JEFCA considered that different 90-day repeated toxicity studies on mineral oils and waxes utilising rat strains other than Fischer 344 showed no adverse toxicological effects (primarily granuloma formation), suggesting that Fischer 344 was an especially sensitive rat strain. Carlton et al. (2001) reported that liver microgranulomas in the Fischer 344 rat were of potential toxicological significance specific to this rat strain. However, in 2012 the EFSA CONTAM Panel was of the view that there was insufficient information to conclude on the specificity of Fischer 344 rats (EFSA CONTAM Panel, 2012).

Recently, new evidence has emerged. In Fischer 344 rats, induction of liver granuloma appeared to be related to *n*-alkanes $\geq$ C25 and not to the accumulated amount of total MOSH (Nygaard et al., 2019). The absence of accumulated *n*-alkanes in human liver and spleen (Barp et al., 2014) confirmed that the accumulation of *n*-alkanes and the resulting granuloma formation observed in Fischer 344 rats is unique for this rat strain and therefore not relevant for humans (Grob, 2018). Highly branched alkanes and alkylated cycloalkanes found in human liver (Biedermann et al., 2015) were similar to those in Fischer 344 rat liver after treatment with a L-C25 oil, a mixture virtually free of *n*-alkanes and with hydrocarbons with carbon number largely higher than C25. This mixture was able to induce changes in liver and spleen weight, but without any histopathological findings (no liver granuloma observed) at any dose tested, 400, 1,000 and 4,000 mg/kg feed for 120 days (Barp et al., 2017; Cravedi et al., 2017). The total MOSH concentrations measured in human liver (Barp et al., 2014) reached a mean content of 131 mg/kg, i.e. about 10% of that measured after treatment of rats with 400 mg/kg of the mixture, able to induce these alterations (Nygaard et al., 2019).

Overall, based on the available evidence, the CEP Panel confirmed that the Fischer 344 rat strain has a particular sensitivity to *n*-alkanes and possibly other wax components and therefore considered the effects observed in this rat strains as being non-relevant for humans.

3.2.4. Reproductive and developmental toxicity[17]

No specific reproductive and developmental toxicity studies on the substance used for migration tests ([redacted] Wax 1) were available.

In recent 90-day repeated oral dose toxicity study (described in Section 3.2.3.1) with the test item EWF FCM 93 58, the following endpoints, recommended for detection of endocrine activity according to the OECD TG 408, were investigated: adrenal glands, pituitary gland, thyroid gland, testes, epididymides, epididymal cauda, seminal vesicles, uterus, ovaries weights; morphology of seminiferous epithelium, sperm count, motility and morphology, oestrus cycle, morphology of cervix, vagina, mammary glands and vaginal smear, and circulating thyroid hormones. All results were negative.

MOAH with 3–7 aromatic rings are known to be related to the induction of developmental effects, including resorption, increased incidence of abnormal development and decreased foetal body weight (Feuston et al., 1994). As discussed in Section 3.1.1 and in Section 3.2.6, the CEP Panel considered the concentration of such MOAH in FCM No. 93 waxes negligible due to several steps of the manufacturing/refining process.

Based on available literature data (reviews from Boogaard et al., 2012 and Dalbey et al., 2014), although with different substances and via different routes of exposure, the CEP Panel noted the lack of reproductive and developmental toxicity of isoalkanes and alkylated cycloalkanes, which was attributed to test items lacking MOAH with more than three aromatic rings. Based on the results

[17] Technical dossier/Consolidated version_Sept22/Appendix B/Section 8.2.3 and Annexes 17, 19, 27.

obtained in the provided 90-day repeated oral toxicity study, the CEP Panel concluded that the FCM No. 93 did not elicit any endocrine activity.

3.2.5. Chronic toxicity and carcinogenicity[18]

No specific long-term toxicity and carcinogenicity studies on the substance used for migration tests ([redacted] Wax 1) were provided.

The carcinogenic potential of the MOAH with three and more rings is well known. However, these compounds are expected to be largely removed during the manufacturing process (Section 3.1.1).

Another substance of concern for carcinogenicity is the 2-ring naphthalene, which is carcinogenic by a non-genotoxic mode of action, involving cytotoxicity and proliferative regeneration (EFSA CONTAM Panel, 2012). However, the presence of a long alkyl side-chain is expected to reduce the formation of toxic intermediates: it has been recently shown that high alkylation on MOAH reduces the chance of formation of intermediate toxic and/or DNA-reactive metabolites, favouring the side-chain oxidation instead of ring oxidation. This was not observed for hazardous MOAH, that are non-alkylated or alkylated MOAH with three or more conjugated rings (Wang et al., 2020, 2022b; Carrillo et al., 2022).

Shubik et al. (1962) evaluated the carcinogenicity of five waxes (two paraffin and three microcrystalline waxes). The carcinogenicity study was performed in Sprague–Dawley rats (50 animals per sex) exposed to the tested waxes *via* the diet at 10% in feed (equivalent to 4,500 and 5,800 mg/kg bw per day in male and female rats, respectively) for 2 years. No treatment-related changes in survival rates and body weights were reported. The treatment did not affect the incidence of the tumours. The authors concluded that the five waxes tested did not induce neoplastic effects. No certificate of analysis of the tested waxes was provided. However, Carrillo et al. (2021) considered Wax 4 (Section 3.1.1) to be representative of one of the paraffin waxes tested in Shubik et al. (1962), based on the same overall physical properties, the chain length range and the content of *n*-, mono-branched and monocyclic alkylated cycloalkanes. Consequently, the authors claimed that the wax tested in Shubik et al. (1962) was worst-case for its MOAH content (0.09% in Wax 4 compared to 0.01% in Wax 1). The Panel identified major limitations for the use of this study in this evaluation, including the lack of the original report and certificate of analysis of the tested waxes as well as deviations from the current and previous OECD TG 453, i.e. the use of only one dose level (10%) and the lack of evaluation of non-neoplastic effects.

The Panel concluded that the available information suggests the lack of carcinogenic potential for FCM No. 93 waxes.

3.2.6. Discussion

As a result of manufacturing and refining steps, the substance almost entirely consists of MOSH, predominantly *n*-alkanes with minor proportions of little branched isoalkanes and cyclohexanes and cyclopentanes (naphthenes) with predominantly straight chain alkyl groups.

The substance also contains a small fraction of MOAH, reported to be up to 3.9% w/w from 27 waxes covering the range of FCM No. 93 specifications. MOAH with three and more conjugated aromatic rings (including PAHs, which can be considered as non-alkylated MOAH) are of main toxicological concern due to the genotoxic properties and carcinogenic potential of some of its constituents (EFSA CONTAM Panel, 2012). The Panel considered the content of MOAH with two or more conjugated aromatic rings to be very low based on the following lines of evidence:

- Steps in the manufacturing process:
    1) The solvent extraction of the mineral oil distillate is expected to remove a large part of the MOAH of concern[redacted].
    2) Crystallisation of the wax, particularly for low melting waxes, is selective for *n*-alkanes, isoalkanes and alkylated cycloalkanes with straight or little branched side chains. This also results in the preferential removal of MOAH with two or more conjugated aromatic rings.
    3) Hydrogenation further preferentially and efficiently reduces the content of MOAH with two or more conjugated aromatic rings.

[18] Technical dossier/Consolidated version_Sept22/Appendix B/Section 8.2.2 and Annexes 17, 19, 24.

As a consequence of the manufacturing process, preferentially MOAH with two and more conjugated aromatic rings are removed.

- Five wax samples (with a MOAH content up to 0.09% w/w) were analysed for their PAH content. The 16 genotoxic PAHs considered by the EFSA CONTAM Panel (EFSA, 2008a) were largely undetected with the middle-bound sum of PAH16 being up to 2.7 μg/kg wax.[19] For the purpose of illustration, considering that the highest measured MOAH content in waxes is 3.9% w/w, the pro rata sum of PAH16 would be up to 117 μg/kg waxes (2.7 μg/kg × 3.9/0.09). Based on the migration of the substance, the pro-rata migration of PAH is expected to be far lower than 1 μg/kg food, the lower bound of maximum levels allowed for benzo[a]pyrene (Regulation 1881/2006)[20]. For a wax migrating at 5 mg/kg food in fatty food, a pro rata migration of the sum of the 16 genotoxic PAH would be up to 0.00059 μg/kg food.
- In the same five waxes, the predominant PAH with more than two aromatic rings was phenanthrene (not a member of PAH16), with the highest content around 21 μg/kg wax.[20] Based on this value and using a batching oil (Section 3.1.1) to cover the content of non-alkylated phenanthrenes, the total content of phenanthrenes, i.e. non-alkylated plus alkylated phenanthrenes, was calculated as 1.17 mg/kg wax. This value would be 51 mg/kg, if corrected pro-rata to the highest measured MOAH content found in all the 27 waxes analysed for MOAH (3.9% w/w). For a migration of 5 mg/kg food, the pro rata migration of 3-ring MOAH would be 0.26 μg/kg. The migration from FCM No. 93 waxes is expected to be much lower.
- In the provided GC×GC analysis of EWF FCM 93 58 wax,[6] [redacted].
- In tests for genotoxicity, DMSO extracts of [redacted] Wax 1 were used to perform *in vitro* genotoxicity assays. The actual concentrations of the test substance in the test solutions were not determined, but the highest technically applicable volumes of extracts were tested. From polarity considerations, it is anticipated that DMSO extracts are enriched in polycyclic aromatic constituents. The results of the assays did not show a genotoxic potential for the [redacted] extracts tested.

The Panel noted that the whole mixture approach followed for the assessment of genotoxicity of the test article in principle does not allow to rule out the presence of low amounts of contaminating genotoxic MOAH, including alkylated MOAH, below the experimental detectable limit. However, the CEP Panel noted that based on the calculation given above, the sum of genotoxic PAHs possibly migrating into food is estimated to be far below the TTC for genotoxic carcinogens, i.e. 0.0025 μg/kg bw or 0.15 μg/kg food under the SCF food consumption scenario (European Commission, 2001; EFSA Scientific Committee, 2019). Concerning the alkylated MOAH, a genotoxic hazard related to their possible presence in the wax cannot be anticipated in view of the lack of their chemical characterisation and the complex influence of alkylation on the genotoxicity of PAHs (Wang et al., 2022a,b). However, even under very conservative assumptions the estimated migration of alkylated PAHs (both genotoxic and non-genotoxic) is sufficiently low (i.e. close to the above cited TTC level) to rule out a genotoxic concern. Overall, the CEP Panel concluded, in agreement with the previous opinion of the AFC Panel (EFSA, 2006), that FCM No. 93 waxes do not raise concern for genotoxicity.

Taking into account the above-mentioned lines of evidence, the CEP Panel concludes that any concern for the potential presence of MOAH with two or more conjugated aromatic rings in FCM No. 93 waxes can be ruled out.

The CEP Panel noted that the use of FCM No. 93 waxes in contact with fatty foods may result in a specific migration exceeding 60 mg/kg food or 10 mg/dm^2.

As regards subchronic toxicity, the results of the 90-day repeated oral toxicity study performed in Sprague–Dawley rats allowed to identify a NOAEL at 9 g/kg bw per day.

Based on data available in the literature, the Panel acknowledged that the induction of reproductive and developmental toxicity is mainly ascribed to the presence of MOAH with at least three conjugated

[19] The highest PAH16 content was determined in Wax 6 (MOAH content = 0.00% w/w wax) and the highest phenanthrene content was determined in Wax 2 (MOAH content = 0.04% w/w wax). No correspondence was found, i.e. it is not the same wax that gave simultaneously the highest MOAH, PAH16 and phenanthrene content. Thus, in the illustration given to estimate migration, it is very exaggerated to apply the highest content of MOAH found in all the wax samples (e.g. 3.9% w/w from the 22 samples). It is then reasonable to allocate the highest values of PAH16 and phenanthrene to the highest content of MOAH in the five tested waxes (0.09% w/w for Wax 5) for the illustration.

[20] The Panel noted that Regulation 1881/2006 sets the maximum levels of benzo[a]pyrene in the range 1–10 μg/kg food (depending on the food type).

aromatic rings. Based on the results obtained in the extended 90-day repeated dose oral toxicity study, including the evaluation of endocrine-related parameters, the CEP Panel concluded that FCM No. 93 waxes does not raise concern for endocrine activity.

The CEP Panel observed no relevant toxicological effects connected to the accumulation of hydrocarbons in MLNs in the 90-day repeated oral toxicity study, with a steady-state concentration during the exposure and rapid elimination in the recovery period. Additionally, data from literature demonstrate that accumulation in humans is limited.

The CEP Panel concluded that the provided information on chronic toxicity and carcinogenicity was limited and inadequate to reach conclusions on these endpoints.

Based on the above-mentioned considerations and following the tiered approach reported in the SCF guidelines (European Commission, 2001) and the relevant EFSA's Note for Guidance (EFSA, 2008b), the CEP Panel concluded that the substance does not raise safety concern if its migration does not exceed 5 mg/kg.

The Panel considered the additional specifications proposed by the applicant:

- Congealing point > 45°C.
- Purity requirements: the content of polycyclic aromatic compounds is verified by analytical purity tests as described in BfR recommendation XXV. 1.1.2002 (similar methods and purity requirements are described in FDA § 172.886) and in the Dutch Food contact materials regulation (Annex B, Chapter II).

Whereas a minimum melting point is relevant for these hydrocarbons to be waxes rather than liquids at ambient temperature, no rationale was provided by the applicant as to why congealing at 45°C should be considered to be an appropriate cut-off point for the safety evaluation and for the EC specifications. With regard to the proposed purity requirement, the proposal did not come with any quantitative data on what substances the tests may control for, the respective detection limits of the method(s) suggested or the limit values that should be observed. Thus, it is not clear in what way the proposed specifications (both the nature of the specification and/or any limit value proposed) would help ensure the safety of FCM No. 93 waxes as currently defined. The CEP Panel therefore does not recommend that these aspects are considered for addition to the existing specifications for FCM No. 93 waxes.

4. Conclusions

Based on the above-mentioned data, the CEP Panel concluded that under the intended and tested conditions of uses, the substance 'waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity' (FCM No. 93) does not raise safety concern for the consumer if used as an additive to a level ensuring that its migration in food is no more than 5 mg/kg in food.

The Panel noted that the migration of the substance is expected to exceed 5 mg/kg food under some of the intended uses proposed by the applicant.

5. Recommendations

The Panel recommends the following specifications for FCM substance number 93.

- Average molecular weight not less than 350 Da.
- Viscosity at 100°C not less than 2.5 cSt (2.5×10^{-6} m^2/s).
- Content of hydrocarbons with carbon number less than 25, not more than 40% (w/w).
- An obligatory hydrogenation step in the manufacturing process.

Documentation provided to EFSA

1) Initial dossier. June 2019. Submitted on behalf of European Wax Federation aisbl.
2) Additional data. May 2020. Submitted on behalf of European Wax Federation aisbl.
3) Additional data. September 2022. Submitted on behalf of European Wax Federation aisbl.

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Abbreviations

AFC	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
BfR	Bundesinstitut für Risikobewertung
bw	body weight
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	Scientific Panel on food contact Materials, Enzymes and Processing Aids
CONTAM	Scientific Panel on Contaminants in the Food Chain
DMSO	dimethyl sulfoxide
FID	flame ionisation detector
FCM	food contact materials
GC	gas chromatography
GLP	Good Laboratory Practice
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDPE	low-density polyethylene
LoD	limit of detection
HPLC	high-performance liquid chromatography
LMPW	low melting paraffin wax
MLN	mesenteric lymph nodes
MOAH	mineral oil aromatic hydrocarbons
MOSH	mineral oil saturated hydrocarbons
MS	mass spectrometry
NOAEL	no observed adverse effect level

OECD	Organisation for Economic Co-operation and Development
OML	overall migration limit
PAH	polycyclic aromatic hydrocarbons
PVC	polyvinylchloride
SCF	Scientific Committee on Food
SML	specific migration limit
w/w	weight by weight