GreenScreen® Substance Assessment

Paraffin Wax

Method Version: GreenScreen® for Safer Chemicals v1.4[1]

Assessment Details[2]:

Assessment Type:	Certified
Assessment Prepared By:	WAP Sustainability Consulting, LLC
Assessment Prepared For:	WA Department of Ecology
Date Assessment Completed:	July 11, 2023
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Assessor Type: (Licensed GreenScreen Profiler or equivalent, Authorized GreenScreen Practitioner or Unaccredited)	Licensed GreenScreen® Profiler

[1] Use GreenScreen® Chemical Hazard Assessment Guidance (Guidance) v1.4 in Section II
[2] **Assessment Type**: GreenScreen reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen Practitioner), or "CERTIFIED" (by Licensed GreenScreen Profiler or equivalent);-**Assessment Prepared By**: Licensed GreenScreen Profilers must provide name of organization; Authorized GreenScreen Practitioners must provide their name;

GREENSCREEN BENCHMARK™ SUMMARY

Paraffin Wax has been reviewed in accordance with GreenScreen® benchmark classification guidelines (CPA, 2018). This chemical assessment report includes a GreenScreen Benchmark™ score and results for **Paraffin Wax (CASRN 8002-74-2)** only.

No marketing claims can be made without licensing through Clean Production Action.

GreenScreen Benchmark Score:

Paraffin wax was assigned a **Benchmark Score of 3** ("Use but Still Opportunity for Improvement") as it has moderate hazard for ecotoxicity based on a moderate classification for chronic aquatic toxicity. Although a data gap exists for endocrine activity (E), as outlined in CPA (2018) Section I, part 11.6.2 (Step b: "Determine the final Benchmark score"), the minimum data requirements to support a GreenScreen® Benchmark Score of 3 are met, since a maximum of one data gap is permitted in Group I Human endpoints without a modification to the Benchmark Score. In a worst-case scenario, if the chemical under review was assigned a hazard level of "high" for endocrine activity, it would be categorized as a Benchmark 1 chemical. Since the aquatic toxicity classifications were assigned with low confidence, further studies on these endpoints might be useful to refine the Benchmark Score.

HAZARD CLASSIFICATION SUMMARY

Table 1. GreenScreen Hazard Summary

GreenScreen Hazard Summary Table for Paraffin Wax																			
Group I Human					**Group II and II* Human**									**Ecotox**		**Fate**		**Physical**	
Carcinogenicity	**Genotoxicity/Mutagenicity**	**Reproductive Toxicity**	**Developmental Toxicity**	**Endocrine Activity**	**Acute Toxicity**	**Systemic Toxicity**		**Neurotoxicity**		**Skin Sensitization***	**Respiratory Sensitization***	**Skin Irritation**	**Eye Irritation**	**Acute Aquatic Toxicity**	**Chronic Aquatic Toxicity**	**Persistence**	**Bioaccumulation**	**Reactivity**	**Flammability**
						single	repeat*	single	repeat*	*	*								
L	**L**	**L**	**L**	DG	**L**	*L*	**L**	*L*	*L*	**L**	**L**	**L**	**L**	*L*	*M*	*L*	*vL*	*L*	**L**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in **BOLD** font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that Group II Human Health endpoints have four hazard scores (i.e., vH, H, M and L) instead of three (i.e., H, M and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints.

SCOPE OF ASSESSMENT

Chemical Name: Paraffin Wax

CAS Registration Number: 8002-74-2 (alternate CAS #: 64742-51-4)

Chemical Structure:

Paraffin waxes and hydrocarbon waxes (hereafter referred to as paraffin wax) is a solid mixture of saturated open-chain hydrocarbons obtained from petroleum characterized by relatively large crystals; it contains solid hydrocarbons of the methane series and a small percentage of other organic entities. It is a solid crystalline mixture of straight-chain (normal) hydrocarbons ranging from C_{20} to C_{30} and possibly higher ($CH_3(CH_2)_nCH_3$) where n ≥ 18 (Speight, 2011). Paraffin waxes and hydrocarbon waxes are highly refined substances that originate from a stream of lubricating base oils that act as feedstocks for most of the dewaxing operations that produce finished paraffin and microcrystalline waxes. Paraffin wax is a substance of unknown or variable composition (UVCB); as such, there is no discrete chemical structure.

Suitable analogs or moieties of chemicals used in this assessment:

The following analogous test compounds were considered relevant to paraffin wax, as they are expected to contain n-alkane components within the appropriate carbon chain length range of C_{20} to C_{30}. As these are UVCB compounds, no chemical structures are available.

- Petroleum distillates, hydrotreated heavy paraffinic (CAS # 64742-54-7)
- Distillates (Fischer-Tropsch), heavy, C_{18}-C_{50} branched, cyclic, and linear (CAS # 848301-69-9)
- Distillates (Fischer-Tropsch), C_{18}-C_{26} branched and linear (CAS # 848301-67-7)
- Microcrystalline wax (CAS # 63231-60-7)

In addition to the above UVCB compounds, data relevant to less relevant test substances were considered for endpoints in which data on paraffin wax and the above related compounds were not available. These compounds include:

- Residual oils (petroleum) solvent, dewaxed (CAS # 64742-62-7), considered for aquatic toxicity only.
- Distillates, petroleum, hydrotreated light naphthenic (CAS # 64742-53-6), considered for aquatic toxicity only.
- Distillates, petroleum, solvent-refined light naphthenic (CAS # 64741-97-5), considered for aquatic toxicity only.

In cases where data on one of these mixtures was relied upon for the hazard classification, the confidence was considered low.

Identify potential applications/functional uses of the chemical:

- Viscosity control agent
- Lubricating agent
- Waterproofing agent
- Use in food contact applications, including use in food coatings, waxed paper, and vegetable protection
- Use in cosmetic and related products, including perfumes, eye and makeup preparations, manicuring products, hair preparations, shaving and skin care products, suntan preparations, ointments, and salves
- Use in other consumer products such as candles, crayons, and polishes

Paraffin wax is regulated for use by the FDA in several food contact applications. For example, Paraffin waxes and hydrocarbon waxes may be used as a component of resinous and polymeric coatings complying with 21 CFR 175.300, as a component of resinous and polymeric coatings on polyolefin films complying with 21 CFR 175.320, as a component of coatings for paper and paperboard complying with 21 CFR 176.170 and 21 CFR 176.180, as a defoaming agent used in the manufacture of paper and paperboard complying with 21 CFR 176.210, and as a component of cellophane complying with 21 CFR 177.1200. Paraffin waxes and hydrocarbon waxes may also be used for direct addition to food as a chewing gum base under 21 CFR 172.615.

Table 2. Physical and chemical properties

Property	Value	Reference
Molecular formula	Not applicable (UVCB)	-
SMILES Notation	Not applicable (UVCB)	-
Molecular weight	Approximately 360 – 540 Da, assuming C_{20} – C_{30}	Speight, 2011
Physical State	Solid at room temperature	Speight, 2011
Appearance	Off-white soft waxy solid	ECHA, 2023
Boiling point	341 – 665 °C	ECHA, 2023
Melting point	43 - 68°C, typically ~55°C	ECHA, 2023
Vapor pressure	0 – 20 Pa at 80°C; negligible at 20°C	ECHA, 2023
Water Solubility	Varies depending on composition (Predicted: 2.96 x 10^{-12} mg/L to 142.1 mg/L at 25°C)	ECHA, 2023
Dissociation constant	Not applicable	-
Density/Specific gravity	0.79 – 0.94 g/cm^3 at 15°C	ECHA, 2023
Partition coefficient Log Pow	3.17 – 18.02 (predicted)	ECHA, 2023
Log Koc	No data identified	-
Henry's Law constant at 25°C	Not applicable	-
Viscosity	3 – 30 mm^2/s at 100°C	ECHA, 2023

GROUP I HUMAN HEALTH EFFECTS (GROUP I HUMAN)

Carcinogenicity (C): *L*

Paraffin wax was assigned a hazard classification level of Low (low confidence) for carcinogenicity. GHS classification is not warranted for paraffin waxes and hydrocarbon waxes based on a lack of observed adverse effects in a pre-guideline carcinogenicity study (at a dose of 5700 mg/kg-day). Furthermore, genotoxicity data indicates a lack of observed mutagenicity, clastogenicity and/or aneugenic activity. The target chemical was not in the OECD QSAR or OncoLogic domains; therefore, modeling data were not available for these two systems. Although the target substance was within the VEGA domain, the chemicals in the carcinogenicity models were not sufficiently similar to provide confidence in the modeled prediction for carcinogenicity (i.e., the compounds are or may be outside the applicability domain), with the exception of the oral classification model (IRFMN) which provides a prediction of Non-carcinogen. Confidence in this classification is low as it is based on pre-guideline experimental data, genotoxicity data and estimated data for the target substance.

Data

- Lists
  - *Authoritative:* Not present on any Authoritative A or B list.
  - *Screening:* Not present on any Screening A or B list.

- Measured Data (oral)
  - Shubik et al., 1962 as summarized in ECHA, 2023a
  - Test substance: Five petroleum waxes, CAS number indicated as “most likely” 8002-74-2

In a pre-guideline carcinogenicity study (Klimisch reliability score of 2), Sprague Dawley rats (approximately 50 animals/sex/dose) were administered dietary concentrations of five paraffin waxes at doses of 0 or 10% for two years. According to the authors of the corresponding ECHA summary, the 10% dietary concentration was equivalent to approximately 5700 mg/kg-day based on reported body weights. The rats were inspected and weighed every second week, and all gross lesions were recorded. This was continued until all the rats died or were sacrificed moribund. The animals were submitted to a complete autopsy followed by histological examination of all abnormal tissues. Survival rates and growth rates were unaffected by oral exposure to paraffin wax. Several tumors were found in all groups at necropsy. The most common tumors were those of the mammary regions (fibrocarcinomas, adenocarcinomas, fibromas, and sarcomas), of the adrenal glands (cortical adenomas with a few carcinomas and pheochromocytomas) and of the pituitary. The number of tumor-bearing animals and the incidence of tumors of each type were similar in both the unexposed and the exposed groups. No other toxic effects were found at histological examination. The study authors concluded that the five paraffin waxes were devoid of carcinogenic or other toxic action when fed at a level of 10% in the diet. Therefore, petroleum wax considered to be non-carcinogenic.

- Measured Data (dermal)

  - Shubik et al., 1962 as summarized in ECHA, 2023b
  - Test substance: Five petroleum waxes, CAS number indicated as “most likely” 8002-74-2

In a pre-guideline carcinogenicity study (Klimisch score 2), Swiss mice (60 females and 30 males/dose) were dermally exposed to petroleum wax (CAS RN 8002-74-2) at doses of 0 (warm benzene), 0.05 ml or 7.5 mg of wax (15 g of wax dissolved in 85 ml of warm benzene), three times per week, over a duration equivalent to the lifetime of the animals. According to the ECHA summary, this study was conducted prior to the implementation of the relevant OECD test guideline and has several deviations (only one dose tested, non-standard vehicle, histopathology was only conducted on grossly abnormal tissues); however, it was scientifically suitable for assessment. No treatment-related toxicity was observed in clinical signs, mortality, gross pathology, or histopathology. Therefore, petroleum wax was considered to be non-carcinogenic, and the NOEL was stated by the study authors as 128 mg/kg-day (using a weekly dose of 7.5 mg, assuming the mice weighed approximately 25 g, this equates to an average daily dose of approximately 128 mg/kg-day).

- Measured Data (dermal)
  - ECHA, 2023c
  - Analog (lubricant base oil, no additional information on identity of test substance)

In a dermal carcinogenicity study (stated as compliant to OECD test guideline 453 with a Klimisch reliability score of 1), male C3H/HeNCrlBR mice (50/group) were dermally exposed to a lubricant base oil (designated MRD-87-016, no CAS provided) at a dose of 150 mg/kg-day. A positive control group and negative control group were also incorporated. Exposures occurred at a frequency of twice per week for 24 months or until observation of carcinoma at which time the animal was sacrificed. Except for the positive control group, there were no statistically significant differences in time to tumor and tumor production between dose groups. Survival analysis indicated that the positive control had the lowest survival rates; however, this finding is related to the fact that animals were euthanized following the appearance of a carcinoma. Gross postmortem examination showed a high incidence of liver masses in all groups, including the vehicle control, except for the positive control group. According to the ECHA dossier, these liver masses as well as other observed histopathological lesions were of the usual type and incidence in this strain and age and were considered unrelated to treatment. Therefore, the test material did not cause local or systemic effects when applied neat. The NOAEL was determined to be ≥150 mg/kg-day, based on the lack of treatment-related histopathology or gross pathology changes observed in the study.

Mutagenicity/Genotoxicity (M): L

Paraffin wax was assigned a hazard classification level of Low for Mutagenicity/Genotoxicity based on a lack of observed mutagenic, clastogenic and/or aneugenic effects in several high-quality guideline compliant studies. Confidence in this

classification is high since results were consistently negative in robust guideline compliant studies.

Data

- Lists
    - *Authoritative:* Not present on any Authoritative A or B list.
    - *Screening:* Not present on any Screening A or B list.

- Measured Data
    - ECHA, 2023d (unpublished test report dated 2005)
    - Test substance: Paraffin waxes and hydrocarbon waxes (CAS number 8002-74-2), 100% purity

In a GLP and OECD TG 476-compliant *in vitro* mammalian cell gene mutation test, mouse lymphoma L5178Y cells were exposed to the test substance at doses of 0 (dimethyl sulfoxide), 0.018, 0.037, 0.074, 0.15, 0.29, 0.59, 1.2, 1.7, 2.4, 3.4, 4.9, 7, and 10 mmol/l for 24 hours, with and without S9 metabolic activation system (derived from Aroclor 1254-induced male Wistar rats). The study was assigned a Klimisch reliability score of 1 (reliable without restrictions). There was no cytotoxicity but the recommended limit dose was achieved. No significant increases in mutagenic frequency were observed in any test concentration. Therefore, the test substance was considered non-mutagenic under the conditions of the assay.

- Measured Data
    - ECHA, 2023e (unpublished test report dated 2005)
    - Test substance: Paraffin waxes and hydrocarbon waxes (CAS number 8002-74-2), 100% purity

A GLP and OECD TG 471-compliant bacteria reverse mutation assay was described in the ECHA database. The study was assigned a Klimisch reliability score of 1 (reliable without restrictions). *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and *E. coli* WP2uvrA were exposed to paraffin waxes and hydrocarbon waxes (purity 100%) at concentrations of 0 (dimethyl sulfoxide), 1.23, 3.70, 11.10, 33.30 and 100% with and without S9 metabolic activation system (derived from Aroclor 1254-induced male Wistar rats). According to the authors of the ECHA summary, these concentrations were equivalent to 62 – 5000 µg/plate. Cytotoxicity results were not described in the available summary; however, the recommended limit dose was achieved. No significant increase in revertant colonies was observed at any test concentration, regardless of metabolic activation status. Therefore, paraffin waxes and hydrocarbon waxes and hydrocarbon waxes were considered non-mutagenic under the conditions of the assay.

- Measured Data
    - ECHA, 2023f (unpublished test report dated 2006)
    - Test substance: Paraffin wax (no further details)

A GLP and OECD TG 471-compliant bacteria reverse mutation assay using the plate incorporation method was described in the ECHA database. The study was assigned a

Klimisch reliability score of 1 (reliable without restrictions). *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and *E. coli* WP2uvrA were exposed to paraffin wax at concentrations of 0 (tetrahydrofuran), 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate with and without the metabolic activation system (derived from phenobarbitone/β-naphthoflavone induced rat liver cells). No cytotoxicity was induced by the test substance; however, the recommended limit concentration was achieved. The vehicle and untreated control plates produced counts of revertant colonies within the normal range, and there was no evidence of induced mutant colonies over background in any test concentration. Therefore, the test substance was considered non-mutagenic under the conditions of the assay.

- Measured Data
    - ECHA, 2023g (unpublished test report dated 1993)
    - Test substance: Paraffin wax (no further details)

A GLP and OECD TG 471-compliant bacteria reverse mutation assay (was described in the ECHA database. The study was assigned a Klimisch reliability score of 1 (reliable without restrictions); however, the assay lacked an *E. coli* strain or other strain sensitive to oxidizing agents (such as TA 102). *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 were exposed to paraffin wax at concentrations of 0 (hexane), 0.1, 0.33, 1.0, 3.3 and 10 mg/plate with and without metabolic activation system (derived from Aroclor-induced rat liver S9). Cytotoxicity results were not described; however, the recommended limit concentration was achieved. No significant increase in revertant colonies was observed in any test strain or concentration, regardless of metabolic activation status. Therefore, the test substance was considered non-mutagenic under the conditions of the assay.

- Measured Data
    - ECHA, 2023h (unpublished test report dated 2005)
    - Test substance: Trade name, Sasolwax 5203, 100% purity. Described as hydrocarbon wax containing alkanes ranging from C_{19}-C_{36}, the main component is C_{26} and the average molecular weight is 360.

A GLP-compliant *in vitro* chromosome aberration test was described in the ECHA database. The study was assigned a Klimisch reliability score of 1 (reliable without restrictions) and “closely adhered to” OECD TG 473. Two separate assays were conducted. Chinese hamster ovary cells were exposed to “hydrocarbon wax” (CAS RN 8002-74-2) (purity 100%) at concentrations of 0 (dimethyl sulfoxide), 0.034, 0.069, 0.138, 0.277, 0.625, 1.25, 2.5, 5, or 10 mmol/l in the first assay, and 0 (dimethyl sulfoxide), 2.78, 4.17, 5.56, 6.94, 8.33, or 10 mmol/l in the second assay. Both assays were conducted with and without S9 metabolic activation system. No cytotoxicity was induced by the test substance; however, the recommended limit concentration was achieved. No significant increase in chromosomal aberration was observed in either assay, at any concentration tested, regardless of metabolic activation status. In both chromosomal aberration tests, the positive control substances mitomycin C (in the absence of a metabolic activation system) and cyclophosphamide (in the presence of a metabolic activation system) induced the expected statistically significant increases in the incidence of structural

chromosomal aberrations. Therefore, the test substance was considered non-clastogenic under the conditions of the study.

- Measured Data
    - ECHA, 2023i (unpublished test report dated 2005)
    - Test substance: Paraffin waxes (Fischer-Tropsch), full range, C_{15-50}-branched and linear)

A GLP-compliant *in vitro* micronucleus test was described in the ECHA database. The study was assigned a Klimisch reliability score of 1 (reliable without restrictions) and was conducted according to OECD test guideline 487 (indicated as "draft" at the time the study was conducted). Human lymphocyte cultures (unspecified cell line) were exposed to the test substance in two experiments. Experiment 1 used a 4-hour exposure in the presence and absence of a standard metabolizing system (S9, at a 2% final concentration) with a 16-hour expression period and cell harvest following 28 hours exposure to Cytochalasin-B. Experiment 2 used a similar 4-hour exposure time with S9 (at a 1% final concentration), and in the absence of metabolic activation, the exposure time was increased to 20 hours. After a total of approximately 96 hours of incubation, the cells were harvested, and microscope slides were prepared. In both experiments, the test material did not induce any statistically significant increases in the frequency of cells with micronuclei in either the absence or presence of S9. The test material was therefore considered to be non-clastogenic and non-aneugenic to human lymphocytes *in vitro*.

- Measured Data
    - ECHA, 2023j (unpublished test report dated 1990)
    - Test substance: Solvent-extracted, dewaxed paraffin oils, sufficiently refined, suspended in corn oil. Sourced from five feedstocks.

An *in vivo* micronucleus test was described in the ECHA database. The study was assigned a Klimisch reliability score of 1 (reliable without restrictions) and "closely adhered" to OECD TG 474. Groups of male and female CD-1 mice (number per group not specified) were administered intraperitoneal doses of one of the five feedstocks of the test substance at concentrations of 0 (corn oil), 1000, 2500 and 5000 mg/kg. According to the authors of the ECHA summary, all but one mouse survived to the scheduled sacrifice, and there was no gross evidence of toxicity. In only one case was the mean micronucleus frequency significantly greater than the concurrent negative control (in one of the male high-dose groups); however, it was stated the negative control was unusually low in that instance (mean of 1.6 micronuclei in the high-dose group compared to 0.0 in the control), and the results in the high-dose groups for the other four feedstocks in both males and females were similar to their corresponding controls. The positive and negative controls responded as expected. Therefore, the test substance was considered non-clastogenic under the conditions of the study.

Reproductive Toxicity (R): L

Paraffin wax was assigned a hazard classification level of Low for reproductive toxicity based on the results of a guideline-compliant reproductive screening assay and two guideline-compliant multigeneration studies showing no evidence of reproductive toxicity at doses up to 1000 mg/kg-day. Confidence in this classification is high. The relevant data are from GLP-complaint and guideline-compliant studies which include results from multiple generations. Although some of the test substances may fall outside the range of hydrocarbon distillates generally classified as “paraffin wax”, the test substances included mixtures relevant to alkanes in the C_{20-30} range.

- Measured Data
    - ECHA, 2023k (unpublished test report dated 1995)
    - Test substance: Petroleum distillates, hydrotreated heavy paraffinic (CAS # 64742-54-7). Described as a lubricant base oil with polycyclic aromatic hydrocarbon content < 3% (trade name: Chevron 100 Neutral).

A reproduction and developmental toxicity screening test was described in the ECHA database. The study was assigned a Klimisch reliability score of 1 (reliable without restrictions) and stated as compliant to GLP and OECD test guideline 421. Sprague Dawley rats (12 animals/sex/dose) were administered gavage doses of 1000 (25%) and 1000 (5%) mg/kg-day for at least 14 days prior to mating and continuing for a total dosing period of 30 days in F0 males, and for a minimum of 14 days prior to mating and continuing until the day prior to the scheduled necropsy on lactation day 4 in F0 females. The animals were observed twice daily for appearance, behavior, morbidity, and mortality. Males and females were also observed during dosing and for one hour thereafter. Male F0 body weights were recorded weekly. Female F0 body weights were also recorded weekly until evidence of mating was observed and then on gestation days 0, 7, 14 and 20 and on lactation days 1 and 4. Food consumption was also recorded in the parental males and females. After parturition, litters were sexed and examined for evidence of gross malformations, numbers of stillborn, and live pups. Litters were examined daily, and each pup received a detailed physical examination on days 1 and 4 of lactation. All abnormalities were recorded. The live litter size and viability index were calculated. All surviving pups were necropsied on post-natal day 4. A complete gross examination was made on all animals at necropsy. There were no clinical findings and growth rates were normal. There were no treatment-related effects on pup body weights, sex ratios, live litter sizes, viability indices, and general physical conditions. Necropsy findings of the pups were unaffected by administration of the test substance in the F0 animals. Therefore, the NOAEL for developmental toxicity was > 1000 mg/kg-day.

- Measured Data
    - ECHA, 2023l (unpublished test report dated 2017)
    - Test substance: Distillates (Fischer-Tropsch), heavy, C_{18}-C_{50} branched, cyclic and linear (CAS # 848301-69-9), dissolved in corn oil

A multigeneration reproduction study was described in the ECHA database. The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) and stated as compliant to GLP and OECD test guideline 416. The stated reason for the Klimisch score

was a lack of detailed documentation. Sprague Dawley rats (25-26 animals/sex/dose/generation) were administered gavage doses of 0 (corn oil), 50, 250 and 1000 mg/kg-day for 70 days prior to mating, and continuing through the mating period, gestation period, and lactation period, until the day prior to euthanasia in F0 generation. In the F1 generation, dosing began at post-natal day 22, and continued through the mating, gestation, and lactation periods. All animals were observed at least twice daily; clinical observations, body weights, and food consumption were recorded at appropriate intervals for males throughout the study and for females prior to mating and during gestation and lactation. Vaginal smears were performed daily for the determination of estrous cycles beginning 21 days prior to pairing until mating occurred. Spermatogenic endpoints, including sperm motility and progressive motility, morphology, and number, were recorded for F0 and F1 males. On post-natal day 4 litters were arbitrarily culled to 10 pups per litter (five per sex, when possible) to reduce variability due to litter size. Offspring (25/sex/group) from the pairing of the F0 animals were selected on post-natal day 21 to constitute the F1 generation. Developmental landmarks (balano-preputial separation and vaginal patency) were evaluated for the selected F1 rats. Ovarian primordial follicle counts and corpora lutea counts were recorded for all F1 females in the control and high-dose groups. Administration of the test substance to male and female rats at doses up to 1000 mg/kg-d had no effect on F1 and F2 litter parameters, postnatal survival, physical condition/mortality, anogenital distance, and pup body weights. There was also no effect on reproductive performance, gestation length, or parturition in both the F0 and F1 generations. Therefore, the NOAEL for developmental toxicity was 1000 mg/kg-day.

- Measured Data
    - ECHA, 2023m (unpublished test report dated 2017)
    - Test substance: Distillates (Fischer-Tropsch), C_{18}-C_{26} branched and linear (CAS # 848301-67-7), dissolved in corn oil

A multigeneration reproduction study was described in the ECHA database. The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) and stated as compliant to GLP and OECD test guideline 416. The stated reason for the Klimisch score was a lack of detailed documentation. Sprague Dawley rats (25-28 animals/sex/dose/generation) were administered gavage doses of 0 (corn oil), 50, 200 and 750 mg/kg-day for 70 days prior to mating, and continuing through the mating period, gestation, and lactation periods in the F0 generation. In the F1 generation, dosing begant on post-natal day 22 and continued through the mating, gestation, and lactation periods. All animals were observed at least twice daily; clinical observations, body weights, and food consumption were recorded at appropriate intervals for males throughout the study and for females prior to mating and during gestation and lactation. Vaginal smears were performed daily for the determination of estrous cycles beginning 21 d prior to pairing until mating occurred. Spermatogenic endpoints, including sperm motility, including progressive motility, morphology, and number, were recorded for F0 and F1 males. On post-natal day 4 litters were arbitrarily culled to 10 pups per litter (five per sex, when possible) to reduce variability due to litter size. Offspring (28/sex/group) from the pairing of the F0 animals were selected on post-natal day 21 to constitute the F1 generation.

Developmental landmarks (balano-preputial separation and vaginal patency) were evaluated for the selected F1 rats. Ovarian primordial follicle counts and corpora lutea counts were recorded for all F1 females in the control and high-dose groups. Tissues (as specified in the relevant test guideline) from all F0 and F1 parental animals were examined microscopically, and a series of additional organs (brains, liver, kidneys, spleen, and thyroid) from both F0 and F1 adults were also microscopically examined. The control males from the F0 generation showed an unexplained low fertility with male mating indices of 69.6%, 80.0%, 95.8%, and 90.9% for controls, 50 mg/kg, 200 mg/kg, and 750 mg/kg dose groups, respectively. To compensate for reduced statistical sensitivity of the first-generation litter data, the sizes of the F1 groups were increased from 25 to 28 rats of each gender. Administration of the test substance to male and female rats at doses up to 750 mg/kg-day had no effect on test item-related effects on F1 and F2 litter parameters, postnatal survival, physical condition/mortality, anogenital distance, and pup body weights. There was also no effect on reproductive performance, gestation length, or parturition in both the F0 and F1 generations. Therefore, the NAOEL for developmental toxicity was 750 mg/kg-day.

- Measured Data
    - Unpublished data summarized by EFSA (2023)
    - Waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity (designated EWF FCM 93 58)

The study was performed according to OECD TG 408 and was compliant to GLP. Three groups of Sprague-Dawley rats (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-day (achieved dose level: 0.19, 0.24; 1.9, 2.0 and 9, 10 g/kg-day in males and females, respectively). A control group received the vehicle (powdered rodent diet). No treatment-related differences between the control and the treated groups were observed in sperm motility, morphology, and concentration (expressed as million sperm/g caudal epididymal tissue). No treatment-related differences in organ weight, including testes, epididymides, epididymal cauda, seminal vesicles, uterus, or ovaries were observed. Histopathological examination revealed no abnormal findings in the morphology of organs/tissues, including cervix, vagina, and mammary glands.

Developmental Toxicity incl. Developmental Neurotoxicity (D): L

Paraffin wax was assigned a hazard classification level of Low for developmental toxicity based on the results of a guideline-compliant assays showing no evidence of developmental effects at doses up to 1000 mg/kg-day. Confidence in this classification is high. Although some of the test substances may fall outside the range of hydrocarbon distillates generally classified as “paraffin wax”, the test substances included mixtures relevant to alkanes in the $C_{20\text{-}30}$ range. No specific data relevant to developmental neurotoxicity were reported in these studies, although given the lack of developmental effects, developmental neurotoxicity is similarly considered to be of low concern.

- Measured Data

  - ECHA, 2023n (unpublished test report dated 2017)
  - Test substance: Distillates (Fischer-Tropsch), C_{18}-C_{26} branched and linear (CAS # 848301-67-7), dissolved in corn oil

A developmental toxicity study was described in the ECHA database. The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) and stated as compliant to GLP and OECD test guideline 414. The stated reason for the Klimisch score was a lack of detailed documentation. Sprague Dawley rats (24 mated females/dose) were administered gavage doses of 0 (corn oil), 50, 200 and 750 mg/kg-day from gestation days 5 to 19. No treatment related effects were observed in any uterine parameter, or in fetal viability or fetal growth and development. There were no treatment related abnormalities in external development or in type or incidence of skeletal or visceral findings. Therefore, the NOAEL for developmental toxicity was > 750 mg/kg-day.

- Measured Data
  - ECHA, 2023o (unpublished test report dated 2017)
  - Test substance: Distillates (Fischer-Tropsch), heavy, C_{18}-C_{50} branched, cyclic and linear (CAS # 848301-69-9), dissolved in corn oil

A developmental toxicity study was described in the ECHA database. The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) and stated as compliant to GLP and OECD test guideline 414. The stated reason for the Klimisch score was a lack of detailed documentation. Wistar Hannover rats (22 pregnant females/dose) were administered gavage doses of 0 (corn oil), 50, 200 and 1000 mg/kg-day from gestation days 6 to 20. It was reported that the mean body weight of the live fetuses was slightly increased (5.1 ±0.2 g) in the highest dose group (1000 mg/kg-day) compared with the controls (4.9 ± 0.2 g) and the low-dose (5.0 ± 0.2 g) and mid-dose (4.9 ± 0.3 g) groups. The minor increase in fetal body weight in the high-dose group was just outside the historical control range but well within the range of normal biological variability. Therefore, it was determined by the study authors that no treatment related toxicity was observed in developmental parameters. The NOAEL for developmental toxicity was > 1000 mg/kg-day.

Endocrine Activity (E): DG

Paraffin wax was assigned a hazard classification level of “data gap” for endocrine activity based on repeated dose studies examining various parameters related to endocrine activity that did not result in observed effects on the endocrine system. Although the weight of evidence supports low concern for endocrine activity, required data are lacking for specific analyses of the estrogen, androgen, and thyroid pathways, such as the 11 assays recommended by the US EPA (2015b) for Tier 1 screening for endocrine activity.

- Measured Data
  - Unpublished data summarized by EFSA (2023)
  - Waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity (designated EWF FCM 93 58)

The study was performed according to OECD TG 408 and was compliant to GLP. Three groups of Sprague-Dawley rats (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-day (achieved dose level: 0.19, 0.24; 1.9, 2.0 and 9, 10 g/kg-day in males and females, respectively). A control group received the vehicle (powdered rodent diet). Thyroxine (T4) 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-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 estrus 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 in organ weight, including adrenal glands, pituitary gland, thyroid gland, were observed. Histopathological examination revealed no abnormal findings in the morphology of organs/tissues, including a vaginal smear to determine stage of estrus cycle.

GROUP II AND II* HUMAN HEALTH EFFECTS (GROUP II AND II* HUMAN)

Note: Group II and Group II endpoints are distinguished in the v1.4 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. See GreenScreen Guidance v1.4, Annex 2 for more details.*

Acute Mammalian Toxicity (AT): L

Paraffin wax was assigned a hazard classification level of low (high confidence) for acute mammalian toxicity based on consistent data from multiple oral gavage single-dose studies in which no mortality or evidence of toxicity was reported. The test substances in these assays included paraffin wax, microcrystalline wax, as well as formulated cosmetic products containing various concentrations of paraffin wax. LD_{50} values were reported as high as 5000 mg/kg. Dermal acute toxicity data similarly resulted in zero mortality at applied doses up to 2000 mg/kg.

- Measured Data
    - ECHA, 2023p (unpublished test report dated 2007)
    - Test substance: Paraffin waxes (Fischer-Tropsch), full range, C_{15-50} branched and linear

An oral acute toxicity study using the fixed dose method was described in the ECHA database. The study was stated as compliant to GLP and OECD test guideline 420 and was assigned a Klimisch reliability score of 1 (reliable without restriction). Five female Sprague-Dawley rats were administered a single gavage dose of 5000 mg/kg. No mortality was reported and there were no treatment-related clinical signs, necropsy findings, or changes in body weight. The oral LD_{50} was determined to be > 5000 mg/kg.

- Measured Data
    - ECHA, 2023q (unpublished test report dated 1975)
    - Test substance: 100% paraffin wax (“most likely” CAS # 8002-74-2)

An oral acute toxicity study was described in the ECHA database and in CIR (1984). The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. Rats (5 animals, strain not specified) were dosed orally (method of dosing not specified) at a dose of 5000 mg/kg. No mortality was reported, although no further details were provided on evidence of toxicity.

- Measured Data
    - ECHA, 2023r (unpublished test report dated 1975)
    - Test substance: Undiluted, melted eye shadow formulation containing 5% paraffin wax (“most likely” CAS # 8002-74-2)

An oral acute toxicity study was described in the ECHA database and in CIR (1984). The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. Beagle dogs (4 animals) were dosed orally (method of dosing not specified) at a dose volume of 25 ml/kg. No treatment related to toxicity and mortality was observed. No abnormalities were observed through the study period. Based on a density of 0.8 g/mL, the dose was equivalent to 20 g/kg test substance or approximately 1000 mg/kg paraffin wax based on a 5% formulation.

- Measured Data
    - ECHA, 2023s
    - Test substance: Blusher formulation containing 4.35% microcrystalline wax (CAS # 63231-60-7)

An oral acute toxicity study was described in the ECHA database and in CIR (1984). The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. Groups of five male and female Sprague-Dawley rats were administered single gavage doses of 25 g/kg of the test substance, equivalent to approximately 1100 mg/kg microcrystalline wax based on a 4.35% formulation. No toxic effects or deaths occurred.

- Measured Data
    - ECHA, 2023t (unpublished test report dated 1975)
    - Test substance: Melted eye shadow formulation containing 5% paraffin wax (“most likely” CAS # 8002-74-2)

An oral acute toxicity study was described in the ECHA database and in CIR (1984). The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for

assessment. Albino Wistar rats (10 animals) were dosed orally (method of dosing unspecified) at a dose volume of 60 ml/kg. Urinary staining of the abdomen and intestines filled with fluid was observed in one animal that died on day 4. All other animals were normal through day 14. No other treatment related toxicity or mortality were observed. Based on a density of 0.8 g/mL, the dose was equivalent to 48 g/kg test substance or approximately 2400 mg/kg paraffin wax based on a 5% formulation.

- Measured Data
    - ECHA, 2023u (unpublished test report dated 1977)
    - Test substance: Eye shadow containing 8% paraffin wax ("most likely" CAS # 8002-74-2)

An oral acute toxicity study was described in the ECHA database and in CIR (1984). The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. Albino rats (5 animals) were orally intubated at a dose volume of 10 ml/kg. No mortality or treatment related toxicity was observed. Based on a density of 0.8 g/mL, the dose was equivalent to 8 g/kg test substance or approximately 640 mg/kg paraffin wax based on an 8% formulation.

- Measured Data
    - ECHA, 2023v (unpublished test report dated 1974)
    - Test substance: Foot cream containing 16% paraffin wax ("most likely" CAS # 8002-74-2)

An oral acute toxicity study was described in the ECHA database and in CIR (1984). The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. Five rats (unspecified strain) were dosed orally (method of dosing not stated) at a volume of 5 ml/kg (purity 100%). No toxicity was reported. Based on a density of 0.8 g/mL, the dose was equivalent to 4 g/kg test substance or approximately 640 mg/kg paraffin wax based on a 16% formulation.

- Measured Data
    - ECHA, 2023w (unpublished test report dated 1976)
    - Test substance: R 9107 paraffin wax ("most likely" CAS # 8002-74-2) dissolved in arachis oil

An oral acute toxicity study was described in the ECHA database. The study was stated as compliant to OECD test guideline 401 with a Klimisch reliability score of 1 (reliable without restrictions). Fasted Wistar rats (5 animals/sex/dose) were administered gavage doses of 1000 and 5000 mg/kg. No mortality or signs of clinical toxicity were observed in either male or female rats in both dose groups during the 7-day observation period. No treatment-related effects were observed in either male or female rats in both dose groups.

- Measured Data

  - ECHA, 2023x (unpublished test report dated 1993)
  - Test substance: Paraffin wax (CAS # 8002-74-2) dissolved in olive oil. Purity not specified.

An oral acute toxicity study using the fixed dose procedure was described in the ECHA database. The study was stated as compliant to OECD test guideline 401 (similar to current OECD guideline 420) with a Klimisch reliability score of 1 (reliable without restrictions). Sprague Dawley rats (5 animals/sex/dose) were administered a single gavage dose of 5000 mg/kg. No mortality was observed, and no treatment-related signs of clinical toxicity were observed in male or female rats when compared with controls. No treatment-related findings were observed during necropsy.

- Measured Data
  - ECHA, 2023y (unpublished test report dated 1972)
  - Test substance: Paraffin wax ("most likely" CAS # 8002-74-2) and petrolatum in a 1:1 ratio.

A dermal acute toxicity study was described in the ECHA database and in CIR (1984). The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. A dose of 4 ml/kg of 50% paraffin in petrolatum was applied to rabbits (number of animals per sex were not reported) under closed patching for 24 hours. No treatment related toxicity was observed, and as such, it is presumed there was no mortality. The LD_{50} was determined as > 4 ml/kg (equivalent to 3600 mg/kg test substance or 1800 mg/kg paraffin wax).

- Measured Data
  - ECHA, 2023z (unpublished test report dated 1993)
  - Test substance: Paraffin wax ("most likely" CAS # 8002-74-2) dissolved in olive oil. Purity not specified.

A dermal acute toxicity study was described in the ECHA database. The study was stated as compliant to GLP and OECD test guideline 402 with a Klimisch reliability score of 1 (reliable without restrictions). Sprague Dawley rats (5 animals/sex/dose) were administered dermally dosed (occlusive) with paraffin wax in arachis oil at a dose of 2000 mg/kg. The test article caused slight skin irritation reactions in a majority of treated animals which persisted throughout the study; however, no mortality was observed. Therefore, the LD_{50} was >2000 mg/kg.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single): *L*

Paraffin wax was assigned a hazard classification level of *Low* (low confidence) for single dose systemic toxicity/organ effects. Based on the weight of evidence, the observed lack of clinical signs of toxicity in the single dose studies described previously do not provide

clear evidence of adverse effects leading to classification under GHS for this endpoint. Confidence is low since the study designs associated with these single dose studies do not provide information relevant to clinical chemistry, hematology, urinalysis, or specific tissue injury.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*): L

Paraffin wax was assigned a hazard classification of low (high confidence) for systemic toxicity/organ effects (repeated dose). Evidence of an inflammatory response in the liver and mesenteric lymph nodes, and in the heart mitral valve lymphoid infiltrate, are consistently reported in multiple repeated dose studies. The effect appears to be strain-dependent and sex-dependent, with the greater sensitivity in Fischer-344 (F-344) rats compared to Sprague-Dawley rats, and in females compared to males. LOAEL values were as low as 2 mg/kg-day in F-344 rats for some studies where several paraffin wax products were evaluated. In a weight-of-evidence evaluation by EFSA (2023), it was concluded that the F-344 rat strain has a particular sensitivity to *n*-alkanes and therefore, these species-specific and strain-specific inflammatory effects were deemed non-relevant to humans. This was based on the results of several related studies. For example, Cravedi et al. (2011) showed that the metabolism of n-alkanes by liver and small intestine microsomes *in vitro* is less efficient in cells derived from F-344 rats compared to Sprague-Dawley rats and Wistar rats. Furthermore, Cravedi et al. (2017) showed that n-alkanes showed the greatest accumulation in the liver compared to other components of paraffin wax mixtures. In a discussion by Grob et al. (2018), it was noted that human liver and spleen contains comparatively few n-alkanes, which is not due to low exposure, but rather due to efficient metabolism. Thus, the granulomatous lesions experimentally induced by MHC-feeding, particularly in the livers of F-344 rats, may reflect an exaggerated toxicological response by that strain due to inefficient metabolism, and therefore, the exaggerated response in the F-344 strain may not be relevant to humans. The definitive 90-day study was summarized by EFSA (2023) in Sprague-Dawley rats (a strain showing a comparatively reduced inflammatory response to exposure), in which the critical effect was an increase in mesenteric lymph node (MLN) weights correlating with granulomas in MLNs, which were noted mainly in females. The MLN granulomas in females showed a dose-dependent trend of increased severity, with minimal severity reported at all dose levels and slight severity being reported at the mid and high doses in females (≥ 1900 mg/kg-day). This effect was deemed non-adverse by EFSA (2023) because it is not associated with a wider inflammatory response and did not progress to necrosis. If this effect at slight severity (but not minimal severity) were considered adverse, the NOAEL would be the low dose of 190 mg/kg-day in females, and thus would not qualify for GHS classification as the guidance value is > 100 mg/kg-day. The confidence in this hazard classification is moderate. As noted, numerous repeated dose studies in rats exposed to a range of refined petroleum mixtures consistently demonstrate an exaggerated inflammatory response in F-344 rats but not in other strains. Carillo et al. (2021) proposed an adverse outcome pathway for this toxicological response that consists of three key events (KEs):

- (KE1) Decreased metabolism of linear n-alkanes primarily in the C_{25}-C_{35} range in the F-344 rat strain. The reason for this strain-specific difference in metabolic rate is unknown and no comparative data for relevant CYP isoforms in rats and humans are available. The unmetabolized compounds accumulate with increasing dose and length of exposure.
- (KE2) Crystallization of accumulated n-alkanes inside hepatocytes which rupture to release them. Liver enzyme activities increase, and an inflammatory response is initiated in response to the foreign crystals. The F-344 strain has an inherent lower population of resident Kupffer cells that may be overloaded.
- (KE3) Granuloma formation. Resident Kupffer cells phagocytize the wax crystals, forming microgranuloma.

Carillo et al. (2021) further summarized experimental evidence suggesting that in humans, KE1 and KE2 are unlikely to occur (as n-alkanes are metabolized relatively quickly in humans), whereas there is no evidence that KE3 occurs in humans as any n-alkane crystals that do form are likely to be quickly metabolized.

Therefore, the weight of evidence indicates that the key events associated with this strain-specific response are unlikely to be relevant to humans, and this conclusion underpins the overall hazard rating for this endpoint.

- Measured Data
    - Unpublished data summarized by EFSA (2023)
    - Waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity (designated EWF FCM 93 58)

The study was performed according to OECD TG 408 and was compliant to GLP. Three groups of Sprague-Dawley rats (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-day (achieved dose level: 0.19, 0.24; 1.9, 2.0 and 9, 10 g/kg-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 euthanized on day 30 and 60 for the evaluation of the accumulation of hydrocarbons in different organs and tissues.

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 sex. 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 hematology evaluation revealed slight, but not dose-related, changes in mean cell hemoglobin 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 differences in alanine aminotransferase, creatinine, potassium, protein and albumin in males, and chloride, calcium, and phosphorus in females, were observed, but these showed no evidence of

dose dependence. MLN weights 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 the presence of granulomas 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 EFSA 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.

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

- Measured Data
    - Griffis et al. (2010)
    - Test substance: Highly refined mineral hydrocarbons (melting point of 55°C; oil content of 0.1%, sulfur content of < 5 ppm; and carbon number of n-alkanes ranging from C_{19}-C_{42}). CAS # 8002-74-2

In a published 90-day study, female F-344 and Sprague-Dawley rats were administered the test substance in the diet at 0, 0.2% or 2% (corresponding to 157, 160 and 1,609, 1,644 mg/kg-day, respectively, for the two strains). The study design included interim sacrifices. Ten control and high-dose rats of each strain were euthanized and necropsied after 30 and 60 days of treatment. Ten control, low- and high-dose rats of each strain were necropsied after 90 days. Additional rats (5/dose) were used to analyze mineral hydrocarbon contents in certain tissues after 30, 60 and 90 days of treatment. Thus, the total number of rats used were 45 in control group, 25 in mid-dose group and 45 in the high-dose group for each strain. Normal repeated-dose toxicity parameters were evaluated in the main study group (clinical signs, hematology, clinical chemistry, organ weight and histopathology). Given knowledge from previous studies, there was a focus on mesenteric lymph nodes and the liver by use of immunohistochemical analyses. In the

additional animal group, the liver, kidney, heart, spleen, and mesenteric lymph nodes were analyzed for mineral hydrocarbon content. There were no effects in either strain at either dose on mortality, clinical signs, body weight gain (there is no mention of effects on body weight) and food consumption. The following effects (hematology and clinical chemistry) were seen in F-344 rats but not in the Sprague-Dawley strain of rats (only those labeled as statistically significant and that are dose-related are reported here):

- Increased neutrophils (both doses)
- Decreased hemoglobin (both doses)
- Elevated liver enzymes (both doses for AST, ALT, GGT, high dose only for alkaline phosphatase (ALP); and decreased serum albumin levels (both doses).
- Increased absolute and relative to body organ weights in liver, spleen, and mesenteric lymph nodes (all at 30, 60 and 90 days) and ovaries (90 days only) at both doses.
- Histopathological changes were noted in the liver (microgranulomas), spleen (extramedullary hematopoiesis) and mesenteric (and cervical) lymph nodes (microgranulomas) and heart (mitral valve-based lymphoid cell infiltrate) - but not the ovaries.

In Sprague-Dawley rats, the only organ weight changes noted were increases in the absolute and relative mesenteric lymph nodes (at 30, 60 and 90 days), some of which were statistically significant for the 30- and 60-day time periods, but more detailed information was not provided. The report further indicated that microgranulomas in the mesenteric lymph nodes were present in the Sprague-Dawley rats. There was also reticuloendothelial cell hyperplasia observed in both rat strains at both doses. Documentation of histopathological changes was reported on a severity scale and there did not appear to be a statistical analysis of these data. In addition to the organ weight data above, results from the immuno-histochemical and tissue mineral hydrocarbon analyses confirmed that the liver and mesenteric lymph nodes were target organs in rats exposed to the test substance, but that F-344 rats were more sensitive in terms of both dose and magnitude of response.

- Measured Data
    - ECHA, 2023aa (unpublished test report dated 1993)
    - Test substances: (1) Paraffin wax (CAS # 8002-74-2), (2) mixture of paraffin wax (CAS # 8002-74-2) and microcrystalline wax (CAS # 63231-60-7), and (3) low melting point wax (CAS # not specified)

A subchronic oral toxicity study was described in the ECHA database. The study was stated as compliant to GLP and OECD test guideline 408 with a Klimisch reliability score of 1 (reliable without restrictions). Groups of Fischer 344 rats (20 animals/sex/dose) were administered dietary concentrations of test substance (1) and (2) at concentrations of 0.02, 0.2 and 2% (equivalent to 19.5, 193.4, and 1949.2 mg/kg-day for test substance (1) and 19.6, 197.6, 1986.2 mg/kg-day for test substance (2)) and 2% only of test substance (3) (equivalent to 2010.3 mg/kg-day). The dosing duration was 90 days. The tests included a main study, a reversal study, and a tissue level study. The reversal group was treated for 90 days followed by 85 days with untreated diet. Group size was 20

rats/sex/untreated control or dose for the main study, 10 rats/sex for the untreated control or high dose (2.0%) for the reversal study, and 5 rats/sex at the high dose only for the tissue level study. There were no effects on food intake, growth rate, or clinical conditions of animals fed paraffin waxes. There were some increases in organ weights which included increased spleen and liver weights (0.2 and 2.0% groups). Although some reductions were noted during the reversal period, the weights were still higher in the 2.0% group. Mesenteric lymph node (MLN) weights were also increased in the high dose group animals, and, like the spleen and liver weights, did not return completely to control weights during the reversal period. There were also changes in hematological parameters with a greater response observed in females. Serum enzymes including alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and gamma glutamyl transferase (GGT) were elevated in females from the 0.2 and 2% groups treated with paraffin wax and a mix of paraffin and microcrystalline waxes and from the 2% group treated with intermediate melting point paraffin wax and in males from the 2% groups. Serum bilirubin was also elevated in females from the 2% dose group. Histopathological changes in most organs were considered consistent with the age of the animals and not treatment-related, but changes in the liver, MLNs, and the cardiac mitral valve were considered to have been treatment-related. These effects were described as dose-related and more severe in females than males; however, these lesions were not described further in the available ECHA summary. As noted in the ECHA entry, NOAEL and LOAEL values were not able to be derived as significant treatment-related changes were observed at the lowest dose tested (0.02%, equivalent to 19.5, and 19.6 mg/kg-day for paraffin wax 64 and micro/paraffin wax mixture, respectively). It is unclear exactly what effects were reported in low-dose animals based on a review of the ECHA summary; however, it appears that changes in the liver, MLNs, and in the cardiac mitral valve were reported in all doses in females.

- Measured Data
    - ECHA, 2023ab (unpublished test report dated 1993)
    - Test substance: Low melting point wax (CAS # “most likely” 8002-74-2)

A subchronic oral toxicity study was described in the ECHA database. The study was stated as compliant to GLP and OECD test guideline 408 with a Klimisch reliability score of 1 (reliable without restrictions); however, assessment was limited to major histocompatibility complex (MHC) content in the liver, kidney, heart, spleen, and MLN. Female Fischer 344 and Sprague Dawley rats (30 animals/group for control and 2% and 10 animals/group for 0.2%) were administered dietary concentrations of 0, 0.2 and 2% for 90 days. This equates to 0, 226, 2258 mg/kg-day for Fischer 344 rats and 0, 196, 1961 mg/kg-day for Sprague Dawley rats. Sections of the heart, liver, and MLN were examined from rats in control and high dose groups at 30 and 60 days and in all groups at 90 days to follow the time course for the development of any lesions in these organs. Treatment related observations included a significant 50% increase in mean MHC level in Fischer 344 and Sprague Dawley rats at 2% compared to levels in the corresponding 0.2% group for both liver and MLN. This effect was strain-specific; the mean MHC level in the 2.0% group was 130% greater in F344 rats compared to Sprague-Dawley rats at 90 days.

- Measured Data
    - ECHA, 2023ac (unpublished test report dated 1993)
    - Test substance: Paraffin wax (CAS # 8002-74-2), trade names SX30, SX50, and SX701

A subchronic toxicity study was described in the ECHA database. The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. Furthermore, evaluation of hematology, clinical chemistry, and urinalysis endpoints was not conducted. Fischer 344 and Sprague Dawley rats (5 Fischer 344 rat/sex/dose and 10 Sprague Dawley rats/sex/dose) were exposed to SX30, SX50 and SX701 at dietary concentrations of 0.002, 0.02, 0.2, or 2% for Fischer 344 rats (SX30, SX50), 0.002, 0.02, 0.2, 2, 5% for Fischer 344 rats (SX701), and 2% for Sprague Dawley rats (SX30, SX50, SX701) for 90 days. This equates to 2, 20, 200, 2000 mg/kg-day and 2.26, 22.6, 226, 2258 mg/kg-day for male and female Fischer 344 rats, respectively (SX30, SX50); 2, 20, 200, 2000, and 5000 mg/kg-day and 2.26, 22.6, 226, 2258 and 5645 mg/kg-day for male and female Fischer 344 rats, respectively (SX701); and 1723 mg/kg-day and 1961 mg/kg-day for male and female Sprague Dawley rats, respectively (SX30, SX50, SX701).

No treatment related toxicity was observed in clinical signs, mortality, body weight, gross pathological observations. Histopathological findings included mesenteric lymph node lesions such as histiocytosis, reactive node, adenitis at 2% (SX30, SX50, SX701) and 5% (SX702) for Fischer 344 rats and various degrees of histiocytosis were observed in lower doses of SX50 and SX701. Heart lesions included thickening, chronic inflammation, increased basophilia on the mitral valve at 2% (SX50), 2% and 5% (SX701) on Fischer 344 rats. Liver lesions included microgranuloma at 0.2% (SX50, SX701) on Fischer 344 rats, periportal vacuolation at 2 & 5% (SX701) on Fischer 344 rats. Foci inflammatory cells were observed at 0.002 and 0.2% (SX701) for Fischer 344 rats and in the same dose levels of (SX50 & SX701) for Sprague Dawley rats. Subsequent accumulation of non-polar hydrocarbons were observed in Fischer 344 rats fed with SX50 & SX701 and accumulation in mesenteric lymph node dosed with all the waxes. The changes in the histopathological parameters were more extensive in Fischer 344 rats fed at 2% than the Sprague Dawley rats. Therefore, the NOAEL was reported to be 0.2% for SX30 (equivalent to 200 and 226 mg/kg-day male and female Fischer 344 rats, respectively) based on lymph node lesions and spleen weight changes, 0.02% for SX50 (equivalent to 20 and 22.6 mg/kg-day male and female Fischer 344 rats, respectively) based on lymph node lesions and spleen weight changes. The LOAEL was reported to be 0.002% for SX701 (equivalent to 2 and 2.26 mg/kg-day male and female Fischer 344 rats, respectively) based on liver and lymph node lesions observed at the lowest dose tested.

- Measured Data
    - ECHA, 2023ad (unpublished test report dated 1993)
    - Test substance: Paraffin wax (CAS # 8002-74-2), trade names SX701, SX702, and SX100

A subchronic toxicity study was described in the ECHA database. The study was assigned a Klimisch reliability score of 2 (reliable with restrictions) as the method did not strictly follow the relevant guideline but was deemed appropriate for assessment. Furthermore, evaluation of hematology, clinical chemistry, and urinalysis endpoints was not conducted. Female Fischer 344 and Sprague Dawley rats (5-10 animals/dose) were fed with paraffin wax SX701, SX702, SX100 at concentrations of 0, 2, 5% for SX701 diet, 0, 0.002, 0.02, 0.2, 2% for SX702 & SX100 diet for 13 weeks. This equates to 0, 2258, 5645 mg/kg-day for female Fischer 344 rats (SX701); 0, 1961, 4902 mg/kg-day for female Sprague Dawley rats (SX701); 0, 2.26, 22.6, 226, 2258 mg/kg-day for female Fischer 344 rats (SX702 & SX100); and 0, 1.96, 19.6, 196, and 1961 mg/kg-day female Sprague Dawley rats (SX702 & SX100). No treatment related toxicity was observed in clinical signs, mortality, body weight, food consumption.

Significant increases in absolute and relative liver weight were observed in Fischer 344 rats at 2% SX701 & SX702 and at 5% SX701, significant increase in absolute and relative weight of mesenteric lymph node in Fischer 344 rats at 2% SX701, 0.2 & 2% SX702 and in Sprague Dawley rats at 2% SX701. Significant increases in absolute and relative spleen weight were observed in Fischer 344 rats at 2% SX701 and at 5% SX701. Increased relative spleen weight was observed at 0.02, 0.2, 2% SX702. Histopathological lesions were observed in mesenteric lymph nodes and liver. A LOAEL of 2% was reported in females for SX701 (equates to 2258 and 1961 mg/kg-day for Fischer 344 and Sprague Dawley rats, respectively). A NOAEL of 0.02% was reported in females for SX702 (equates to 22.6 and 19.6 mg/kg-day for Fischer 344 and Sprague Dawley rats, respectively), and a NOAEL of 2% was reported in females for SX100 (equates to 2258 and 1961 mg/kg-day for Fischer 344 and Sprague Dawley rats, respectively).

- Measured Data
    - ECHA, 2023ae (unpublished test report dated 1992)
    - Test substance: (1) Hydrotreated paraffin wax (CAS # 64742-51-4), (2) hydrotreated microcrystalline wax, (3) clay-treated microcrystalline wax (CAS # 64742-42-3)

A subchronic toxicity study was described in the ECHA database. The study was stated as compliant to GLP and OECD test guideline 408, with a Klimisch reliability score of 1 (reliable without restriction). The test substances were administered to Fischer 344 rats via the diet (20/sex/dose for waxes, 60/sex for control) at concentrations of 0.002, 0.02, 0.2, or 2.0% (equivalent to approximately 1.5, 15, 150, 1500 mg/kg-day) for 90 days. Almost without exception, the effects seen during the study were much more severe in females than in males. In the liver, the only male groups showing a statistically significantly increased incidence of microgranuloma or granuloma were those receiving test substance 1 at the top two dose levels. The severity of the lesion increased with dose and at the highest dose, centrilobular vacuolation was also present. In the mesenteric lymph node, histiocytosis was increased compared with controls in males given test substance 1 at 0.02% dose level. Histiocytosis was seen in females given test substance 1 at all doses. In the ileum and jejunum, a statistically significantly increased incidence of macrophage accumulation in Peyer's patches was observed in both male and female animals receiving test substance 1 at the top two dose levels. A significant increase in

macrophage infiltration of the lamina propria was observed in the high-dose females receiving test substance 1. In the heart, the high-dose group receiving test substance 1 had 11/20 male and 11/20 female animals showing focal inflammatory lesions within the cusps of the mitral valve. The lesion was characterized by an increased cellularity of the valve with destruction of the fibrous core. The increased cellularity was composed of a mixed population of macrophages, plasma cells and lymphocytes. Pyknotic nuclei and cell debris were scattered throughout the lesions. After the observation of the lesion in the high-dose group, the 0.2, 0.02 and 0.002% groups receiving test substance 1 were examined. The only statistically significantly increased incidence of the lesion was observed in the female 0.2% group (5/20 animals). The lesion was present in 1/60 male control animals and in occasional males from the high-dose treatment group. In animals treated with test substance 1, effects in the mesenteric lymph node were comprised of focal histiocytosis and increased organ weights. Effects on the liver included an increase in the organ weight and formation of granulomas. Increased ALP, ALT, and GGT activities were also reported and deemed indicative of hepatic damage. The significance of an inflammatory lesion of the mitral valve of the heart in females dosed with 0.2% of test substance 1 is not known. Spleen weight was also significantly increased, but no concomitant histopathological changes were observed, and the toxicological significance of this effect was deemed unclear. Hematological effects appeared to be related to histopathological findings in the lymph node and liver. The increase in the serum glucose levels were not considered to be clinically significant.

Therefore, for test substance 1, the NOAEL is equal to 0.002% in diet (equivalent to 1.5 mg/kg-day) for males and females based on histiocytosis in the mesenteric lymph nodes, granuloma in the liver, and inflammation of cardiac mitral valve. For the other two test substances, the NOAEL is greater than or equal to 2% in diet (equivalent to 1500 mg/kg-day) for males and females.

- Measured Data
    - Cravedi et al. (2017)
    - Test substance: Mineral oil saturated hydrocarbon mixtures (MOSH) ranging from about C_{14} to $C_{50.}$ Several mixtures evaluated, including L-C25 (free of n-alkanes, mainly > C25), and S-C25 mixture, containing n-alkanes with only 27% exceeding C25), and L-C25W mixture (L-C25 fraciton mixed with wax largely consisting of n-alkanes >C25)

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. Rats were exposed to three mixtures, including a mixture containing 1:1 of C_{25}–C_{45} 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-day. Dietary exposure to MOSH at the highest level tested resulted in a significant increase in both absolute and relative liver weight. At the highest dose tested, no steady state was reached at the end of the experiment. After 120 days of exposure to 40 mg/kg feed, ca 10% of the ingested MOSH were recovered from the whole body; after 90 days followed by a depuration period of 30 days, approximately 40% of the MOSH

body burden was eliminated. The depuration period resulted in a significant decrease of the MOSH concentration in the liver, but not in the adipose tissue. Accumulation of MOSH occurred mainly in the liver and to a lesser extent in adipose tissue and spleen.

Concentrations in the tissues increased far less than proportionally with the dose. The histological analyses indicated that dietary exposure to the MOSH mixture affects the granuloma formation in the liver of rats, but this was only evident at the highest dose (4000 mg/kg feed) tested. This effect was not observed after 30 or 60 days of treatment, but appeared after 90 or 120 days of treatment. Hepatic granulomas formed in the group exposed to the highest dose for 90 days were not reversible within the 30 days recovery period. MOSH were analyzed in liver, spleen, adipose tissue, and the carcass. In liver and spleen, accumulation of the C26-30 MOSH was higher than that of the C20-25 fraction. Wax constituents, such as n-alkanes and n-alkyl monocyclic naphthenes, were generally well retained in adipose tissue. No increase in granuloma formation was observed in any of the three dose groups given the L-C25 mixture. However, the groups fed 4000 mg/kg of the S-C25 mixture demonstrated significantly increased levels versus the control feed group. For the groups fed the L-C25W fraction, the granuloma density was significantly higher than in the control group for all doses tested. The numbers of lymphoid cell clusters in the liver parenchyma and in the liver portal tract were not affected by any dose of L-C25, but were significantly increased for the highest dose of the S-C25 mixture. For the L-C25W mixture (L-C25 mixed in a 1:1 ratio with a wax of similar mass range (n-alkanes ranging from C23 to C45), a significant increase of the numbers of lymphoid cell clusters in the parenchyma was observed at low and medium doses, whereas the effect was significant only at the highest dose in the portal tract.

Thus, in addition to clinical effects, liver microgranulomas, hepatic inflammation, and disruption of the immune function were the main toxicological endpoints investigated. 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 > C_{25}, assumed to be due to particularly slow metabolism and crystallisation in Fischer 344 rats.

- Measured Data
    - Hoglen et al. (2008)
    - Test substance: Low melting point wax (CAS # “most likely” 8002-74-2)

This published study examined differential effects of dietary exposure to 2% low melting point wax (LMPW) for 60 days on female Fischer-344 and Sprague-Dawley (SD) rats (9-10 animals/group). All animals were monitored for weight gain, food uptake and clinical condition throughout the study. After the 60-day exposure period, rats were sacrificed, whole blood was collected for hematology evaluation, and serum was prepared for serum liver enzyme analysis. Half of the medial and caudal lobes of the liver were collected for MHC analysis, and the remaining tissue from each lobe was fixed in 10% buffered formalin for histopathological evaluation. In addition, Kupffer cells (KC) were isolated from

livers of Fischer-344 and Sprague-Dawley rats for morphological and functional evaluations because KC can be involved in granuloma formation.

While mean body weights were not affected in either strain by 60 days of LMPW exposure throughout the course of the study, several changes within the livers of Fischer-344 rats in the LMPW group were observed, but not in livers from Sprague-Dawley rats. Histopathological analysis revealed the presence of microgranulomas in livers of all LMPW-treated Fischer-344 rats, but only in one of the treated Sprague-Dawley rats. Lymphoid cell aggregates were found only in livers of LMPW-treated Fischer-344 rats. ALAT, ASAT, and GGT activities were greater in the serum of LMPW-treated Fischer-344 rats compared to that of controls and LMPW-treated Sprague-Dawley rats. Total white blood cell and neutrophil counts were significantly elevated in blood of Fischer-344 rats following LMPW treatment. Detectable amounts of MHCs were present only in livers of LMPW-treated Fischer-344 rats, where 3.64 mg/g liver was found.

Electron microscopy of Kupffer cells (KC) revealed the presence of large, irregularly shaped, membrane-associated vacuoles in over half of cells isolated from Fischer-344 rats exposed to LMPW. These vacuoles were not seen in KC from control rats and rarely detected (<5%) in KC isolated from LMPW-exposed Sprague-Dawley rats. Indices of KC function including phagocytosis and nitric oxide and superoxide anion production were significantly increased by KC isolated from Fischer-344 rats exposed to LMPW over untreated controls. In contrast, lipopolysaccharide-stimulated production of tumor necrosis factor-α and leukotriene B4 was significantly decreased only in KC of LMPW-fed Fischer-344 rats. The observed differences in KC function may account for the strain differences in the response to LMPW. No significant changes in these functions were observed in KC isolated from Sprague-Dawley rats exposed to LMPW or from control rats.

Neurotoxicity (N-single): *L*

Paraffin wax was assigned a hazard classification level of *Low* (Low confidence) for single-dose neurotoxicity effects. Based on the results of single-dose acute toxicity studies described previously, there were no clinical signs suggestive of neurotoxicity (such as tremors, ataxia, reduced or increased motor activity, etc,) following single oral or dermal exposures to paraffin wax or related substances. Confidence in this designation is low because no single-dose studies designed to specifically target neurological endpoints were identified. Additionally, this endpoint should be based primarily on human data and only animal data are available for review.

Neurotoxicity (N-repeated) (Group II*): *L*

Paraffin wax was assigned a hazard classification level of Low (low confidence) for repeated dose neurotoxicity based on a lack of effects observed in a guideline-compliant 90-day feeding study in Sprague-Dawley rats that included a functional observation

battery. Confidence is low; although the hazard classification is based on the results of a functional observation battery in a robust 90-day study, no other repeated-dose studies with assessment of neurological endpoints were located, and there are no relevant human data on this endpoint. Additional functional observation batteries or more targeted neurological studies in other rat strains and other species would increase the confidence in the hazard classification.

Data

- Lists
  - *Authoritative:* Not present on any Authoritative A or B list.
  - *Screening:* Not present on any Screening A or B list.

- Measured Data
  - Unpublished data summarized by EFSA (2023)
  - Test substance: Waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, low viscosity (designated EWF FCM 93 58)

The study was performed according to OECD TG 408 and was compliant to GLP. Three groups of Sprague-Dawley rats (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-day (achieved dose level: 0.19, 0.24; 1.9, 2.0 and 9, 10 g/kg-day in males and females, respectively). A control group received the vehicle (powdered rodent diet). 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 in any dose tested.

Skin Sensitization (SnS) (Group II*): L

Paraffin wax is assigned a hazard classification of low (high confidence) for skin sensitization. GHS classification is not warranted based on lack of sensitization reactions in two robust Guinea pig maximization tests conducted with paraffin wax formulations.

- Measured Data
  - ECHA, 2023af (unpublished test report dated 1997)
  - Test substance: Paraffin wax (CAS # 8002-74-2), purity not stated.

A Guinea pig maximization test was summarized in the ECHA database. The study was stated as compliant to GLP and “closely followed” OECD TG 406, with a Klimisch reliability score of 1 (reliable without restriction). Dunkin Hartley guinea pigs (5-10 animals/sex/dose) were exposed to paraffin wax at doses of 0.1 ml of 3% w/v in propylene glycol for intradermal induction, 0.25 ml of 50% in propylene glycol for topical induction and 10 & 50% in propylene glycol for topical challenge. At the topical challenge phase, no skin reactions were observed at 24 or 48 hours in both the controls and groups treated with 10% or 50% paraffin wax in propylene glycol. There was a 0% sensitization rate. Therefore, paraffin was determined to be non-sensitizing under the conditions of the assay.

- Measured Data
  - ECHA, 2023ag (unpublished test report dated 2007)
  - Test substance: Paraffin waxes (Fisher-Tropsch), full range, C_{15-50} branched and linear (purity not stated)

A Guinea pig maximization test was summarized in the ECHA database. The study was stated as compliant to GLP and OECD TG 406, with a Klimisch reliability score of 1 (reliable without restriction). Dunkin Hartley guinea pigs (5-10 animals/sex/dose) were exposed to paraffin wax at doses of 0.1 ml of 3% w/v in propylene glycol for intradermal induction, 0.25 ml of 50% in propylene glycol for topical induction and 10 & 50% in propylene glycol for topical challenge. At the topical challenge phase, no skin reactions were observed at 24 or 48 hours in both the controls and groups treated with 10% or 50% paraffin wax in propylene glycol. There was a 0% sensitization rate. Therefore, paraffin was determined to be non-sensitizing under the conditions of the assay.

- Measured Data
  - Unpublished data cited in CIR (1984)
  - Test substances:
    - Smoothening cream formulation containing 15% paraffin
    - Eye shadow formulation containing 5% paraffin

The CIR (1984) review briefly described the results of patch-testing assays conducted in human volunteers. Few details were provided in the available summaries. In a 1980 repeated insult patch test, smoothening cream formulation containing 15% paraffin was applied to 48 human subjects, and no signs of sensitization reaction were reported. Additionally, in a 1975 maximization test, eye shadow formulation containing 5% paraffin was applied to 25, 30 and 29 human subjects, and no signs of sensitization were observed.

Respiratory Sensitization (SnR) (Group II*): L

Paraffin wax is assigned a hazard classification of low (high confidence) for respiratory sensitization. No relevant toxicological data are available to evaluate this endpoint. However, since paraffin waxes have boiling points well above 300°C, inhalation is not expected to be a relevant route of exposure in humans under typical environmental conditions. Thus, recognizing the lack of exposure by the relevant pathway, respiratory sensitization is of low concern for these compounds and additional studies addressing this endpoint are not warranted.

Skin Irritation/Corrosivity (IrS): L

Paraffin wax is assigned a hazard classification of low (high confidence) for skin sensitization/corrosivity. GHS classification is not warranted based on a weight of evidence approach evaluating human and animal studies for the target substance. In animals, no signs of irritation were observed in an OECD 404 guideline compliant study

in rabbits. This is supported by no signs of irritation in a skin irritation study with 100% paraffin wax under closed patching and reversible erythema in rabbits exposed to 50% paraffin. In addition, two studies in humans evaluated neat paraffin. In these studies, barely perceptible to mild pink/pink-red erythema was observed in 1 of 20 human subjects. The remaining human and animal studies evaluated product formulations. In three of the human studies, mild pink/pink-red erythema was observed in 5-50% of the participants; in all cases the primary irritation score was determined as 0.75 or lower (when reported). No skin irritation was observed in the remaining human studies. In animals, minimal, mild, moderate, and severe irritation were observed in foot care formulations, and eye shadow formulations containing paraffin; however, as these formulations contain multiple chemicals, they are unable to provide insight into classification for the target substance. Therefore, although slight erythema was observed in five of the human studies (three of which evaluated product formulation), given the description of the erythema and the low primary irritation score, the observed erythema does not warrant GHS classification. This is supported by the lack of observed irritation in an OECD 404 guideline study in rabbits. Confidence in this classification is high as it is based on human and animal studies for the target chemical.

- Measured Data
    - ECHA, 2023ah (unpublished data, 2003).
    - Test substance: Paraffin wax (CAS # 8002-74-2); 90-94% n-alkanes, 6-10% iso-alkanes (trade name: Sarawax 50)

A dermal irritation study was summarized in the ECHA database. The study was stated as compliant to GLP and OECD TG 404. 0.5 g of paraffin wax was applied semi-occlusively to the shaved flanks of New Zealand white rabbits (3 animals) for 4 hours. Animals were observed for 72 hours, and skin irritation or corrosion was scored by the method of Draize at 1, 24, 48, and 72 hours. At the end of the 4 -hour test period, excess paraffin wax was removed with water. Slight erythema was observed at 1 hour in all three animals which was fully reversible by 24 hours. Therefore, under conditions of the study, paraffin wax is considered non-irritating.

- Measured Data
    - ECHA, 2023ai and 2023aj
    - Test substance: 50% paraffin wax (“most likely” CAS # 8002-74-2) in petrolatum

In a series of 1972 skin irritation/corrosion studies assigned a Klimisch reliability score of 2 (reliable with restrictions), albino rabbits (6 animals) were dermally exposed to 0.5 ml of test chemical containing 50% of paraffin wax under open patching. In two studies, erythema was observed in 4 animals until day 3, while in the third study erythema was observed in 1 animal until day 2. No further details were provided.

- Measured Data
    - ECHA, 2023ak and 2023al (unpublished test reports dated 1977)
    - Test substance: Eye shadow products containing 8% paraffin

In a series of skin irritation/corrosion studies similar to OECD TG 404, four eye shadow formulations containing 8% paraffin in petrolatum were applied to albino rabbits (9 animals) under closed and open patching. The closed patches produced primary irritation scores that ranged from 0.17 (minimal) to 3.66 (severe), and open patches produced primary irritation scores that ranged from 0 to 0.17 (minimal). No further details were provided.

- Measured Data
    - ECHA, 2023am (unpublished report dated 1980)
    - Test substance: Foot care product containing 15% paraffin wax

In a skin irritation/corrosion study stated as similar to OECD TG 404 and assigned a Klimisch reliability score of 2, 0.5ml of the test substance was applied to albino rabbits (9 animals) under open insult patching. The primary irritation score was reported as 0.61 (minimal). No further information was provided.

- Measured Data
    - Unpublished data summarized in the CIR (1984) report

Several human patch test studies on cosmetic products containing various concentrations of paraffin wax were conducted and summarized in the CIR (1984) report.

- In a 1972 24-hour patch test, 20 human subjects were dosed with 100% paraffin. Barely perceptible erythema was observed in 1 human subject.
- In a 1972 24-hour patch test, 20 human subjects were dosed with 100% paraffin. Pink uniform erythema was observed in 1 human subject.
- In a series of 1977 24-hour patch tests, 8% paraffin showed no signs of irritation in 18, 19 and 20 human subjects.
- In a 1981 24-hour patch test, foot cream formulation containing 15% paraffin showed no signs of irritation on 19 human subjects (CTFA as cited in CIR, 1984).
- In a 1974 24-hour patch test, a foot cream formulation containing 16% paraffin was applied to 17 human subjects. Mild pink erythema was observed in 1 subject and the primary irritation score was found to be 0.15.
- In a 1980 24-hour patch test, foot cream formulation containing 16% paraffin was applied to 18 human subjects. Pink-red erythema was observed in 2 subjects and the primary irritation score was found to be 0.24.
- In a 1981 24-hour patch test, foot cream formulation containing 16% paraffin was applied to 18 human subjects. Pink to pink-red erythema was observed in 9 human subjects and the primary irritation score was found to be 0.75.
- In a 1981 24-hour patch test, foot cream formulation containing 16% paraffin was applied to 10 human subjects. The primary irritation score was found to be 0.35.

  - In a series of 1975 maximization tests, eye shadow formulation containing 5% paraffin was applied to 25, 30 and 29 human subjects. No signs of irritation were observed.
  - In a 1975 21-day cumulative irritancy test, eye shadow formulation containing 5% paraffin was applied to 10 human subjects for 21 days. No signs of irritation were observed and the primary irritation score was found to be 18 out of 630.
  - In a 1975 in-use test, eye shadow formulation containing 5% paraffin was applied to 187 females for 14 days. No signs of irritation were observed.

- Measured Data
  - Unpublished data summarized in the CIR (1984) report

Several patch test studies on cosmetic products containing various concentrations of paraffin wax were conducted in rabbits and summarized in the CIR (1984) report.

- In a 1980 skin irritation/corrosion study, albino rabbits (9 animals) were dermally exposed to 0.5 ml of test chemical containing 100% paraffin wax under closed patching. No signs of irritation were reported.
- In a series of 1974, 1979, 1980 skin irritation studies, three-foot care formulations containing 16% paraffin was dermally applied to albino rabbits (9 animals) under closed insult patching. The primary irritation score ranged from 0.95 (minimal) to 1.22 (mild). No further information was provided.

Eye Irritation/Corrosivity (IrE): L

Paraffin wax is assigned a hazard classification of low (high confidence) for eye irritation/corrosivity. GHS classification is not warranted for paraffin wax based on the results of three eye irritation studies that were similar to or compliant with OECD test guideline 405. In two OECD 405-compliant studies, minimal irritation was initially observed 1 to 24 hours after instillation; however, this irritation was scored low (mild) and was fully reversible. In a third study conducted similarly to OECD TG 405, two of four solutions of 50% paraffin elicited mild irritation in 1 of 6 rabbits one day after instillation (without rinsing); no further irritation was noted by the study authors. In addition to these three studies, there were multiple short data summaries available for cosmetic products containing paraffin (5-16%); however, as the remainder of these formulations are of unknown composition, these studies cannot be relied upon to determine GHS classification. Therefore, based on the three eye irritation studies that were similar to or compliant to OECD TG 405, GHS classification is not justified as the observed signs of irritation were not sufficient to warrant classification under GHS criteria. Confidence in this classification is high as well-conducted studies were available for the target chemical.

- Measured Data
  - ECHA, 2023an
  - Test substance: Paraffin waxes (Fisher-Tropsch), full range, C_{15-50} branched and linear (purity not stated)

In a 2007 GLP-compliant and OECE TG 405-compliant eye irritation study (assigned a Klimisch reliability score of 1), 0.1 ml of paraffin wax was instilled into the eyes of New Zealand white rabbits (3 animals). Washing was not performed. Animals were then observed for 72 hours. Irritation was scored by the Draize method. The mean scores for cornea and iris were 0. Slight conjunctival irritation was observed at 1 and 24 hours after treatment (conjunctivae score was 2, max score of 20). The conjunctival reaction was fully reversible after the 24 hour timepoint. Therefore, paraffin wax was a non-irritant to the eyes.

- Measured Data
    - ECHA, 2023ao
    - Test substance: Paraffin waxes (Fisher-Tropsch), full range, C_{15-50} branched and linear (purity not stated)

In a 1993 GLP-compliant and OECD TG 405-compliant eye irritation study (assigned a Klimisch reliability score of 1), 0.1 ml of the paraffin wax was instilled into the conjunctival sac of three young adult female New Zealand White rabbits. Eyes remained unwashed. Animals were then observed for 72 hours. Redness of the conjunctiva was observed in rabbits 1 hour after treatment. This observation was not noted again at any time over the study period. No other effects due to treatment were reported. The maximum group irritation score was 1.3 at the 1-hour observation period. On a scale of 1 to 8, a score of 1.3 was considered non-irritating. Therefore, paraffin waxes and hydrocarbon waxes was a non-irritant to the eyes.

- Measured Data
    - ECHA, 2023ap, aq
    - Test substance: 50% paraffin wax (“most likely” CAS # 8002-74-2) in petrolatum

In a series of eye irritation studies (conducted between 1972 and 1989, similar to OECD TG 405, Klimisch reliability score of 2), four solutions of 50% paraffin in petrolatum were instilled into the eyes of albino rabbits (6 animals) without rinsing. Rabbits were then observed for three days. Two of the formulations produced eye irritations (mild) in one rabbit on day 1, whereas the remaining solutions were non-irritants.

- Measured Data
    - Unpublished data summarized in the CIR (1984) report

Several eye irritation studies were conducted on cosmetic products containing various concentrations of paraffin wax and were summarized in the CIR (1984) report:

- In a 1980 eye irritation study (similar to OECD TG 405), 0.1 ml of foot cream formulation containing 15% paraffin was instilled into the eyes of albino rabbits (6 animals). Mild irritation was observed in one animal after 48 hours.

- In a 1975 eye irritation study, 0.1 ml of an eye shadow product containing 5% paraffin wax was instilled into the left eyes of rhesus monkeys (6 animals). The eyes were

washed with 20 ml tap water after 30 minutes of instillation. Observation up to 72 hours after instillation showed no signs of irritation or corneal damage.

- In a second 1975 eye irritation study, 0.1 ml eye shadow formulation containing 5% paraffin wax was instilled into the eyes of rhesus monkeys (6 animals). No signs of irritation or damage in the eyes were observed.

- In a 1975 eye irritation study, 0.1 ml of an eye shadow formulation containing 5% paraffin wax was instilled into one eye of albino rabbits (9 animals). In three of the animals the treated eyes were washed 30 second after instillation with 20 ml of deionized water. The eyes were inspected at 24, 48 and 72 hours, and four and seven days after instillation. Minimal conjunctival redness was observed in the unwashed eyes of 4/6 animals 48 hours after installation. Minimal conjunctival redness was also observed in the washed eyes of 2/3 animals 48 hours after instillation.

- In a series of 1977 eye irritation studies, 0.1 ml of four eye shadow formulations containing 8% paraffin wax was instilled into the eyes of albino rabbits (6 animals) at full strength with no rinse. Mild irritation was observed in one animal at 24 hours with three formulations, whereas mild irritation was observed in one animal after 48 hours with a fourth formulation.

- In a 1974 eye irritation study, 0.1 ml of a foot cream formulation containing 16% paraffin wax was instilled into the eyes of albino rabbits (6 animals). Mild irritation was observed in one animal at 48 hours.

- In a 1980 eye irritation study, 0.1 ml of a foot care formulation containing 16% paraffin was instilled into the eyes of albino rabbits (6 animals). Mild irritation was observed in two animals after 24 hours.

ECOTOXICITY (ECOTOX)

Acute Aquatic Toxicity (AA): *L*

Paraffin wax is assigned a hazard classification of low (low confidence) for acute aquatic toxicity. The GHS category is “not classified” due to a lack of evidence of acute toxicity to fish (LL50 > 100 mg/L), a lack of evidence of acute toxicity to invertebrates (EL50 > 100 mg/L), and a lack of evidence of acute toxicity to algae (EL50 > 100 mg/L) It should be noted that these values are above the solubility limit for the test compound. Confidence in this assessment is low. The test substances consisted of highly refined base oils and petroleum distillates which may contain longer chain hydrocarbons, branched alkanes, or unsaturated components. Therefore, the similarity of these test substances to paraffin wax is unclear, which reduces confidence in the overall assessment.

- Measured Data
    - ECHA, 2023ar

    - Test substance: Solvent dewaxed residual oil (basestock solvent neutral 600) (CAS # 64742-62-7)

In an acute aquatic toxicity study (stated as compliant to OECD TG 203, Klimisch score of 1, GLP) 96-hour short-term fathead minnow (*Pimephales promelas*) limit test, 10 animals/loading were exposed to the Water Accommodated Fraction (WAF) of a lubricant base oil, Basestock Solvent Neutral 600 (MRD-94 -981) at a nominal concentration of 100 mg/L. The LL50 was > 100 mg/L and the NOEL was ≥100 mg/L.

- Measured Data
    - ECHA, 2023as (unpublished test report dated 1988)
    - Test substance: Distillates, petroleum, hydrotreated/solvent refined light naphthenic (CAS # 64742-53-6 / 64741-97-5)

In an aquatic toxicity study (similar to OECD TG 202, Klimisch score 2) 48-hour short-term Daphnia magna toxicity test, 10 animals/loading were exposed to the Water Accommodated Fraction (WAF) of a lubricant base oil, MVI(N) 40 base oil (CAS # 64742-53-6 or 64741-97-5), at nominal concentrations of 0, 10, 100, 1000, and 10,000 mg/L. The EL_{50} was >10,000 mg/L and the NOEL was ≥ 1000 mg/L (ECHA, 2023bb).

- Measured Data
    - ECHA, 2023at (unpublished test report dated 1988)
    - Test substance: Distillates, petroleum, hydrotreated/solvent refined light naphthenic (CAS # 64742-53-6 / 64741-97-5)

In an aquatic toxicity study (similar OECD TG 202, Klimisch score 2) freshwater shrimp (Gammarus pulex, 10 animals/loading) were exposed to the test substance at nominal concentrations of 0, 10, 100, 1000, and 10,000 mg/L for 96 hours. The LL50 was >10,000 mg/L and the NOEL was ≥ 10,000 mg/L (ECHA, 2023bc).

- Measured Data
    - ECHA, 2023au (unpublished test report dated 2008)
    - Test substance: Lubricant base oil (no further details provided)

In an aquatic toxicity study (similar OECD TG 202, Klimisch score 2) the freshwater alga Pseudokirchneriella subcapitata was exposed to the test substance at a nominal concentration of 100 mg/L for 72 hours. The NOEL was ≥ 100 mg/L based upon average specific growth rate and cell yield.

- Estimated Data
    - ECHA, 2023av

The aquatic toxicity of paraffin wax to Oncorhynchus mykiss (based on a 96-hour exposure) was estimated using the PETROTOX computer model, which combines a partitioning model (used to calculate the aqueous concentration of hydrocarbon components as a function of substance loading) with the Target Lipid Model (used to

calculate acute and chronic toxicity of non-polar narcotic chemicals). PETROTOX computes toxicity based on the summation of the aqueous-phase concentrations of hydrocarbon block(s) that represent a hydrocarbon substance and membrane-water partition coefficients (K_{MW}) that describe the partitioning of the hydrocarbons between the water and organism. Results of computer modelling to estimate aquatic toxicity show no acute toxicity to freshwater fish at or below its maximum attainable water solubility (predicted LL50 > 1000 mg/L).

Chronic Aquatic Toxicity (CA): *M*

Paraffin wax is assigned a hazard classification of moderate (low confidence) for chronic aquatic toxicity, based on the results of a 21-day exposure study in Daphnia magna that resulted in a NOEL of 10 mg/L. Confidence is low since the test substance (distillates, petroleum, hydrotreated light naphthenic) is of unclear similarity to paraffin wax. Paraffin wax is comprised mainly of n-alkanes in the C_{20} to C_{30} range and no information on the hydrocarbon composition is available for the test substance. Furthermore, only one study relevant to this endpoint that measured chronic aquatic toxicity data was identified, and measured data for vertebrates (fish) and algae is missing. Estimated data using a proprietary model was provided in the ECHA database but the reliability of the model utilized is uncertain.

- Measured Data
  - ECHA, 2023aw (unpublished test report dated 1995)
  - Test substance: Distillates, petroleum, hydrotreated light naphthenic (CAS # 64742-53-6)

In a semi-static 21-day long-term Daphnia magna reproductive test (compliant to GLP and OECD TG 211, Klimisch reliability score of 2), 10 animals/loading were exposed to the water accommodated fraction of the test substance at nominal concentrations of 1, 10, 100 and 1000 mg/L. Concentrations of the test substance were not determined during the study. After 21 days, ≥ 80% of those daphnia exposed to 1 and 10 mg/L survived; all daphnia exposed to 100 mg/L died; 40% of those exposed to 1 mg/L died. The number of offspring produced per female per day at 1 mg/L was 4.3 offspring/day; at 10 mg/L was 4.2 offspring/day; at 100 mg/L was 0 offspring/day; and at 1000 mg/L was 1.3 offspring/day. The time to first brood release or time to hatch was 7 days for control and 1 mg/L, was 8 days for 10mg/L, and was 9 days for 1000 mg/L. The NOEL was 10 mg/L with respect to effects on reproduction. The loss of all daphnids in the 100 mg/L concentration is attributed to a non-treatment related effect, the cause of which is unknown.

- Estimated Data
  - ECHA, 2023ax

The aquatic toxicity of paraffin wax was estimated using the PETROTOX computer model, which combines a partitioning model (used to calculate the aqueous concentration of hydrocarbon components as a function of substance loading) with the Target Lipid Model (used to calculate acute and chronic toxicity of non-polar narcotic chemicals).

PETROTOX computes toxicity based on the summation of the aqueous-phase concentrations of hydrocarbon block(s) that represent a hydrocarbon substance and membrane-water partition coefficients (K_{MW}) that describe the partitioning of the hydrocarbons between the water and organism. Results of computer modelling to estimate aquatic chronic toxicity in a 28-day freshwater fish study show no toxicity to freshwater fish at or below its maximum attainable water solubility. The predicted NOEL associated with a 28-day exposure was > 1000 mg/L.

ENVIRONMENTAL FATE (FATE)

Persistence (P): *L*

Paraffin wax was assigned a hazard classification level of low (low confidence). In a manometric respirometry test (compliant to OECD TG 301 F), a lubricant base oil met the criteria for inherent biodegradability (≥ 60% in 28 days). Confidence is low since the test substance is of unclear similarity to paraffin wax. Paraffin wax is comprised mainly of n-alkanes in the C_{20} to C_{30} range and no information on the hydrocarbon composition is available for the test substance. Unpublished data in ChemView (2023) suggests that paraffin wax may be readily biodegradable; but due to the very limited information available on the test substance used and the results of this study, it was only used as supporting information.

- Measured Data
    - Unpublished data in ChemView (2023)

According to EPA ChemView (2023), in an OECD TG 301B:CO_2 evolution test, paraffin waxes and hydrocarbon waxes achieved 80% degradation after 28 days and is considered to be readily biodegradable.

- Measured Data (unpublished report dated 1995)
    - ECHA, 2023ay
    - Test substance: Lubricant base oil, trade name Solvent Neutral 600 Base Oil (no further details provided)

The ready biodegradability test was carried out according to OECD 301F guidelines, Manometric Respirometry. The test substance was added to an aqueous solution of mineral salts and exposed to relatively low numbers of microorganisms under aerobic conditions for a period of 28 days. A few minor deviations from the protocol of the manometric respirometer test were: No abiotic sterile or toxicity control systems were tested. Test medium was prepared on a large volume basis, aerated and aliquoted into each test container, instead of preparation in the individual test systems. The commercial phosphate buffer had a pH of 7.2 instead of 7.4. The percent biodegradability of the test substance was determined to be 31%. The test substance was determined to be

inherently biodegradable. This study received a Klimisch score of 1 and is classified as reliable without restriction because it followed well established guidelines.

Bioaccumulation (B): *vL*

Paraffin wax was assigned a hazard classification level of very low (Low Confidence) for bioaccumulation No empirical data on paraffin waxes was identified. A BCF of < 100 was identified based on modelling with EPI Suite using the SMILES notation for paraffin waxes and hydrocarbon waxes (C20 alkane and C30 alkane). Confidence in this classification is low based on modelled results.

- Estimated Data
    - Modeling (EPISuite)

	BCF from regression-based method	BAF from Arnot-Gobas upper trophic	Kow from KOWWIN Program
A: C20 alkane	94.94	9.271	10.16
B: C30 alkane	0.5	0.8936	15.07

PHYSICAL HAZARDS (PHYSICAL)

Reactivity (Rx): *L*

Paraffin wax was assigned a hazard classification level of Low (low confidence). Conifdence is low due to the lack of empirical data. However, paraffin waxes have extremely low vapor pressures and are unlikely to be explosive. Further, the substance unlikely to react exothermically with combustible materials based on its n-alkane composition.

Flammability (F): L

Paraffin wax was assigned a hazard classification of low (high confidence) for flammability based on robust test data for several related compounds.

- Measured Data
- ECHA, 2023az (unpublished report dated 2018)
    - Test substance: Paraffin waxes and hydrocarbon waxes (CAS # 8002-74-2)

In the preliminary test, the test substance melted and did not ignite and propagate combustion either by burning with flame or smoldering along 200 mm of the powder train within the 2-minute test period. Therefore, the test substance does not meet the GHS criteria for flammability. Similar results were obtained for hydrotreated paraffin wax (CAS # 64742-51-4), paraffin waxes and hydrated waxes, microcrystalline, hydrated (CAS # 92045-76-6), hydrocarbon waxes (petroleum) hydrotreated microcrystalline (CAS # 64742-60-5), paraffin waxes (petroleum), clay-treated (CAS # 64742-43-4), hydrocarbon waxes (petroleum), clay-treated microcrystalline (CAS # 64742-42-3), and paraffin waxes and hydrocarbon waxes, microcrystalline (CAS # 63231-60-7).

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