ROBUST SUMMARY
OF INFORMATION ON

Substance Group:

WAXES
And
RELATED MATERIALS

Summary prepared by: American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : Petroleum product
Physical status : Solid

Remark : This robust summary covers the waxes and related products which includes:
Slack wax
Petrolatum
Paraffin wax
Microcrystalline wax

Petroleum waxes are obtained from paraffinic refinery streams in lubricating oil manufacture.
The wax is separated by filtering a chilled solution of waxy oil in a selected solvent (usually a mixture of methyl ethyl ketone and toluene).

SLACK WAX is obtained from the dewaxing of refined or unrefined vacuum distillate fractions. If the material has been separated from residual oil fractions it is frequently called PETROLATUM.
The slack waxes are de-oiled by solvent crystallization or "sweating" processes to manufacture commercial waxes with low oil content. The oil that is separated from these processes is known as FOOTS OIL.
The refined petroleum waxes are known as PARAFFIN WAXES.
MICROCRYSTALLINE WAXES have higher molecular weights than the paraffin waxes and consist of substantial amounts of iso- and cycloalkanes.

1.2 SYNONYMS AND TRADENAMES

Remark : Paraffin wax
Slack wax
Petrolatum
Microcrystalline wax

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : TLV (US)
Limit value : 2 mg/m³

Remark : The UK HSE have established an occupational exposure limit of 2 mg/m³ (8 hour TWA) and a 15 minute Short Term Exposure Limit (STEL) of 6 mg/m³.

(2) (51)

1.13 REVIEWS

Memo : EU SCF

Remark : The EU Scientific Committee for Food (SCF) reviewed the available information on mineral hydrocarbons, which included the petroleum waxes. Their opinion was published in 1995.
The SCF reached the following conclusion:

There are sufficient data to allow a full Group ADI of 0-20 mg/kg bw for waxes conforming to the following specification: -

Highly refined waxes derived from petroleum based or synthetic hydrocarbon feedstocks, with

viscosity	not less than 11 mm³/s (cSt) at 100 °C
Carbon number	not less than 25 at the 5% boiling point
Average molecular weight	not less than 500

(47)

Memo : WHO JECFA

Remark : The WHO Joint Expert Committee on Food Additives (JECFA) reviewed the available information on food grade mineral hydrocarbons. Their evaluation was published in 1996.
With respect to waxes they made the following conclusions:

Substance	**ADI (mk/kg bw)**
Paraffin waxes	
LMPW (Low melting point wax)	ADI withdrawn
IMPW (Intermediate melting point wax)	ADI withdrawn
Microcrystalline waxes	
HSW (High sulfur wax)	0-20
HMPW (High Melting Point Wax)	0-20

(36)

Memo : CTFA

Remark : An independent expert panel reviewed data supplied to them by the Cosmetics, Toiletries & Fragrances Association (CTFA). A report of the evaluation was published in 1984. However, few experimental details are available and the conclusions of the panel cannot be verified.
Their overall conclusion was:

Toxicological test data on Ozokerite, Ceresin, Montan Wax, Paraffin, Microcrystalline Wax, Emulsifying Wax N.F., and Synthetic Beeswax are presented. Based on the documented animal and clinical test data, it is concluded that these waxes are safe for use as cosmetic ingredients in the present practices of concentration and use.

(21)

2.1 MELTING POINT

Value : 36 - 60 °C
Method : ASTM D127
Year : 1999
GLP : No data
Test substance : Petrolatum

(7) (15) (23) (38)

Value : 43 - 63 °C
Method : ASTM D127
Year : 1999
GLP : No data
Test substance : Slack wax

(7) (15) (23) (38)

Value : 43 - 68 °C
Method : ASTM D127
Year : 1999
GLP : No data
Test substance : Paraffin wax

(7) (15) (23) (38)

Value : 60 - 95 °C
Method : ASTM D127
Year : 1999
GLP : No data
Test substance : Microcrystalline wax

(7) (15) (23) (38)

2.2 BOILING POINT

Value : ca. 350 - 500 °C

Remark : In a survey of the composition of food grade waxes and oils the boiling range for paraffin wax was reported to be 350-485°C. Microcrystalline waxes boiled in excess of 500 °C. While boiling points for slack wax and petrolatum are not available, because their constituent hydrocarbons are produced from vacuum distillation, they will have boiling points above 300°C.

(12) (14)

2.3.1 GRANULOMETRY

Remark : Not relevant

2.4 VAPOUR PRESSURE

Remark : All the materials in the category are solid or semi-solid at room temperature. Any vapor pressure attributable to these materials would be from the oil component of the material (if it is present). As discussed in the Lubricating Oil Basestocks test plan, the vapor pressures of lubricating base oils are expected to be negligible and have been determined in one study to be 1.7×10^{-4} Pa.

2.5 PARTITION COEFFICIENT

Log pow : 4.7 -≥ 6.
Method : Calculated: KOWWIN Version 1.65 (EPIWIN)
Year : 2001
Test substance : Wax and related materials

Remark : As hydrocarbon number increases above C13, as is the case for the majority of the wax constituents, Log P values >6 are predicted. Substances having Log P estimates greater than 6 are characterized by extremely large molecular weight and subsequent hydrophobicity, therefore no significant aqueous exposures or bioaccumulation are expected to occur.

Result : Octanol-water partition coefficients (log P or Kow) were modeled with isomers of the lowest molecular weight component (C13 hydrocarbons) in waxes. These partitioning estimates are characteristic of only a small fraction of component molecules in a given wax. Because of the diversity of compounds encompassing waxes, it is not feasible to model the physicochemical endpoints for each potential compound. Since molecular weight and structural conformation determines in large part the solubility and vapor pressure characteristics of the hydrocarbons, modeling focused on the lower molecular weight hydrocarbons. These would be selected C13 and C20 hydrocarbons since waxes consist mostly of C20 to C85 compounds, with some minimal percent of C13 through C20 hydrocarbons. Therefore, the majority of the physicochemical modeling was performed on various paraffinic, naphthenic and aromatic representatives containing 13 and C20 carbon atoms.
The Log pow ranges from 4.7 to ≥6.7

Reliability : (2) Valid with restrictions

(44)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Value : 0.027 - 5.96 mg/l at 25 °C
Method : WSKOW Version 1.36 (EPIWIN)
Year : 2001
Test substance : Wax and related materials

Remark : The water solubility of waxes cannot be determined due to their complex mixture characteristics. Therefore, the water solubility of individual C13 hydrocarbons was modeled. The highest solubilities would be exhibited by only a small fraction of the hydrocarbon molecules present in waxes.

Increasing carbon number results in rapidly decreasing solubility, so that the most-soluble (predominantly methyl-substituted diaromatic) C18 and C20 analogues yield model values of 0.01195 and 0.00125 mg/l, respectively. Higher molecular weight (higher carbon number) components are even less water soluble. Based on water solubility modeling for C13 components of complex mixtures, aqueous solubilities of these waxes are typically much less than 1 ppm, due to differential partitioning of components between the aqueous and organic phases.

Reliability : (2) Valid with restrictions

(16)

2.8 AUTO FLAMMABILITY

Remark : Not relevant

2.9 FLAMMABILITY

Result : Non flammable

2.10 EXPLOSIVE PROPERTIES

Result : Not relevant

2.11 OXIDIZING PROPERTIES

Result : Not relevant

2.14 ADDITIONAL REMARKS

Memo : The information given in this section represent the range of values that are found for the various waxes and related products.

Remark :

Physico chemical properties for typical grades of wax and petrolatum are shown in the following table (CONCAWE, 1999). See also Bennet (1975), Kauffman et al (1993) and EWF (1990).

Melting Point (°C)	Kinematic viscosity at 100 °C	Oil content (%m/m)	Carbon number range	Penetration (25°C) (mm^2/sec)
ASTM D127	ASTM D445	ASTM D721 or D3235	ASTM D2505	ASTM D1321 or D937*
Slack wax				
45-85	3-30	2-30	12-85	9-80*
Lower Melt Paraffin Wax				
43-74	3-10	<2.5	18-75	9-50*
Microcrystalline Wax				
60-95	10-30	<5	23-85	3-60*
Petrolatum				
36-60	3-30	>10	12-85	>6

NB * The second value given for penetration was determined using method D937

(7) (15) (23) (38)

3.1.1 PHOTODEGRADATION

Type	:	Atmospheric oxidation
Method	:	Calculated: AOPWin Version 1.89 (EPIWIN)
Year	:	2001
Test substance	:	Wax and related materials
Remark	:	Although waxes typically have low vapor pressures, volatilization of some lower molecular weight components exhibit relatively high atmospheric oxidation half-lives. Therefore, those compounds that may partition to the atmosphere will be removed through indirect photochemical degradation. All modeled components exhibited rapid degradation in the atmosphere; the value presented represents both the most volatile component and the longest modeled half-life. All other modeled C13 components had both lower volatility and shorter half-lives.
Result	:	t½ = 0.913 days (10.96 hr) for most volatile C13 component modeled
Reliability	:	(2) Valid with restrictions

(43)

3.1.2 STABILITY IN WATER

Remark	:	Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. Materials in the waxes category are not subject to hydrolysis, as they lack these reactive groups.
Reliability	:	(1) Valid without restriction

(31)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	Calculated according to Mackay Level I
Media	:	Soil, air, water, suspended sediment, and sediment
Year	:	2000
Remark	:	Fugacity-based computer modeling indicated that the majority of high molecular weight hydrocarbons with carbon numbers of C20 and greater in waxes would be distributed to soil. Percent distribution estimates were modeled with C13 to C29 branched paraffins as this class of wax hydrocarbons shows the greater distribution to air. Aromatic compounds with carbon numbers from C13 through C85 will partition principally to soil. Linear paraffins and naphthenes distribute to both soil and air, with increasing partitioning to soil for hydrocarbons greater than C20 as vapor pressure decreases. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. The default model assumptions were used when performing the fugacity estimates. Since the majority of hydrocarbon components in waxes are primarily normal paraffins of C20

and greater, with moderate to minimal amounts of naphthenics, isoparaffins and trace amounts of aromatics, volatility is not a significant fate process for these petroleum substances due to negligible vapor pressures at ambient temperatures and their high molecular weight. As hydrocarbon number increases above C20, partitioning to soil is the predominant behavior of these compounds.

Result : **Carbon No. Isoparaffin** **% Distribution**

	Air	Soil	Water	Sediment	Susp. Sediment	Biota
C13	98	1.9	$7E^{-3}$	$4E^{-2}$	$8E^{-3}$	$1E^{-4}$
C18	69	30	$4E^{-4}$	0.68	$2E^{-2}$	$2E^{-3}$
C20	33	65	$2E^{-5}$	1.4	$3E^{-2}$	$4E^{-3}$
C21	18	80	$5E^{-6}$	1.8	$5E^{-2}$	$4E^{-3}$
C22	12	86	$2E^{-6}$	1.9	$6E^{-2}$	$4E^{-3}$
C24	6	92	$2E^{-7}$	2.1	$6E^{-2}$	$5E^{-3}$
C26	1	97	$2E^{-8}$	2.1	$7E^{-2}$	$5E^{-3}$
C29	0.1	98	$9E^{-10}$	2.2	$7E^{-2}$	$6E^{-3}$

Reliability : (2) Valid with restrictions

(41)

3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Oil-contaminated soil from land-farming project
Contact time : 84 day(s)
Result : 80% in 28 days; inherently and extensively biodegradable
Deg. Product : No
Method : Modified OECD 301B (significant modification, actually shake flask test)
Year : 1989
GLP : Yes
Test substance : Paraffin wax CAS 8002-74-2

Remark : Paraffin wax residue analysis showed less than 10% parent hydrocarbons and some hydrocarbon enrichment from contaminated soil inoculum after 28 days.

Result : Degradation % after time 80% of $ThCO_2$ after 28 days;
87% after 84 days (paraffins)

66% of $ThCO_2$ after 28 days;
77% after 84 days (intermediate wax)

Kinetic (for sample, positive and negative controls)
Reference (sodium acetate) - Not Reported
Test substance - 80% (paraffin, 28 days),
66% (intermediate wax, 28days)

Breakdown Products No other than residual HCs

Test condition : Inoculum: Soil was collected from land-farm used by the investigators to treat oil-contaminated soil. Soil contained 2200 mg/kg mineral oil (generally at greater retention times than wax components, based on chromatograms provided in report), and was a sandy loam comprising 68% sand, 14.2% clay and 10.2% silt with 5.4% OC. Elevated levels of heavy metals were measured in the soil but not considered to be inhibitory to the test. Soil was suspended in mineral medium prior to distribution to test vessels at a loading rate of approximately 80 mg/l. No microbial enumeration was

undertaken but performance of the inoculum in degrading a reference standard (sodium acetate at 100 mg/l) provided evidence of inoculum adequacy.

Concentration of test chemical: Test substance loading was approximately 20 mg/l of medium.

Temp of incubation: 20 ±2ºC

Dosing procedure: Each 2-liter vessel contained 1 liter of inoculated medium. The wax was dissolved in heated carbon tetrachloride, then the solution applied to glass fiber filters (13 mm) to obtain about 20 mg wax/filter after evaporation of the solvent. One filter was added to each test material vessel. Controls and reference standards also received glass fiber filters to which CCl_4 was added and allowed to evaporate.

Sampling frequency: Carbon dioxide production was monitored weekly through day 28, and then every other week to day 84. Wax residues were measured only at test termination.

Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium acetate was used as the positive control.

Analytical method: Carbon dioxide production was measured by titrating residual base with 0.1 N HCl. Wax residues were measured by extracting filters with warm heptane and the volume of extract adjusted prior to GC-FID analysis.

Method of calculating biodegradation: Wax was assumed to have a mean composition of $[CH_2]$ for the purpose of calculating $ThCO_2$ (3.14 mg CO_2/mg wax). The report does not include the mechanics of calculation of the mineralization endpoint. Total hydrocarbon remaining at 84 days was determined by area integration of the chromatograms, and primary biodegradability was determined by comparing the amount of hydrocarbons at the end of the test with the amount on wax-dosed filters prepared at the start of the test.

Other: Two grades of paraffin wax, 52/50 and 58/60 were tested; only the 52/50 grade was tested for 84 days, and in all, three tests were carried out for 52/50. Result below for 28 days is mean of 52/50 average and 58/60 result. An intermediate wax was also tested as noted in results.

Test substance was incubated in the inoculated mineral medium in sealed vessels containing a vial of 0.4 M NaOH (5 ml) suspended in the headspace above the medium (similar to EPA 835-3100). Carbon dioxide evolution resulting from mineralization of the test substance was trapped in the base for periodic quantitation. Base was renewed at each sampling period. GC analysis for parent compound was carried out on the solid phase of the test medium at study termination.

Reliability : (2) Valid with restrictions

(30)

Type	:	Aerobic
Inoculum	:	Oil-contaminated soil from land-farming project
Contact time	:	84 day(s)
Result	:	Inherently biodegradable
Method	:	Modified OECD 301B (significant modification)
Year	:	1989
GLP	:	Yes
Test substance	:	Microcrystalline wax CAS 63231-60-7
Remark	:	Wax residue analysis showed 65% parent hydrocarbons (mostly n-alkanes greater than C43) remained after 84 days. Most iso-alkanes were degraded regardless of carbon number.
Result	:	Degradation % after time: 21% of $ThCO_2$ after 28 days; 25% after 84 days Kinetic (for sample, positive and negative controls: Reference (sodium acetate) -Not Reported Test substance - 21% (28d) Breakdown Products: None
Test condition	:	Inoculum: Soil was collected from land-farm used by the investigators to treat oil-contaminated soil. Soil contained 2200 mg/kg mineral oil (generally at greater retention times than wax components, based on chromatograms provided in report), and was a sandy loam comprising 68% sand, 14.2% clay and 10.2% silt with 5.4% OC. Elevated levels of heavy metals were measured in the soil but not considered to be inhibitory to the test. Soil was suspended in mineral medium prior to distribution to test vessels at a loading rate of approximately 80 mg/l. No microbial enumeration was undertaken but performance of the inoculum in degrading a reference standard (sodium acetate at 100 mg/l) provided evidence of inoculum adequacy. Concentration of test chemical: Test substance loading was approximately 20 mg/l of medium. Temp of incubation: 20 ± 2ºC Dosing procedure: Each 2-liter vessel contained 1 liter of inoculated medium. The wax was dissolved in heated carbon tetrachloride, then the solution applied to glass fiber filters (13 mm) to obtain about 20 mg wax/filter after evaporation of the solvent. One filter was added to each test material vessel. Controls and reference standards also received glass fiber filters to which CCl_4 was added and allowed to evaporate. Sampling frequency: Carbon dioxide production was monitored weekly through day 28, then every other week through day 84. Wax residues were measured at test termination. Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium acetate was used as the positive control. Analytical method: Carbon dioxide production was measured by titrating residual base with 0.1 N HCl. Wax residues were measured by extracting filters with warm heptane and the volume of extract adjusted prior to GC-FID analysis.

Method of calculating biodegradation: Wax was assumed to have a mean composition of [CH_2] for the purpose of calculating $ThCO_2$ (3.14 mg CO_2/mg wax). The report does not include the mechanics of calculation of the mineralization endpoint. Total hydrocarbon remaining at test termination was determined by area integration of the chromatograms, and primary biodegradability was determined by comparing the amount of hydrocarbons at the end of the test with the amount on wax-dosed filters prepared at the start of the test.

Other: Test substance was incubated in the inoculated mineral medium in sealed vessels containing a vial of 0.4 M NaOH (5 ml) suspended in the headspace above the medium (similar to EPA 835-3100). Carbon dioxide evolution resulting from mineralization of the test substance was trapped in the base for periodic quantitation. Base was renewed at each sampling period. GC analysis for parent compound was carried out on the solid phase of the test medium at study termination.

Reliability : (2) Valid with restrictions

(30)

Type : Aerobic
Inoculum : Naturally-occurring leaf-litter and soil biota (microbes and invertebrates)
Contact time : 6 month
Year : 1989
GLP :
Test substance : CAS 8002-74-2 and CAS 63231-60-7

Result : Decomposition in the 5 mm mesh bag, which were exposed to invertebrates as well as microbes, proceeded at a higher rate than in the 45 µm bags. Decomposition in the 5 mm mesh bags was nearly complete within 13 weeks in the autumn/winter test and within 26 weeks in the spring/summer test, while in the 45 µm bags 25 - 50% was still left after 6 months, based on visual observation. Wax residue analyses also indicated more rapid degradation in the cold-weather experiment.

Waxed and non-waxed (control) paper decomposed at the same rate.

Paraffin wax residue analysis showed after 6 months a complete or nearly complete degradation of the samples in the 5 mm mesh bags (the 52/54 paraffin wax showed 10% residues remaining after the spring/summer experiment and 0% after the autumn/winter experiment.

In the 45 µm bags, wax residues remaining at the end of the summer exposure were 30 - 50% for the paraffins and intermediate wax, and 60% for the microcrystalline wax. After winter exposure, paraffin wax residues were 10 - 30% of initial, intermediate wax is reported as 80% of initial, and microcrystalline wax residues were 40% of initial. The winter value for the intermediate wax appears incorrect based on the chromatograms, which show smaller peaks for the winter vs the summer analyses (same scale for both).

Test condition : Inoculum: Waxed paper was placed in nylon bags of different mesh size (45 µm or 5 mm) to allow colonization by either microbes alone or by microbes and soil fauna. Leaf litter

served as the source of the inoculum, and was placed in a layer over the mesh bags at the start of the test.

Concentration of test chemical: Approximately 20 mg of wax per mesh bag.

Temp of incubation: Ambient forest litter layer temperatures. Testing was carried out during two different seasons: spring/summer (April - October 1989) and autumn/winter (November 1989 - May 1990)

Dosing procedure: Each mesh bag contained four 2 x 2 cm squares of waxed paper, which were dried and weighed before they were placed in the bags. The squares were arranged in a single layer within the bags (10 x 10 cm) to avoid sticking together.

Sampling frequency: Samples were retrieved monthly and decomposition of the squares was estimated visually. The remaining sample material was then removed from the bags, cleaned, dried (50 ºC) and weighed.

Controls: Non-waxed paper was used as a negative control.

Analytical method: 1) physical decomposition of paper: Each piece of paper was assessed visually according to the scale 100%, 75%, 50%, 25%, 5%, and 0% decomposition. 2) Wax residues were measured by extracting paper with warm heptane and the volume of extract adjusted prior to GC-FID analysis. To prevent interference of the analysis by the mesh bags, soil particles, and base paper, a cleanup step with aluminum oxide was used and as much of the bag material as possible was removed before extraction. The squares (or remnants thereof) from each treatment were pooled before extraction.

Method of calculating biodegradation: The extent of paper decomposition was determined by averaging the visual percent decomposition scores of the four squares. The degradation of the wax was calculated from the analysis of samples taken at the start of the test, combined with analyses of uncoated paper and of field blanks for determination of background interference. Weight differences were not used as artifacts such as soil particles could not be removed from the waxed surfaces without removing the wax or destroying the paper.

Other: Two grades of paraffin wax, 52/50 and 58/60, intermediate wax, and microcrystalline wax were tested.

Conclusion : Waxed paper decomposes at about the same rate as unwaxed paper. Soil invertebrates contribute significantly to the decomposition of waxed paper in leaf litter. Decomposition of waxed paper occurs more rapidly during the autumn/winter, when there is a fresh layer of leaf litter on the ground, than during the spring/summer, when the last fall's leaf litter has been largely reduced to humus.

Reliability : (2) Valid with restrictions, since positive control data not reported

(29)

Type	:	Aerobic
Inoculum	:	Unacclimated domestic sewage sludge supernatant and forest soil
Contact time	:	137 day(s)
Deg. Product	:	No
Method	:	Shake flask test
Year	:	1989
GLP	:	No data
Test substance	:	Paraffin wax CAS 8002-74-2

Result :

Degradation % after time: 55 % of $ThCO_2$ after 31 days; 98.5% after 137 days

Kinetic (for sample, positive and negative controls):
Reference (cellulose) 88.7% after 31 days
Test substance - 55% (31d); 98.5% (137 d)

Test condition :

Inoculum: Soil was collected from a state park in central NJ, and sewage sludge was obtained from a domestic sewage treatment plant in Pennington, NJ. The sludge was aerated for 30 minutes and allowed to settle for an additional 30 minutes before the supernatant was withdrawn and filtered through #1 filter paper prior to use as the sewage inoculum. Filtrate was used at a rate of 25 ml/l of test medium (2.5%). Soil was added directly to each test flask at a rate of 0.1 g/l.
Concentration of test chemical: Test substance loading was approximately 10 mg carbon/l of medium.

Temp of incubation: 25 ± 2 ºC

Dosing procedure: Test material was added by direct addition of 11.8 mg grated wax to each test flask. Reference material (cellulose) was also weighed (25 mg) and added to the reference flasks to provide 10 mg C/l.

Sampling frequency: Carbon dioxide production was monitored after 2, 4, 7, 10, 17, and 24 days, and approximately weekly thereafter through day 137.

Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Cellulose was used as the positive control.

Analytical method: Carbon dioxide produced by mineralization of the test substances was absorbed in 0.2 N KOH solution in cuvettes in the headspace of the test vessels. CO_2 production was measured by titrating residual base with 0.2N HCl.

Method of calculating biodegradation: Wax was assumed to contain 85% carbon for the purpose of calculating $ThCO_2$ wax). Average titration volumes at each sampling point were corrected for average blank volumes and then the amount of carbon dioxide produced was divided by $ThCO_2$ to determine percent biodegradation.

Conclusion : Not readily biodegradable; inherently biodegradable and extensively biodegradable in long-term exposures

Reliability : (2) valid with restrictions. Unable to determine GLP status. Study report is in the form of a memo from which some details are lacking. Same details (e.g., temperature log) are also lacking from the raw data provided with the report
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(5)

Type	:	Aerobic
Inoculum	:	Unacclimated domestic sewage sludge supernatant and forest soil
Contact time	:	137 day(s)
Result	:	Extensively biodegraded in long-term test
Deg. Product	:	No
Method	:	Shake flask test
Year	:	1989
GLP	:	No data
Test substance	:	Microcrystalline wax CAS 63231-60-7

Result :

Degradation % after time: 27 % of $ThCO_2$ after 31 days;
67.2% after 137 days

Kinetic (for sample, positive and negative controls):
Reference (cellulose) 88.7% after 31 days
Test substance - 27% (31d);
67.2% (137 d)

Test condition :

Inoculum: Soil was collected from a state park in central NJ, and sewage sludge was obtained from a domestic sewage treatment plant in Pennington, NJ. The sludge was aerated for 30 minutes and allowed to settle for an additional 30 minutes before the supernatant was withdrawn and filtered through #1 filter paper prior to use as the sewage inoculum. Filtrate was used at a rate of 25 ml/l of test medium (2.5%). Soil was added directly to each test flask at a rate of 0.1 g/l.
Concentration of test chemical: Test substance loading was approximately 10 mg carbon/l of medium.

Temp of incubation: 25 ± 2 ºC

Dosing procedure: Test material was added by direct addition of 11.8 mg grated wax to each test flask. Reference material (cellulose) was also weighed (25 mg) and added to the reference flasks to provide 10 mg C/l.

Sampling frequency: Carbon dioxide production was monitored after 2, 4, 7, 10, 17, and 24 days, and approximately weekly thereafter through day 137. Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Cellulose was used as the positive control.

Analytical method: Carbon dioxide produced by mineralization of the test substances was absorbed in 0.2 N KOH solution in cuvettes in the headspace of the test vessels. CO_2 production was measured by titrating residual base with 0.2N HCl.

Method of calculating biodegradation: Wax was assumed to contain 85% carbon for the purpose of calculating $ThCO_2$ wax). Average titration volumes at each sampling point were corrected for average blank volumes, then the amount of carbon dioxide produced was divided by $ThCO_2$ to determine percent biodegradation.

Reliability : (2) Valid with restrictions. Unable to determine GLP status. Study report is in the form of a memo from which some details are lacking. Same details (e.g., temperature log) are also lacking from the raw data provided with the report
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(5)

Inoculum	:	Activated sludge, domestic
Contact time	:	28 day(s)
Method	:	OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year	:	1995
GLP	:	Yes
Test substance	:	Slack wax (petroleum), hydrotreated CAS 92062-09-4

Result : By day 28, 40% degradation of the test material was observed and indicated that the test material was inherently biodegradable. By day 5, >60% biodegradation of positive control was observed, which meets the guideline requirement. No excursions from the protocol were noted.Biodegradation was based on net oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
SN 60	50.20, 34.54, 33.92	39.55
Na Benzoate	82.04; 72.88	77.46

* replicate data

Test condition : Fresh activated sludge was obtained one day prior to test initiation, and homogenized in a blender for two minutes.
After allowing the sample to settle for approximately 30 minutes, the homogenated supernatant was decanted, avoiding carry-over of solids. Microbial activity of an aliquot of the filtered supernatant was $1E^6$ CFU/ml which was determined using microbial agar dip slides. Activated sludge supernatant was added to the test medium at 10 ml/l, and the inoculated medium was continuously aerated with CO_2-free air until the next day when the test systems were prepared.
Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride). Test vessels were 1L glass flasks located in a waterbath and electronically monitored for oxygen consumption. Test material was tested in triplicate, controls and blanks were tested in duplicate. Test material (Slack wax (petroleum), hydrotreated) concentration was approximately 37 mg/l, equivalent to a theoretical oxygen demand (ThOD) of 127 mg/l. Test material was weighed onto a Gelman type A/E 13 mm glass fiber filter, which was then added to each respirometer flask. Sodium benzoate (positive control) concentration was 53.54 mg/l, and was added using an aliquot of a stock solution.
Test temperature was 22 ±1 °C. All test vessels were stirred constantly for 28 days using magnetic stir bars and plates.

Remark Although this specific slack wax process stream is not among the HPV-sponsored materials in this category, the hydrotreating procedure (i.e., removal of sulfur) does not substantially alter the component hydrocarbon character from the source slack wax material (CAS No. 64742-61-6).

Reliability : (1) Valid without restriction

(25)

Type	:	Aerobic
Inoculum	:	Domestic sewage, non-adapted
Concentration	:	20 mg/l related to Test substance
Method	:	OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year	:	1984
GLP	:	No data
Test substance	:	Two materials were tested White mineral oil CAS 8042-47-5 Technical white oil CAS 8042-47-5 The test materials were not characterized any further
Remark	:	To assist in the evaluation of petrolatum and slack waxes, information on two white oils is included in this robust summary
Result	:	Degradation after 28 days was 0% for the white oil 24% for the technical white oil

(6) (45)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Remark : See remarks in section 4.9 below
27.12.2001

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Remark : See remarks in section 4.9 below

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Remark : See remarks in section 4.9 below

4.9 ADDITIONAL REMARKS

: The physical size and number of carbon atoms in petroleum waxes and related materials severely limits the ability of these materials to be taken up into living organisms. It is accepted that the ecotoxicity of alkanes of carbon number greater than C10 are not acutely toxic to aquatic organisms at their limit of solubility in water (Adema, 1986). The petroleum waxes, containing hydrocarbons greater than C13, would not be expected to cause acute toxicity to aquatic organisms. The results of toxicity tests with lubricant base oils, which have similar hydrocarbon ranges and some structures in common, show no acute toxicity to freshwater fish, invertebrates, or algae and no chronic effects to aquatic life at concentrations below 1 mg/l. (CONCAWE, 1997) Representative acute toxicity data for selected base oils presented in the CONCAWE (1997) review are shown below

Test species	Exposure method*	Endpoint**	Value (mg/l)
Hydrotreated, heavy paraffinic distillate			
Fish: Oncorhyncus mykiss	OWD	96h LL_{50}	>6 000
Solvent refined, heavy paraffinic distillate			
Fish: Oncorhyncus mykiss	OWD	96h LL_{50}	>1 000
White mineral oil			
Fish: Oncorhyncus mykiss	OWD	96h LL_{50}	>10 000
Solvent refined, light naphthenic distillate			
Invertebrate: Daphnia magna	WAF	48h EL_{50}	>10 000

Solvent refined, heavy paraffinic distillate
Alga: Scenedesmus subspicatus

WAF	96h i_rL_{50}	>1 000
	96h i_vL_{50}	>1 000

Solvent refined residual oil
Alga: Scenedesmus subspicatus

WAF	96h i_rL_{50}	>1 000
	96h i_vL_{50}	>1 000

* OWD = Oil-Water dispersion
WAF = Water Accomodated Fraction

** LL_{50} = Lethal loading rate required to kill 50% of test organisms
EL_{50} = Effective loading rate required to immobilize 50% of the test organisms
i_rL_{50} = Inhibitory loading rate required to reduce algal growth rate by 50%
i_vL_{50} = Inhibitory loading rate required to reduce area under growth curve (biomass) by 50%

(4) (14) (16)

: The values of log Kow for individual hydrocarbons increase with increasing carbon number within homologous series of generic types. Quantitative structure activity relationships (QSAR), relating log Kow values of single hydrocarbons to toxicity, show that water solubility decreases more rapidly with increasing Kow than does the concentration causing effects (Abernathy, et al, 1988; Donkin, et al, 1991). This relationship varies somewhat with species, but it follows that there is a log Kow limit for hydrocarbons, above which, they will not exhibit acute toxicity; this limit is at a log Kow value of about 4 to 5 (Abernathy, et al, 1988; Donkin, et al, 1991). It has been confirmed experimentally that for fish and invertebrates, paraffinic hydrocarbons with a carbon number of 10 or higher (log Kow >5) show no acute toxicity (Adema, 1986) and that alkylbenzenes with a carbon number of 14 or greater (log Kow >5) similarly show no acute toxicity (Adema, 1991) From these well-demonstrated solubility 'cut-offs' for acute toxicity of hydrocarbon substances, which directly relate to their physico-chemical properties, it is clear that the same should hold for complex petroleum substances. QSAR equations for chronic toxicity also suggest that there should be a point where hydrocarbons with high log Kow values become so insoluble in water that they will not cause chronic toxicity, that is, that there is also a solubility cut-off for chronic toxicity (McCarty, L.S. et al, 1991; European Union,1996). Thus, paraffinic hydrocarbons with carbon numbers of greater than 14 (log Kow >7.3) should show no measurable chronic toxicity. The existence of this cut-off for chronic toxicity is supported for petroleum substances by the numerous chronic toxicity studies reported on lubricant base oils, which demonstrate that for these substances which are composed primarily of alkanes and naphthenes of C15 and greater, no evidence of chronic toxicity is seen (Concawe, 1997). Further evidence to support this generalisation is provided by a lack of chronic toxicity for hydrocarbon based solvents (CEFIC, 2000)
Representative chronic aquatic toxicity data for selected base oils

presented in the CONCAE (1997) review are shown below

Test species	Exposure method*	Endpoint	Value (mg/l)
Hydrotreated, heavy paraffinic distillate			
Fish: Pimephales promelas			
	OWD	Reproduction/ surviva	>1 000
Solvent dewaxed, heavy paraffinic distillate			
Fish: Pimephales promelas			
	OWD	Reproduction/ survival	>5 000
Solvent refined, heavy paraffinic distillate			
Invertebrate: Daphnia magna			
	WAF	Reproduction/ survival	>1 000
Hydrotreated, heavy paraffinic distillate			
Invertebrate: Ceriodaphnia dubia			
	WAF	Reproduction/ survival	>5 000
Hydrotreated, light naphthenic distillate			
Invertebrate: Daphnia magna			
	WAF	Reproduction/ survival	>1
Solvent refined residual oil			
Invertebrate: Daphnia magna			
	WAF	Reproduction/ survival	>1 000

* OWD = Oil-Water dispersion
WAF = Water Accomodated Fraction

(1) (3) (4) (11) (14) (19) (22) (42)

: In February of 2001 discharge of slack wax to national parks along British Columbia (Canada) coastline occurred during tank washing activities, impacting approximately 100 km of Pacific Rim National Park beach. Canadian Wildlife Service (a branch of Environment Canada) and the Department of Fisheries and Oceans biologists agreed that the risk of acute toxicity to aquatic life in the area was minimal based on the low solubility of the components in the wax and given that the BC Parks staff observed no significant environmental impacts. Generally the consensus was that the material was relatively inert and would likely pose little environmental damage.

(24)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	No data
Sex	:	Male/female
Number of animals	:	10
Vehicle	:	Arachis oil
Year	:	1976
GLP	:	No data
Test substance	:	R 9071 is described as paraffin wax, without further characterization. R 9071 was prepared as solutions in arachis oil for oral dosing. Two concentrations (20 and 100 mg/ml) were prepared for the two dose levels tested.
Method	:	Paraffin wax was administered orally as a solution in arachis oil to groups of 5 male and 5 female rats at dose levels of 1 and 5 g/Kg. The rats were observed for clinical signs of toxicity for the following 7 days. On the seventh day the animals were weighed, then killed and autopsied.
Result	:	There were no clinical signs of toxicity during the seven day observation period and growth rates were normal. There were no mortalities and no macroscopic changes were observed at autopsy. The LD_{50} was found to be greater than 5g/Kg.
Reliability	:	(1) Valid without restriction. Although there is no indication that the study was carried out according to GLP, it nevertheless is a reliable study and full details are provided in the laboratory report.

(34)

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	No data
Sex	:	Male/female
Number of animals	:	10
Vehicle	:	Arachis oil
Year	:	1976
GLP	:	No data
Test substance	:	R 9269 is described as microcrystalline wax, without further characterization. R 9269 was prepared as solutions in arachis oil for oral dosing. Two concentrations (20 and 100 mg/ml) were prepared for the two dose levels tested.
Method	:	Microcrystalline wax was administered orally as a solution in arachis oil to groups of 5 male and 5 female rats at dose levels of 1 and 5 g/Kg. The rats were observed for clinical signs of toxicity for the following 7 days. On the seventh day the animals were weighed, then killed and autopsied.
Result	:	There were no clinical signs of toxicity during the seven day observation period and growth rates were normal. There were no mortalities and no macroscopic changes were observed at autopsy. The LD_{50} was found to be greater than 5g/Kg.
Reliability	:	(1) Valid without restriction.

Although there is no indication that the study was carried out according to GLP, it nevertheless is a reliable study and full details are provided in the laboratory report.

(35)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD_{50}
Value	:	> 4000 mg/kg bw
Species	:	Rabbit
Strain	:	No data
Sex	:	No data
Vehicle	:	Petrolatum
Year	:	1972
GLP	:	No
Test substance	:	Paraffin wax administered as a 50% solution in petrolatum
Method	:	Method is not described.
Remark	:	The report does not provide sufficient information to fully evaluate the study.
Reliability	:	(4) Not assignable This information is taken from a published safety review conducted by an expert panel. Few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified.

(21)

5.2.1 SKIN IRRITATION

Species	:	Rabbit
Concentration	:	Undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	9
Result	:	Not irritating
Year	:	1984
GLP	:	No data
Test substance	:	Paraffin wax and Microcrystalline wax
Remark	:	An expert panel on cosmetics reviewed the skin irritation data and reported: * An undiluted paraffin wax was non-irritant in a 24 hour occluded patch test in rabbits * A microcrystalline wax was slightly irritating in a 24 hour occluded patch test
Result	:	The report contains the following statement: A sample of 100% paraffin wax was applied full strength under a single closed patch to the skin of 9 rabbits. No irritation developed. Three samples of 50% paraffin in petrolatum were tested in repeated, open patch applications to 6 rabbits. Two samples produced erythema in four animals that lasted three days, and one produced erythema in one rabbit that lasted two days. No other details are provided.
Reliability	:	(4) Not assignable. This information is taken from a published safety review

conducted by an expert panel. Few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified
.

(21)

5.2.2 EYE IRRITATION

Species	:	Rabbit
Concentration	:	50 %
Dose	:	0.1 ml
Exposure time	:	72 hour(s)
Comment	:	Not rinsed
Number of animals	:	6
Vehicle	:	Petrolatum
Result	:	Slightly irritating
Year	:	1984
GLP	:	No data
Result	:	The publication states: Four 50% solutions of paraffin in petrolatum were each instilled into the eyes of six albino rabbits with no rinse. Eyes were observed for irritation for three days. Two of the samples caused mild irritation in one rabbit on day 1; the other samples were not irritating.
Reliability	:	(4) Not assignable. This information is taken from a published safety review conducted by an expert panel. Few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified.

(21)

5.4 REPEATED DOSE TOXICITY

Species	:	Rat
Sex	:	Male/female
Strain	:	Fischer 344
Route of admin.	:	Oral feed
Exposure period	:	90 days
Frequency of treatm.	:	Continuous in food
Post exposure period	:	28 days
Doses	:	0.002, 0.02, 0.2 & 2.0% in the diet
Control group	:	Yes, concurrent no treatment
Method	:	OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year	:	1992
GLP	:	Yes

Test substance : This study was carried out on six mineral oils and three petroleum waxes (a paraffin wax and two microcrystalline waxes). Only information on the waxes is included in this robust summary. For additional details on the oils, see the Lubricating Oil Basestocks Test plan.

The waxes were:

Paraffin wax

LMPW A hydrotreated low melting point paraffin wax

Microcrystalline waxes

HSW A clay-treated microcrystalline wax (High Sulfur Wax)

HMPW Hydrotreated microcrystalline wax, high melting point (High Melting Point Wax)

The characteristics of the three waxes are as follows (CONCAWE, 1993)

Property	Unit	Method (ASTM)	LMPW	HSW	HMPW
Color		D1550	L0.5	L0.5	L0.5
Penetration at 25°C	0.1 mm	D1321	18	27	13
Penetration at 40°C	0.1 mm	D1321	83	101	29
Congealing point	°C	D938	53.5	74.5	85.0
Drop meltingpoint	°C	D127	55.6	82.0	91.4
Oil content	%	D721	0.1	1.8	1.3
Distillation ranges					
	°C	D86			
	5%		369	411	510
	50%		414	551	564
	95%		467	698	721
Viscosity at 100 °C	mm^2/s	D445	3.3	13.7	15.4
Density at 100 °C	kg/m^3	D1298	751.5	794.4	789.2
Ash content	%	D482	<0.01	0.01	<0.01
Refractive index at 100 °C		D1747	1.4230	1.4404	1.4393
Sulfur	ppm	D2622	5	2100	77
Acidity/alkalinity		USP XXIII	----------Pass----------		
UV absorbance		FDA 172.806	----------Pass----------		
Arsenic	ppm	AAS	<1	<1	<1
Chromium	ppm	AAS	<1	<1	<1
Cadmium	ppm	AAS	<1	<1	<1
Lead	ppm	AAS	<1	<1	<1
Carbon no. distribution		EWF/GC	19-42	20-74	22-80

Method : The study consisted of three components each of which is described below.

Main study
Groups of 20 male and 20 female rats were fed diets containing one of three different waxes at dietary concentrations of 0.002, 0.02, 0.2 & 2.0 % for 90 days. Groups of 60 male and 60 females were fed untreated control diet for the same period of time.
A further group of 20 rats of each sex were fed diets containing 2.0 % coconut oil.

Reversal study
Groups of ten rats of each sex were fed diets containing each test material at the 2.0 % level or coconut oil at the 2 % level for 90 days, followed by a 28 day period on control diet. Groups of 300 rats of each sex were fed control diet for the same time period.

Tissue level and reversal study
Groups of ten rats of each sex were fed either control diet, or diet containing 2 % of each of the test materials or coconut oil at 2 % for 90 days. At the end of the 90-days, five rats of each sex were sacrificed and their tissues analyzed for mineral hydrocarbons. The remaining five animals of each sex were then fed control diet for a further 28 days, at the end of which they also were sacrificed and their tissues analyzed for mineral hydrocarbons.

The entire study consisted of 40 different treatment groups and their organization is summarized in the following table.

Group	Treatment*	Main M/F	Reversal M/F	Tissue level and reversal M/F
1	Control	20/20	10/10	10/10**
2	Control	20/20	10/10	
3	Control	20/20	10/10	
4-27 incl.	groups fed diets containing the mineral oils			
28	LMPW (0.002%)	20/20	10/10	10/10
29	LMPW (0.02%)	20/20	10/10	
30	LMPW (0.2%)	20/20	10/10	
31	LMPW (2.0%)	20/20	10/10	
32	HMPW (0.002%)	20/20	10/10	10/10
33	HMPW (0.02%)	20/20	10/10	
34	HMPW (0.2%)	20/20	10/10	
35	HMPW (2.0%)	20/20	10/10	
36	HSW (0.002%)	20/20	10/10	10/10
37	HSW (0.02%)	20/20	10/10	
38	HSW (0.2%)	20/20	10/10	
39	HSW (2.0%)	20/20	10/10	
40	Coconut oil (2.0%)	20/20	10/10	10/10

* For a description of each wax see "test substance" section

** 5 animals were for tissue level analysis after 90 days and five were for tissue level after a 28 day reversal period.

All animals were monitored for weight, food intakes and

clinical condition throughout the study. An ophthalmic examination was performed prior to treatment and prior to necropsy on the animals in the main study and those for the study of reversibility.

Necropsy

Main study and reversal animals

A full necropsy was performed and any abnormalities were recorded. The following organs were weighed:
adrenal glands
brain
caecum (with and without contents
heart
kidney
liver ovaries
spleen
testes
thymus.

Samples of the following tissues were fixed for subsequent microscopic examination:
adrenal glands, artery (aorta), bladder, brain, caecum, colon, cervix uteri, diaphragm, duodenum, epididymis, extra orbital lachrymal glands, eye, femur, Harderian gland, heart, ileum (including Peyer's patches), jejunum, kidneys, liver (representative samples from each lobe), lungs, (with main stem bronchi), lymph nodes (axillary, cervical & mesenteric), mammary gland (inguinal region), nasal bones, nerve (sciatic taken together with surrounding muscle), oesophagus, ovaries, pancreas, perirenal fat, pinnae (retained for identification only), pituitary, prostate, rectum, salivary gland, seminal vesicles, skeletal muscle, skin (inguinal region), spinal cord, spleen, sternum, stomach, testes, thymus, thyroid/parathyroid glands (retained on trachea), tongue, uterine horns, vagina and vein (posterior vena cava).
In addition, samples of the following tissues from the high dose and control animals only were retained in formol calcium: liver, spleen, small intestine & mesenteric lymph node.

Histological examination of tissues
A microscopic examination was made of H&E sections of all preserved tissues from the control and high dose group and from the lung, liver, kidney, spleen, small intestine and mesenteric lymph node of all other groups. All lung sections were examined for evidence of infection.

Hematology
Blood samples collected from all animals on the main study and the reversal study were examined for: total erythrocyte count, total leucocyte count, hemoglobin concentration, mean cell volume, hematocrit (by calculation), platelet count, differential leucocyte count, reticulocyte count and prothrombin time.

Clinical chemistry
Serum from main and reversal study animals was examined for: concentrations of glucose, urea, total protein, albumin, creatinine, calcium, phosphorus (as phosphate), chloride,

total bilirubin, sodium and potassium. Activity of the following enzymes was also determined: alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and gamma glutamyl transferase.

Tissue level and tissue level reversal animals
Animals designated to provide tissues for analysis for mineral hydrocarbons were killed and the following tissues weighed and taken for analysis:
Liver (random samples from the periphery of all lobes)
Mesenteric lymph nodes (all tissue)
Kidney (one kidney)
Spleen (approximately half)
Perirenal fat (random sample)

Tissue analysis for mineral hydrocarbon content
Tissue samples (approximately 1 g of tissue) from those animals designated for tissue analysis were homogenized in 70 % KOH solution. The homogenate was sonicated for 10 minutes at 60 °C. CCl_4 was added to each sample and sonicated for 30 minutes, also at 60 °C, occasionally mixing by hand. The layers were separated using centrifugation if necessary.
An aliquot of the lower organic phase was poured onto an extraction column (Florosil) and the eluate was collected and the column washed with CCl_4 to a known final volume. The infra-red absorbance, in the C-H stretching region, of the eluate was measured against a CCl_4 background using a Fourier Transform infra-red spectrometer. The concentration of mineral hydrocarbon in the tissue was calculated by comparison with appropriate standards.

Statistical analysis
The continuous variable data from the control and test groups were tested for normality using the Kolmogorov-Smirnov (K.S.) test and homogeneity of variance using Bartlett's test.
Statistical significance was determined to be at $p<0.05$ in a K.S. test and at $p<0.01$ in a Bartlett's test. If both tests were non significant, the control and test groups were compared using analysis of variance followed by the least significant difference (L.S.D.) test.
If either test produced a significant result, a suitable transformation was attempted. If the transformation data resulted in a non-significant Bartlett's test but a significant K.S. test, the Wilcoxon Mann-Whitney test was used. If the transformed data resulted in a non-significant K.S. test but a significant Bartlett's test, an appropriate t-test was used, based on whether a pooled variance was suitable or not.
If no suitable transformation could be made, one of the above tests was selected as the most appropriate based on the nature and distribution of the data.
Where levels of significance were reported in the tables for transformed data the means and standard deviations were reported for the untransformed data.
The results of the Mann-Whitney and t-tests were compared with the L.S.D. test. In most cases, the L.S.D test was reported. However, if large differences were evident, other test results were reported as appropriate unless the data

was deemed to be highly variable and there was no evidence to justify the removal of outliers.
Incidence data from the histopathogical examination was tested for differences between treated and control animals using Fischer's exact test. Mann-Whitney tests were performed on incidence data graded by severity.
In all test comparisons, a probability level of $p<0.05$ in a two sided test was taken to indicate statistical significance.

Result : **Main study**

Microcrystalline waxes (HSW and HMPW)

Growth rates, food intakes and clinical condition of animals fed either HSW or HMPW were unaffected by exposure
No effects were observed at necropsy for either test material.
Although there were minor organ weight changes, the authors did not consider them to be treatment-related unless a dose-related trend was apparent. The % increases (+%) or decreases (-%) at the various dietary concentrations are summarized below:

	Dietary concentration (%)			
Treatment	**0.002**	**0.02**	**0.2**	**2.0**
HMPW				
Abs. Male kidney	+5			
Rel. Male kidney	+4			
Abs. Male liver		+4		
Rel. Male liver		+3		
Abs. Female spleen		-5		
Rel. female spleen	-5			
HSW				
Abs. Female kidney	-3			
Rel. Male liver		+4	+3	
Rel. Female liver	-5			

The only minor hematological difference recorded was a 2% increase in hemoglobin concentration in males in the highest dose groups of both HSW and HMPW. Females were unaffected.

Serum glucose levels were raised in all dose groups of animals fed HMPW and in all but the highest dose group of animals fed HSW.
The % increases were:

Dietary concentration (%)	**HMPW**	**HSW**
0.002	13	9
0.02		8
0.2	10	11
2.0	8	

No treatment-related histological changes were observed in either the HSW or the HMPW group animals.

Main, reversal and tissue level studies
Paraffin wax (LMPW)

Although growth rates, food intakes and clinical condition of animals fed LMPW were unaffected by exposure, there was a spectrum of changes that occurred as follows.
Organ weight changes were recorded in both sexes. Liver and spleen weights (absolute & relative) were increased at the 2 and 0.2% dose levels. Although some reduction was observed after the reversal period in the 2% dose groups, they were still higher than the corresponding controls.
Mesenteric lymph node weights were only available for the high dose level animals and these were increased following exposure to LMPW. Although the lymph node weights had reduced in the reversibility group they had not returned to normal by the end of the reversibility period.
The % increase (+) or decrease (-) in the hematological parameters are shown in the following table. The statistical significance of the differences are also indicated (* p<= 0.05, ** p<= 0.01, *** p<= 0.001).

Parameter	**Dietary concentration (%)**			
	0.002	**0.02**	**0.2**	**2.0**
Males				
RBC		+2*		
Hemoglobin		+2*	-2*	-2**
MCH			-2***	-2***
WBC	+16*	+20*	-3	+9
Neutrophils			+22**	+23**
Platelets	-3	-3	-7**	-13***
Females				
RBC				-4***
Reticulocytes				+43***
Hemoglobin content				-6***
Hematocrit				-4***
MCH				-2***
WBC			+26***	+48***
Neutrophils			+45***	+89***
Lymphocytes		+21*	+18*	+29***
Monocytes			+35**	+103***
Eosinophils				+41*
Basophils Actual value (Control value = 0)			0.003***	0.004***
Platelets			-14***	-16***

There were raised serum liver enzyme levels in the highest two dose groups of females but only at the highest dose in males. The enzymes affected were ALA, ALAT, ASAT and Gamma-GT. Serum bilirubin was also elevated in the highest dose group of females. Albumin/globulin ratios were reduced in the females at the highest 2 dose levels and in the highest dose level only for the males.

Histopathological lesions were observed in many tissues and were of a severity and nature consistent with the age of the animals and were not considered to be treatment-related. However lesions in the liver, mesenteric lymph node, Ileum & jejunum and heart were considered to be compound-related. These were as follows:

Liver
Granulomas were observed in the livers of male and female rats at the highest 2 dose levels. At the highest dose centrilobular vacuolation was also observed. After the one month reversal period, granulomas were still present at the same incidence but their severity was less.

Mesenteric lymph node
The lymph node lesions comprised focal collections of slightly vacuolated macrophages in the cortical region and after one month's reversal these were reduced in severity. Such lesions occurred to varying degrees of severity at all dose levels.

Ileum & jejunum
There was an increased incidence in macrophage accumulation in Peyer's Patches in both sexes at the highest two dose levels. There was also an increase in macrophage infiltration of the lamina propria in the high dose females.

Heart
A focal inflammatory lesion was observed within the cusps of the mitral valve. The lesion was characterised by an increased cellularity of the valve with destruction of the fibrous core. The lesion was observed in 11/20 males and 11/20 females at the highest dose level and 5/20 females at the 0.2% group. Following the 28 day reversal period there was still an increased incidence of the lesion but this was less than that at the end of the 90-day feeding study.

Analysis of tissues for mineral hydrocarbons.
In the tissue level studies, no mineral hydrocarbons were found in the kidneys of rats fed LMPW. However it was found in the perirenal fat, liver and lymph nodes.
After the 28-day reversal period, mineral hydrocarbon was still found in these tissues, albeit at lower concentrations.
No mineral hydrocarbons were found in any of the tissues of animals fed microcrystalline wax.

Remark : The purpose of this study was to investigate the biological effects of six mineral oils and three petroleum waxes representative of those used in food processing and food contact applications.
This robust summary only describes the results from the three petroleum waxes that were examined.
For additional details on the oils see the Lubricating Oil Basestocks Test Plan.

Reliability : (1) Valid without restriction.
Study conducted to GLP and thoroughly reported.
(8) (13)

Species : Rat

Remark : The purpose of this study was to assess the safety in use of a variety of oils and waxes for food contact applications.
As a follow up to this study, additional studies were carried out on other finished wax samples and the results are summarized in the table below.

The severity and incidence of the responses were related to the average molecular weights of the materials tested; the lower molecular weight materials causing the most severe effects (CONCAWE 1993).

Sample	Viscosity @ 100°C (cSt)	Carbon Chain Length	Average Mol. Weight	NOAEL (mg/kg/day)
LMPW	3.3	19-42	375	<2
Blend	8	19-80	470	<2
IMPW	6.3	21-49	480	<2
HSW	13.7	20-74	600	2000
HMPW	15.4	22-80	630	2000

LMPW: Low melting point finished wax
Blend: Blend of LMPW & HMPW
IMPW: Intermediate melting point finished wax
HSW: High sulfur wax
HMPW: High melting point finished wax

The findings from all the above studies allowed the EU Scientific Committee for Food (SCF 1995) to set ADIs for the high sulphur (HSW) and high molecular weight waxes (HMPW), but not for the lower molecular weight materials since for these NOELS had not been established.

A further study has also been carried out in which Low Melting Point Wax was fed to F-344 and Sprague Dawley rats at dietary concentrations of 0.2 and 2.0% in the diet for 90 days.

The findings in the F-344 rats were essentially similar to those found in the studies summarized above but the Sprague Dawley rat was found to be a less sensitive strain.

The only effects of treatment seen were an increase in mesenteric lymph node weight and microscopic findings in the same tissue (microgranulomas and reticuloendothelial cell hyperplasia). These effects were less severe and less frequent than those seen in the F-344 rats.

(9) (10)

5.5 GENETIC TOXICITY 'IN VITRO'

: No data available

5.6 GENETIC TOXICITY 'IN VIVO'

: No data available

5.7 CARCINOGENICITY

Species	:	Mouse
Sex	:	Male
Strain	:	C3H
Route of admin.	:	Dermal
Exposure period	:	80 weeks
Frequency of treatm.	:	Twice weekly
Doses	:	50 mg/application
Result	:	Negative
Control group	:	Untreated control and positive control (BaP)
GLP	:	No
Method	:	50 mg melted slack wax was painted on the skin of 50 individually housed male mice, twice weekly for 80 weeks. The animals were shaved bi-weekly with electric clippers and the test material applied to the shaven intrascapular region. Treatment was continued for 80 weeks. A concurrent negative untreated control and a positive control (benzo-a-pyrene) was included in the study. The study was repeated using 25 mg/application, twice weekly.
Remark	:	This report is a summary of results from an extensive program of studies. Consequently all the experimental details have not been presented. The authors state that such details are available in the original laboratory reports.
Result	:	No skin tumors developed in any of the mice to which slack wax had been applied in either of the studies. The responses in the control groups is not reported.
Test substance	:	Slack wax CAS No. 64742-61-6 The sample was tested twice in the study summarized by Kane et al.
Reliability	:	(2) Not assignable. The report sumarises data from many studies and does not contain suficient detail for a full evaluation.

(37)

Species	:	Mouse
Sex	:	Male
Strain	:	C3H
Route of admin.	:	Dermal
Exposure period	:	80 weeks
Frequency of treatm.	:	Twice weekly
Doses	:	50 mg/application
Result	:	Negative
Control group	:	Untreated control and positive control (BaP)
GLP	:	No
Method	:	50 mg petrolatum was painted on the skin of 50 individually housed male mice, twice weekly for 80 weeks. The animals were shaved bi-weekly with electric clippers and the test material applied to the shaven intrascapular region. Treatment was continued for 80 weeks. A concurrent negative untreated control and a positive control (benzo-a-pyrene) was included in the study. The study was repeated using 25 mg/application, twice weekly.
Remark	:	This report is a summary of results from an extensive program of studies. Consequently all the experimental

details have not been presented. The authors state that such details are available in the original laboratory reports.

Result : No skin tumors developed in any of the mice to which petrolatum had been applied in either of the studies. The responses in the control groups is not reported.

Test substance : Petrolatum CAS No. 8009-03-8

Reliability : (3) Not assignable.
The report summarizes data from many studies and does not contain sufficient detail for a full evaluation.

(37)

Species : Mouse
Sex : Male/female
Strain : Swiss
Route of admin. : Dermal
Exposure period : Lifetime
Frequency of treatm. : Twice weekly
Doses : Approximately 60 microlitres per application
Result : Negative
Control group : Yes, concurrent vehicle
Year : 1966
GLP : No data
Test substance : 15% solution of Amber Petrolatum (NF Grade) in isooctane.
Method : Three drops (approximately 60 microlitres) of a 15% solution of amber petrolatum in isooctane was applied to the shaven skin of the mice, twice weekly for their lifetimes.
30 male and 40 female mice were treated in this way.
A group of 50 males and 50 females served as vehicle controls and received 60 microlitres of isooctane twice weekly for the lifespan of each animal. Animals were housed in groups of not more than 10 per cage.
The occurrence of skin tumors and other lesions in the treated area and other visible lesions was noted and their progression recorded.
Histological confirmation of each lesion was confirmed after autopsy of the respective animals.

Result : Treatment with petrolatum caused moderate epidermal hyperplasia.
The authors state that the incidence of internal tumors appeared within the limits observed in the control animals.
Treatment did not appear to affect survival when compared to controls as follows:

	Survival(%) at weeks		
Group	**30**	**50**	**70**
Petrolatum			
Females	90	77	53
Males	93	83	35
Controls			
Females	90	80	64
Males	90	54	32

The skin tumor incidence is summarised below for the control and petrolatum groups. No data are included here for the various extracts of petrolatum that were tested, even though such data were given in the publication reviewed.

	Animals with tumors	Total number of Tumors	Carcinomas	Regressions	Latency (weeks)
Petrolatum					
Females	1	2*	-	1	100
Males	2	3**	-	2	69
Solvent					
Females	-	-	-	-	-
Males	2	2	1 -		63

* one papilloma on eyelid
** one papilloma under chin

Test substance : 15% solution of Amber Petrolatum (NF Grade) in isooctane.
Reliability : (2) Valid with restrictions.
The study was designed only to investigate skin carcinogenicity and consequently detailed pathological findings are not available. Detailed findings (histopathological) are not included in the paper, but the authors make reference to a source of such data.

(39)

Species : Mouse
Sex : Male/female
Strain : Swiss
Route of admin. : Dermal
Exposure period : Lifetime
Frequency of treatm. : 3 times weekly
Doses : 3 drops
Result : Negative
Control group : Yes, concurrent no treatment
Year : 1962
GLP : No data
Test substance : 5 waxes were selected from 36 samples on the basis of their ultraviolet absorptivity, representing the range of aromatic contents Each of the 5 waxes was dissolved in warm benzene to achieve 15% solutions. These were warmed in a water bath prior to application to the skin.
Additionally a benzene solvent control was included in the study as well as an aromatic extract (in is-octane) of one of the waxes and a 15% solution in benzene of a chromatographed wax.
Method : 3 drops (approximately equivalent to 0.05 ml) of the solution of wax or the solvent control was applied to the skin of the intrascapular region over an area of approx. 2 X 2 cm. This treatment was continued 3 times weekly to groups of mice throughout the experiment. Observation was continued until spontaneous death or until the animals were killed when dying. All mice were subjected to a complete autopsy followed by an histological examination of all abnormal tissue.
Group sizes were approximately 60 male and 30 female for each wax sample and 140 mice of each sex for controls.
Result : Survival rates of the mice were similar for treated and control animals with a better survival among females than males.
Some desquamation and epilation occurred in the treated areas of skin after the first few applications and this

persisted throughout the study.
Histologically, moderate epidermal hyperplasia was observed in both treated and control animals. The wax treated animals also had some focal areas of hyperplasia of the sebaceous glands. No degenerative or necrotic changes were observed.

The skin tumor incidences are shown in the following table.

Sample	No. of mice	Benign papillomas	Malignant carcinomas	Sebaceous gland adenomas	Other
Wax 2	61 M 30 F	1			
Wax 8	61 M 31 F	3 1	1		
Wax 12	58 M 34 F	4 1		1 1	1
Wax 15	57 M 30 F	2 1			
Wax 20	61 M 36 F	1 1		2 2	
Benzene	59 M 35 F	 1	1		

A number of other tumors were also observed at autopsy (mainly lung adenomas, mammary carcinomas and malignant lymphomas) but these were found in all groups and their incidence was similar in wax treated groups and controls.
The authors judged that these studies were negative.

Reliability : (2) valid with restrictions
Although not conducted to GLP, the study was nevertheless, robust and is acceptable for the purpose of assessing the skin carcinogenicity potential of paraffin wax solutions in benzene.

(49)

Species : Mouse
Sex : Male
Strain : White albino
Route of admin. : Dermal
Frequency of treatm. : Three times weekly for lifetime
Year : 1951
GLP : No
Test substance : Eight slack waxes and eight aromatic hydrocarbon extracts derived from the slack waxes were tested.
[Because of the lack of detail in the publication it is not possible to establish whic aromatic extract from which specific slack wax].
The extracts were obtained by eluting, with an unspecified solvent, silica gel columns charged with the individual slack waxes. No additional information was provided on the preparation of the aromatic test materials.
[However, in parallel studies on aromatic extracts collected from catalytically cracked oils, the investigators reported that the silica gel columns were eluted first with n-heptane to collect non-aromatic components of the oils and then with acetone to recover the aromatic components. In the parallel studies the recovered aromatics were tested on mice after evaporation of the acetone.]

Method : Approximately 15 mg of warmed test material were applied as a thin film by means of a small brush on Monday, Wednesday and Friday to the shorn scapular region of groups of 30 albino male mice. Test material application was continued until death. After tumors had appeared the test materials were applied around the viable base of the growths, not on their often "dead tops".

For each material at autopsy, sections were taken of representative tumors and any internal lesions of interest. These tissue sections were then examined microscopically. For each test material a cancer and a tumor index was calculated as follows:

Tumor index =
$$100 \times \frac{\text{Total No of animals in which tumors developed}}{\text{Original No. animals less No dead at 90 days without tumors}}$$

Cancer Index =
$$100 \times \frac{\text{Total No animals in which cancer developed}}{\text{Original No less No. dead at 90 days from causes other than cancer}}$$

Potency was calculated:= $\frac{\text{Cancer index}}{\text{Tumor index}}$

Result : Results are summarized in the following two tables:

Slack waxes

Wax Sample	Oil (%)*	CI/TI at Days 250	450
145	25	4/23	8/10***
147	17	0/3	7/7
150	20	0/0	4/4
141	10	0/3	0/7
142	21	0/4	0/4
144	21	0/4	0/4
140	20	4/7	4/4***

146	12	0/0	4/4

Aromatic extracts

Sample	Aromatic (%)**	CI/TI at Days 250	450
231	18	14/38	24/38****
233	0	19/30	23/35****
235	12	17/35	17/43****
228	7	3/17	14/34
229	0	0/0	0/13
230	12	0/42	8/30***/*****
231	11	4/22	4/30
232	8	0/8	4/10

* Oil content of the slack waxes (w/w)
** Aromatics content of the slack wax (w/w)
*** The lower tumor index (TI) at the later date is due to the spontaneous disappearance of some papillomas
**** The experiment was discontinued after 335 days
***** The experiment was discontinued after 490 days

The authors concluded that the slack waxes showed only a low order of carcinogenicity at 250 days. However by 450 days every sample of slack wax had elicited papillomas and for 5 of them cancers as well.
The aromatic extracts on the other hand exhibited a greater potency. At 250 days all but one sample had produced papillomas and 5 samples had produced cancers. At 450 days all but one sample had elicited cancers and all had elicited papillomas.

The authors concluded that the carcinogenicity of slack wax
1. Can be attributed to the aromatic compounds found in the oils from which the waxes were pressed and which are retained on the waxes as impurities.
2. Is not due to paraffins.

Another study from the same laboratory (Dietz et al, 1952) on 11 slack waxes (it is unclear whether some were the same samples as in Smith et al, 1951) produced similar results. The tumor potency of each sample was low to marginal.

Reliability : (3) Not assignable.
The study summarized here was conducted to identify the carcinogenic component(s) of slack waxes.
Although not conducted to GLP and lacking experimental deatils the study is important since it identifies the residual oil in the slack wax and not the paraffins as being responsible for carcinogenic activity

(18) (50)

Species	:	Rabbit
Sex	:	Male/female
Strain	:	New Zealand white
Route of admin.	:	Dermal
Frequency of treatm.	:	Three times weekly
Control group	:	Yes, concurrent vehicle
Year	:	1962
GLP	:	No
Test substance	:	5 waxes were selected from 36 samples on the basis of their ultraviolet absorptivity, representing the range of aromatic contents Each of the 5 waxes was dissolved in warm benzene to achieve 15% solutions. These were warmed in a water bath prior to application to the skin. Additionally a benzene solvent control was included in the study.
Method	:	Solutions of the waxes as well as the benzene alone were applied three times weekly to the shorn skin of the intrascapular region (approximately 10 X 10 cm) of 4 male and 4 female rabbits. Each application consisted of approximately 0.08 ml. The authors state that a few rabbits were added in some groups to compensate for death of other rabbits before one year of treatment. Specific details are not provided.
Remark	:	This study had not been completed at the time of publication of a paper on the toxicity of petroleum waxes (Shubik et al). However, the information is useful in assessing the skin carcinogenicity of petroleum waxes since it provides data from an additional species.
Result	:	Some reddening, desquamation and epilation of the painted skin area occured after a few paintings with the wax solutions and the benzene alone; these changes persisited throughout the study without any notable modifications. 2 small skin papillomas were observed in the male group painted with one of the waxes. One of these papillomas developed after 48 weeks of treatment and was still present at the 105th week. The other papilloma developed after 93 weeks and regressed at the 110th week. No other skin lesions were found in any of the groups.
Reliability	:	(4) Not assignable. This study was not reported thoroughly, nor was it complete at the time of publication. However it does provide supportive information from a species other than the mouse.

(49)

Species	:	Rat
Sex	:	Male/female
Strain	:	FDRL
Route of admin.	:	Oral feed
Exposure period	:	2 years
Frequency of treatm.	:	Ad libitum
Doses	:	5% in the diet
Result	:	Negative
Control group	:	Yes, concurrent no treatment
Year	:	1965
GLP	:	No data
Test substance	:	Three blends of petrolatum were examined. They were as follows:

Blend A, a snow-white grade meeting USP XVI specifications. This sample was a blend in equal proportions of six commercially available materials, each meeting the US specification.

Blend B, a white petrolatum, somewhat darker than Blend A, but nevertheless meeting the USP XVI specification. This blend was also prepared as a mixture of six commercially available materials in equal proportions.

Blend C, a yellow petrolatum meeting NF XI specification. This blend was prepared as a mixture in equal proportions of 5 commercially available products.

The three blends were kept with minimum air space refrigerated in metal containers for the duration of the study.

Analytical characteristics of the blends were as follows:

Blend	UV absorptivity (290 micron)	Lovibond color (2 in. cell)	Specific gravity (60 °C)	Melting point (°C)
A	0.136	2Y	0.830	53.5
B	0.424	12Y 0.5R	0.835	52.2
C	1.48	35Y 10R	0.844	51.3

Method : 50 rats of each sex, individually housed were fed diets containing 5% of one of three blends of petrolatum ad-libitum for two years. A group of 100 rats of each sex served as controls and were fed normal diet ad-libitum that had been supplemented with 1% vitamin mix and 0.2% Aurofac 10.

The animals were observed daily for appearance, behavior and survival.

Weekly measurements were made of body weight for the first 12 weeks of the study and biweekly thereafter. Weekly measurements were also made of food intake for the first 12 weeks for 10 rats of each sex fed the diets containing petrolatum and for 20 rats of each sex fed control diet.

At 12, 26, 52, 72 & 100 weeks the following determinations were made on representative animals from each of the groups:

red cell count and/or hematocrit, total and differential white cell counts, hemoglobin content, blood glucose and blood urea nitrogen levels.

Rats that died and survivors at the end of the study were autopsied and the following organ weights were recorded: liver, kidneys, spleen, heart, adrenals, thyroids and pituitary.

For all rats that died, that were killed in a moribund state or from representative surviving animals at the end of the 2 year feeding period (10 of each sex in the petrolatum groups, 20 of each sex controls) the following organs were fixed and examined histologically: liver, spleen, stomach, large and small intestine, pancreas, kidney, urinary bladder, adrenal, thyroid gland, testis or ovary, salivary gland, lymph node, heart, lung, muscle, skin, spinal cord, brain, thymus, bone marrow and "growths of any description".

Result : Growth rates were unaffected by exposure to petrolatum when compared to controls.
Although there were small statistically significant differences in food utilization values between control and some petrolatum exposed animals these were not of biological significance.
Survival at two years was unaffected when compared to controls. Survival of males was approximately 68% and that for females was 58%.
Neither hematological nor clinical chemical measurements were affected by exposure to any of the petrolatum samples either during or at the end of the study.
No differences were found at autopsy between petrolatum exposed and control animals. Furthermore there were no histological changes that could be attributed to dietary exposure to petrolatum. Histological changes that occured did so in both sexes and in all treatment and control groups and were considered to be ageing related.
Neither of the 3 petrolatum blends caused an increased tumor incidence in any tissue/organ examined.

Reliability : (2) Valid with restrictions.
This study is well conducted and reported, but was carried out prior to the need for GLP. Nevertheless the study is valid

(46)

Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Oral feed
Exposure period : 2 years
Frequency of treatm. : Continuous
Doses : 5000mg/kg bw/day
Result : Negative
Control group : Yes, concurrent no treatment
Year : 1962
GLP : No
Test substance : 5 waxes were selected from 36 samples on the basis of their ultraviolet absorptivity, representing the range of aromatic contents
Each of the 5 waxes was ground into a powder and added to powdered diet and mixed in the proportion 1:9 w/w.

Method : Each of the five waxes were fed ad-libitum to male and female rats at a dietary concentration of 10% for 2 years.
An additional group of 140 male and 157 females were fed

control diet.
The rats inspected and weighed every second week and all gross lesions were recorded. This was continued until the rats died or were killed when dying and were then submitted to complete autopsy followed by histological examination of all abnormal tissue.

Result : Survival rates and growth rates were unaffected by oral exposure to any of the waxes tested.
A number of tumors were found in all groups at autopsy. The incidence of each tumor type was reported. The number of tumor bearing animals was similar to that of controls and furthermore the incidence of the various tumor types was also similar in treated and control animals.
No other toxic effects were found at histological examination.
The authors concluded that the five waxes were devoid of carcinogenic or other toxic action when fed at a level of 10% in the diet.

Reliability : (2) Valid with restrictions.
Study not carried out acording to GLP and only "abnormal" tissue examined histologically.
Study provided supportive information only and coul not be used as a definitive study.

(49)

Species : Rat
Strain : BD I, BD III and W
Route of admin. : Various
Exposure period : Up to approximately 2.5 years
Frequency of treatm. : Various
Year : 1953
GLP : No
Test substance : Various including yellow vaseline

Remark : The following is taken from the method section of an English translation of the German report:

> "Liquid paraffin (DAB. 6) was injected into 30 rats, 2.5 ml once subcutaneously and intraperitoneally in a total dose of 9 ml per animal divided over 15 individual injections over a period of 40 weeks. Another 30 rats obtained the liquid paraffin in the food. The total dose was 136 ml/animal in 500 days.
>
> Yellow vaseline (DAB. 6) was also injected after warming. Eight rats obtained 3 ml intraperitoneally and 26 rats 1 ml subcutaneously besides. All animals were observed until spontaneous death....."

The following is taken from the results section of the publication.

> "In the experiment with vaseline a tumor developed at the injection point after a latent period of 658 days. Histologically this tumor turned out to be an osteo-sarcoma. ....."

Reliability : (4) Invalid.
This study is of historical interest only and is included for completeness only.

(48)

Species	:	Mouse
Sex	:	Male/female
Strain	:	Swiss Webster
Route of admin.	:	s.c.
Frequency of treatm.	:	Single subcutaneous dose
Post exposure period	:	18 months
Doses	:	100 mg
Result	:	Negative
Control group	:	Yes
Year	:	1965
GLP	:	No
Test substance	:	Three blends of petrolatum were examined. They were as follows: Blend A, a snow-white grade meeting USP XVI specifications. This sample was a blend in equal proportions of six commercially available materials, each meeting the US specification. Blend B, a white petrolatum, somewhat darker than Blend A, but nevertheless meeting the USP XVI specification. This blend was also prepared as a mixture of six commercially available materials in equal proportions. Blend C, a yellow petrolatum meeting NF XI specification. This blend was prepared as a mixture in equal proportions of 5 commercially available products. The three blends were kept with minimum air space refrigerated in metal containers for the duration of the study. Analytical characteristics of the blends were as follows:

Blend	UV absorptivity (290 micron)	Lovibond color (2 in. cell)	Specific gravity (60 °C)	Melting point (°C)
A	0.136	2Y	0.830	53.5
B	0.424	12Y 0.5R	0.835	52.2
C	1.48	35Y 10R	0.844	51.3

Stripped lard was used as negative control substance.

Method	:	A single dose of 100 mg of one of the three petrolatum blends or stripped lard was administered subcutaneously into the intrascapular region of 28 day old mice. 50 male and 50 female mice were used for each group and these were housed individually for the following 18 month observation period. The mice were allowed food and water ad-libitum. Growth, physical appearance and behavior were observed throughout the study and special attention was paid to the injection site. Representative mice sacrificed at 9 months and all mice that died or were sacrificed at the end of the 18 month observation period were examined at autopsy for evidence of pathological change. Weights of liver, spleen and kidneys were recorded. After fixation, histological examination was made of: liver, spleen, stomach, small and large intestine, pancreas, kidney, urinary bladder, adrenal, thyroid, testis or ovary, salivary gland, lymph node, heart, muscle, lung,

skin, spinal cord, brain, thymus and bone marrow and any macroscopically observed growths.

Result : Growth rates, food intakes and food utilization was unaffected by s.c. administration of any of the petrolatum samples when compared to the control group. The males consumed slightly more food than the females, but there were no differences between the various treatment groups.
Mortality was similar in the control and petrolatum groups and overall survival ranged between 12 and 24% at the end of the study (78 weeks).
Liver, kidney and spleen weights were not affected by exposure to any of the petrolatum blends.
Gross observations at autopsy were spread equally amongst all groups and were not specifically related to exposure to petrolatum.
At about 7-9 months, there had been a significant rise in mortality in all groups and histopathlogical examination confirmed widespread leukemic infiltration with secondary septicemic involvement in some animals in all groups.
Gross findings at the end of the study were consistent with ageing animals. The responses were largely either of a chronic inflammatory or fibrotic nature. Many of the observations in the lymphatic system showed chronic changes associated with the clearance of the foreign material that had been injected subcutaneously.
There was no specific relationship between tumor incidence and the test material injected.

In conclusion, no toxic or carcinogenic response resulted as a consequence of the s.c. injection of a 100 mg dose of either of the 3 petrolatum blends.

Reliability : (2) Valid with restrictions.
This study is well conducted and reported, but was carried out prior to the need for GLP. Although survival of mice was poor, nevertheless the study is considered valid.

(46)

Species : Mouse
Sex : Male/female
Strain : Swiss
Route of admin. : s.c.
Exposure period : Lifetime
Frequency of treatm. : Once only administration of test material
Post exposure period : Lifetime
Year : 1962
GLP : No
Test substance : paraffin wax

Method : A single wax disc (2 cm. diameter, 2 mm. thick and weighing 0.5 g) was implanted subcutaneously in groups of approximately 45 male and 50 female Swiss mice. This was done for 5 different waxes.
Additionally, 0.5 g of one of the waxes was implanted as a powder in a further group of 48 and 46 female Swiss mice.
The animals and their controls were observed for their lifetimes.

Result : Tumors developed at the implantation sites of the wax discs.
No tumors developed at the site s of the powdered wax.

This finding is consistent with other reports on the tumorigenicity of implanted inert materials. It is generally believed that tumorigenicity at subcutaneous implantation

sites is a function of the physical form of the material rather than of the material itself. If however, the material had been tumorigenic it would be expected that tumors would have developed at the site of the implanted powder.

Reliability : (2) Valid with restrictions.
Although the study was not GLP compliant it nevertheless was properly conducted and reported.

(49)

5.10 EXPOSURE EXPERIENCE

: **Slack wax**
There are no published reports of acute effects in humans with slack waxes, but they are expected to be essentially non-toxic because both the residual oil and the wax components themselves are not acutely toxic.

There have been several reports of human occupational cancer amongst wax pressmen, during the preparation of paraffin wax (Hendricks et al, 1959; Lione and Denholm, 1959). In the process of wax pressing the unrefined or poorly refined oil was chilled and the solidified crude wax (slack wax) removed from the viscous oil on filter presses. This crude wax may have contained as much as 20-40% unrefined/poorly refined oil, which was reduced to less than 0.5% in subsequent processing. It should be noted that wax pressing is no longer used as a process and has been replaced by more modern techniques.

(32) (40)

: **Paraffin wax and Microcrystalline wax**
A review of the clinical studies with two undiluted paraffin waxes and formulated products containing various concentrations of paraffinic (5-16%) and microcrystalline (4.35-15%) waxes was published (Anon, 1984). These studies include a range of acute and repeat application tests in groups of humans for skin irritation and skin sensitization. All products gave, at most, slight erythema and none caused skin sensitization.

The widespread use in cosmetic and in cosmetic surgery over many years demonstrates the low toxicity of refined waxes and many guidelines exist for their safe use (Hjorth, 1987). Notwithstanding this, there are occasional reports of adverse effects with these products. Subcutaneous deposits often referred to as paraffinoma, have been described frequently following injection of these materials under the skin but these are not normally associated with other progressive changes.

There has been one report where an outbreak of skin rashes was attributed to occupational exposure to wax fume (Halton & Piersol, 1994).

(21) (28) (33)

: **Petrolatum**
Despite the widespread use of petrolatum for many years as a vehicle in human skin patch testing, isolated cases of allergy to petrolatum have been reported.

Neverthelesss, petrolatum is still considered to be a good vehicle for patch testing. Fisher has concluded that although allergic reactions to petrolatum are rare, white, and not yellow petrolatum should be used as a vehicle in human skin patch testing.

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Reproductive/Developmental Toxicity

Type	:	One generation study
Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Gavage
Frequency of treatm.	:	Daily
Doses	:	1.15 ml/kg (approximately 1000 mg/kg)
Control group	:	No
Method	:	OECD Guideline 421, Reproductive/Developmental Toxicity screening test
Year	:	1995
GLP	:	Yes
Test substance	:	100 neutral (refined) CAS 64742-54-7

Method :

The method used was as described in OECD guideline 421.

The base oil was administered by gavage at a dose of 1.15 ml/kg (bw) to a group of 12 male and 12 female Sprague Dawley rats. Rats designated F0 animals were dosed for a minimum of 14 days prior to mating. Dosing was continued after mating until a total dosing period of 30 days had elapsed for males and until day 4 of lactation for females (39 days). The animals were observed twice daily for appearance, behavior, moirbundity 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 for F0 both sexes.
Animals were paired on a 1:1 basis. Positive evidence of mating was confirmed either by the presence of sperm in a vaginal smear or a vaginal plug. The day when evidence of mating was identified was termed Day 0 of gestation.

The following Fertility indices were calculated:

- Female mating index
- Male mating index
- Female fertility index
- Male fertility index

All females were allowed to deliver their young naturally and rear them to post-natal day 4. Females were observed twice daily during the period of expected parturition for initiation and completion of parturition and for signs of dystocia. 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. Any 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.
Selected organs of parental animals were weighed and a wide range of tissues was fixed for subsequent histopathological examination.

Result :

Only the results for the base oil control group are reported below.

There were no clinical findings and growth rates and food consumption values were normal.
Fertility indices and mating indices for males and females were both 100%.
At necropsy, there were no consistent findings and the animals were

considered to be normal.
Organ weights and histopathology was considered normal.

Reliability : (2) valid with restrictions
The study was on an oil additive in base oil at two concentrations. The base oil alone was used as the control. Therefore, no control was available with which to compare the study control group. However, since all the recorded values were within normal limits, it could be concluded that the base oil was without effect.

REFERENCE: WIL Research Laboratories Inc. 1995. An oral reproduction/developmental toxicity screening study of **** in finished oil in rats. Laboratory Study No. WIL-187007. Ashland, Ohio.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	64742-04-7
Test Substance:	64742-04-7; Aromatic Extract (AE); Furfural Extract ; 318 Isthmus Furfural Extract.
Test Substance Purity/Composition and Other Test Substance Comments:	Aromatic Extract (CRU No 86187) PAC Content – report no. 64349 ZA (Mobil, 1991) 1) Percent of DMSO-extractable materials (mostly PACs), determined by the PAC 2 method as described in API (2008). 2) ARC is "aromatic ring class". "ARC 1 (%)" is the weight percent of PACs that have 1 aromatic ring within the total sample. "ARC 2 (%)" is the percent of PACs with 2 aromatic rings, and so forth to 7 aromatic rings.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

Sample #	DMSO wt.% [1]	1-ARC (%)[2]	2-ARC (%)	3-ARC (%)	4-ARC (%)	5-ARC (%)	6-ARC (%)	7-ARC (%)
86187	20.30	0.00	0.00	4.06	8.12	6.09	2.03	0.41

METHOD

Route of Administration:	Dermal, non-occluded
Other Route of Administration:	
Type of Exposure:	Developmental toxicity study
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley (Charles River, Kingston, NY)

Other Strain:	Not applicable
Gender:	Females, presumed pregnant (non treated males used for mating)
Number of Animals per Dose:	10- 15 per dose, except for an additional group of 3 animals exposed at 500 mg/kg on GD 10-12 used to obtain bioavailability data
Concentration:	
Dose:	Developmental study: 0, 8, 30, and 125, 500 and 1000 mg/kg/day Bioavailability study: 500 mg/kg/day
Year Study Performed :	1990
Method/Guideline Followed:	Similar to OECD 414 (Prenatal Developmental Toxicity Study). Main difference was that fewer females were used (10/group versus 20).
GLP:	No information
Exposure Period:	GD 0-19 (7 groups); GD 0-16 (1group; see explanation below) ; GD 10-12 (2 groups)
Frequency of Treatment:	Once per day
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The study was designed to detect the effects of exposure to AE (318 Isthmus furfural extract) on parameters of reproductive performance during gestation (implantation, litter size) and viability and development of the embryo/fetus. An experimental group in which AE was administered only on GD 10-12 was included in the study to complement the results of the bioavailability study. The study was also designed to include clinical chemistry analyses of maternal sera, bioavailability/ bioaccumulation of AE in maternal tissues, placentae, and fetuses, and postnatal survival of neonates. The female animals used in the study were approximately 9 weeks old at receipt and approximately 11 weeks old at exposure initiation. Prior to the initiation of dosing with the test material, females were placed with untreated males (approximate 1:1 ratio). Once mating occurred and confirmed by detection of a vaginal plug (<u>in situ</u> or expelled), the individual, presumed pregnant females were randomly assigned to eight treatment groups and dosing began for that animal. The treatment groups and time exposure periods were as follows, where designation as GD 0 followed detection of a vaginal plug, and spermatozoa in the vaginal lavage fluid: <u>Prenatal Group</u> 1. 0 mg/kg/day (control) – GD 0-19 – 15 animals

2. AE 8 mg/kg/day – GD 0-19 – 15 animals
3. AE 30 mg/kg/day – GD 0-19 – 15 animals
4. AE 125 mg/kg/day – GD 0-19 – 15 animals
5. AE 500 mg/kg/day – GD 0-16– 15 animals*
6. AE 1000 mg/kg/day – GD 10-12 – 15 animals; included as an additional group because was anticipated that administration throughout the complete gestation period may result in a high incidence of fetal lethality. This is a period during which fetuses are susceptible to abnormal development.

Postnatal Group

7. Proximately-housed dermal control (0 mg/kg/day) – GD 0-19 - 10 animals
8. AE 125 mg/kg/day –10 animals – GD 0-19 – 10 animals

Bioavailability Group

9. Radiolabeled AE 500 mg/kg/day – GD 10-12 – 3 animals; bioavailability group

* The treatment was discontinued after day 16 because of the suspected high incidence of resorption as indicated by the increased incidence of red vaginal discharge.

The exposure levels were based on results of a thirteen week study previously conducted on this material.

Developmental/Pre and Post-natal study (Groups 1-8):
The test material was administered to groups 2-4 and 8 on GD 0-19. Hair was clipped from the dorsal trunk of each animal on GD 0, and once weekly during the study. Each treatment day, animals were dosed by even application of the test material to their shaved backs, using the tip of a syringe. The test material dose, calculated from each rat's most recent body weight, was measured by weight. Rats were fitted with Elizabethan collars to minimize ingestion of test material. All control animals were handled in the same manner, minus application of the test material. They were clipped and collared and the intact dorsal skin of each rat was stroked with the tip of a syringe, but no test material was applied.

Group 5 females were treated with test material as described above during GD 0-16. This group was originally scheduled to be administered GD 0-19. However, treatment was discontinued after GD 16 because a high incidence of resorption was suspected (as indicated by a red vaginal discharge observed in many of the females in this group). Treatment was terminated in an attempt to salvage some offspring exposed at this dosage. Group 6 (1000 mg/kg/day) animals were were similarly treated but were administered the test material on GD10-12, an interval during which the developing conceptus is sensitive to teratogenic insult.

Each rat was observed at least once a day throughout gestation until sacrifice for 1) changes in appearance, behavior, and excretory function, and 2) signs of ill-health, mortality, abortion and premature delivery. Dams and their litters were observed on postpartum days 0-4 for signs of pathosis and/or death. On postpartum day, pups were examined for external malformations and variations. Pups were observed daily for the presence of milk in their stomachs; absence of milk was recorded. All unusual findings were noted.

Individual body weights were recorded on days 0, 3, 6, 10, 13, 16, and 20 of gestation. Individual food consumption was

measured during the study was calculated for GD intervals 0-3, 3-6, 6-10, 10-13, 13-16, and 16-20. Additionally, the body weights of the postnatal group (Groups 7 and 8) were measured on postpartum days 0 and 4, and recorded. Food consumption was not measured during the postpartum period.

For the prenatal groups (groups 1-6) each female was sacrificed by overexposure to ether on day 20 of its presumed gestation. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The thymus and liver of each animal were removed, trimmed of excess tissue, weighed to the nearest 0.001 gram, and preserved in 10% formalin. Microscopic evaluation was performed on the thymus in the control (group 2) and 500 mg/kg/day groups. No histopathology was completed for these tissues.

The ovaries and uterus of each rat were excised and examined grossly. Corpora lutea in each ovary were recorded. The weight of the intact uterus was measured to the nearest 0.1 gram and recorded. The number and distribution of implantations, early and late resorptions, and live and dead fetuses were noted. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. The uterus of each female rat that appeared non-gravid was pressed between two glass slides and examined grossly for evidence of implantation.

Blood samples from groups 1-4 were collected at the time of sacrifice from the aorta of each rat and serum was analyzed for almandine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, lactate dehydrogenase, iron, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, total protein, triglycerides, urea nitrogen, and uric acid. The globulin and albumin/globulin ratios were calculated.

Each fetus was gendered, weighed and grossly examined. The following definitions and terminology were used in describing fetal findings:

1) Anomaly: Any deviation (malformation or variation) from "normal."
2) Malformation: A permanent structural deviation which generally is incompatible with, or severely detrimental to, normal postnatal survival or development. Absence structures which should have been present, as well as deviations in tail development, are also classified as malformations.
3) Variation: A variation is a divergence beyond the usual range of structural constitution. It has an indeterminate effect on health and generally has no effect on survival.

Approximately half of the fetuses were randomly assigned for examination of soft tissues (viscera) following fixation in Bouin's solution, using a modification of the Wilson's technique. The other half were fixed in 95% ethanol, differentially stained for cartilage and bone, cleared in glycerin and examined for skeletal abnormalities.

For the postnatal groups (group 7 & 8), female rats and their offspring were sacrificed on postpartum day 4. Animals which did

not deliver were sacrificed on GD 25. Thoracic and abdominal organs were examined, and all organs were examined grossly for evidence of pathosis. The uterus was excised and examined for the total number of implantations. The thymus and liver of each animal were removed, trimmed of excess tissue, weighed to the nearest 0.001 gram, and preserved in 10% formalin. All pups were preserved in 95% ethanol at the time of sacrifice; no evaluations were performed.

<u>Bioavailability Study (Group 9)</u>
From GD 0-9, pregnant females were housed in stainless steel cages with wire bottoms and fronts. On GD 10, 11, and 12, the rats were housed in metabolism cages. The AE used in the bioavailability study contained two radioactive surrogates, carbon-14 radiolabeled carbazole and hydrogen-3 radiolabeled benzo(a)pyrene (BaP). On GD 10, the hair was clipped from the dorsal trunk of each animal and the radiolabeled test material was applied to the skin within a protective device designed to contain the administered dose. A mesh screen was attached to the protective device, and each rat was fitted with an Elizabethan collar. The same procedure was repeated on GD 11 and 12, except the needle tip with the test material was inserted through the mesh screen in order to apply the test material.

On GD 13, 24 hrs after the administration of the last AE dose, animals were sacrificed and maternal blood was collected. Necropsies were performed and the uterine contents located and examined for the number of normal and resorbed fetuses for each dam. The individual fetal units were removed, and the amniotic fluid was collected from the isolated placenta. The embryo was separated from the yolk sac and rinsed with water to remove residual amniotic fluid. Placentas, embryos, amniotic fluid and yolk sacs were pooled for each dam and the weights or volumes of the pooled samples determined. Maternal tissues collected for radioactivity analysis included the following: thymus, liver, heart, brain, small intestine, large intestine, kidneys, spleen, stomach, ovaries, urinary bladder, lungs, muscle, retroperitoneal fat, femur bone and residual carcass.

Determination of radioactivity in blood, urine and cage wash was accomplished by measuring the amount of carbon-14 labeled carbon dioxide and H-3 labeled water produced from direct combustion of duplicate samples. Samples were oxidized for three minutes and the carbon dioxide and water generated from the combustion were separated and trapped in a cocktail fluid. Carbon-14 and hydrogen-3 radioactivities were measured. Fecal samples were homogenized, combusted and the radioactivity measured.

The placentae, uteri, embryos, and yolk sacs were homogenized in an equivalent volume of water, and aliquots of the homogenate were combusted. Maternal tissues were treated in the same manner, although six tissues including the ovaries, urinary bladder, muscle, fat, bone and residual carcass were combusted directly without homogenization or dilution. In all cases, the trapped carbon dioxide and water were measured for radioactivity by liquid scintillation counting. Samples of the amniotic fluid were also combusted directly without dilution. Duplicate analyses were performed whenever possible. The sensitivity of the radioactivity allowed for the detection of 0.005% of the applied dose.

The systemic dermal absorption of the two radiolabeled surrogates was determined by summing the total carbon-14 or hydrogen-3 radioactivities found in the urine, urine/cage washings, feces and collected maternal and embryonic tissues at the end of 72 hours. Tissue concentrations of carbazole and benzo(a)pyrene (BaP) were calculated based on the radioactivity found per gram or per ml. The total amount of a radiolabeled surrogate in the tissues was calculated as a percent of the total applied radioactive

	dermal dose over three days. Statistical analysis: Data from the maternal biophase, caesarean section, and gross fetal examinations were evaluated by ANOVA, followed by group comparisons using Fisher's Exact or Dunnett's Test. Data from skeletal and visceral examination were evaluated by ANOVA followed by group comparisons using Fisher's Exact Test. Thymus and liver weights were evaluated using Duncan's multiple range test. Statistical analyses of clinical chemistry data were performed separately on individual serum components using SAS procedures. First the F-test was employed to do an analysis of variance on the serum data obtained from the control and exposed groups. Next the Student-Newman-Keul's multiple comparison test was employed to identify the specific group subsets within the serum data sets identified as having nonrandom variance. Differences between control and treated groups were considered statistically significant only if the probability of the differences being due to chance was less than 5% ($p<0.05$).

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)*

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL – Dermal	Maternal	=	8		mg/kg/day
NOAEL- Dermal	Maternal		Not identified <8		mg/kg/day
LOAEL - Dermal	Offspring (F1)	=	30		mg/kg/day
NOAEL - Dermal	Offspring (F1)		8		mg/kg/day

***Determined by reviewer**

Results Remarks:	Incidental findings included red nasal exudate and chromodacryorrhea. These observations are common in rats that are collared and/or stressed and were noted in both control and treated groups. Scratches were observed on the backs of several of the animals at the time of the first clipping and probably occurred during mating activity. A few females developed neck lesions in spite of the protective soft rubber tubing that lines the inner surface of the cardboard collar. Findings attributable to AE exposure included vaginal bleeding (generally a sign of some degree of litter resorption) observed in the treated groups. The incidence of this finding increased in a dose-related manner. The vaginal bleeding may have contributed to the paleness observed in some of the animals exposed at the 125 and 500 mg/kg/day dose level since this finding was noted either during or following vaginal bleeding for these animals. Several animals exposed at levels of 125 and 500 mg/kg/day also had decreased stool. The mean body weights and body weight of females exposed to AE at dose levels of 125 (both prenatal and postnatal groups at

21.8 and 28.6 %, respectively) and 500 mg/kg/day (27.8 %) were significantly lower than that of their respective control groups throughout most of the gestation period per the table below. Animals administered the test material for a limited period of gestation (GD 0-12, 1000 mg/kg/day) gained significantly less ($p<0.05$) weight during and following the period of exposure; however, when considering the net maternal body weight gain for this group, no differences were noted. The animals exposed to AE at a level of 8 mg/kg/day gained significantly less weight than the control animals during gestation days 0-3, but maintained weight gain comparable to the control group for the remainder of the gestation period. Gravid uterine weight, carcass weight, and net maternal body weight gain were all significantly reduced ($p<0.01$) in the 125 and 500 mg/kg/day groups.
Regarding the postnatal group, only one female delivered viable offspring (125 mg/kg/day); therefore evaluation of these data was not performed for practical reasons.

In general, animals exposed to AE at dose levels of 125 and 500 mg/kg/day consumed less food than the control group during most of gestation. The 8 mg/kg/day group consumed significantly less during GD 3-6 and animals exposed to 30 mg/kg/day consumed significantly less during the first half of gestation; at all other intervals, their food consumption values were comparable to control values. The animals in the 1000 mg/kg/day (GD 10-12) group consumed significantly less during and immediately following exposure to the test material.

Summary of Selected Maternal Weight Parameters

Dose (mg/kg/day)	**0**	**8**	**30**	**125**	**500 (GD 1-16)**	**1000 (GD 10-12)**	**0 (Post natal Gp)**	**125 (Post natal Gp)**
Body wt –at delivery (gr)	399.8	403.9	378.5	312.5b	288.5b	372.1	426.4	304.2b
GD 0-3 wt gain (gr)	13	5	-2b	-16b	19b	14	12	-21b
GD 3-6 wt gain (gr)	14	11	15	16	12	16	20	13
GD 6-10 wt gain (gr)	17	17	18	17	16	18	20	9a
GD 10-13 wt gain (gr)	19	29	16	11a	7b	1b	19	11a
GD 13-16 wt gain (gr)	21	24	17	0b	-10b	19	30	7b
GD 16-20 wt gain (gr)	60	63	51	20b	21	37b	54	12b
GD 0-20 wt gain (gr)	144	141	119	50b	27b	105b	154	31b
Postpartum day 0	--	--	--	--	--	--	332	315
Postpartum day 4	--	--	--	--	--	--	345	297
Gravid uterus (gr)	74.5	77.8	61.3	14.2b	4.5b	44.1b	--	--

Carcass (gr)/Final body weight	325.3	326.1	317.2	298.2b	284.0b	328.0	345	299
Net wt change from day 0 (e)	69.3	62.9	57.3	35.4	22.0b	61.0	--	--
Thymus weight (g)-absolute	0.246	0.255	0.205	0.142b	0.081b	0.114b	0.204	0.185
Thymus weight (g)-relative	0.0760	0.0773	0.0646	0.0475b	0.0284b	0.0315b	0.0585	0.0611
Liver weight - absolute (g)	15.2394.	15.982	16.280	15.557	16.798	17.741a	14.800	12.881
Liver weight (g)-relative	4.6752	4.8992	5.1284	5.2006a	5.9129b	5.3999b	4.2801	4.2929

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)
c)Statistically different from matched control ($p<0.05$)
d) Statistically different from matched control ($p<0.01$)
e) = Carcass weight minus day 0 body wt

A reduction in thymus weight was observed at the time of maternal necropsy; the incidence was dose-related. A significant reduction in absolute and relative thymus weight was noted in prenatal animals exposed to AE at dose levels of 125 mg/kg/day ($\geq$42.3% for absolute and $\geq$37.5% for relative) and higher. Relative liver weights were significantly increased in prenatal group animals exposed at these same dose levels ($\geq$11.3%). Only the 1000 mg/kg/day (GD 10-12 group) showed a significant increase in absolute liver weight. No other findings were observed

Reproductive parameters evaluated appeared to be adversely affected by AE exposure The number of dams with resorptions at the 125 and 500 mg/kg/day dose levels significantly increased ($p<0.05$). At the 125 mg/kg/day level, the number/percent resorptions also increased ($p<0.01$) significantly. The greater than two-fold increase in resorptions (compared to control) in the 30 mg/kg/day group was considered to be of biological significance. The litter size also decreased ($p<0.0l$) at 125 mg/kg/day. The 500 mg/kg/day group had a mean preimplantation loss of approximately 4.3%. This finding was not unexpected since some of the females in the 500 mg/kg/day group had embryos which were apparently resorbed very early in gestation such that some of the corresponding corpora lutea were regressed and could not be counted. Consequently, the number of implantation sites exceeded the number of corpora lutea and the result is a negative value for implantation loss. It appears that one control female had only one implantation site (resorbed) which accounted for the increased variation in (mean percent) resorptions for this group. Also noteworthy is the decrease in percent of females that were pregnant (67%) in the 500 mg/kg/day group. Whether this finding is due to interference with implantation or is stress-induced is uncertain; no dose-response relationship was noted.

Summary of Mean Selected Reproduction Data (Groups 1-6)

Dose (mg/kg/day)	0	8	30	125	500 (GD 1-16)	1000 (GD 10-12)
Implantation sites – mean	14.5	16.1	15.7	14.0	15.5	16.3
Viable fetuses	18.9	20.9	220	182	155	212
Litter size (e)	13.9	14.6	11.6	2.1b	0.2b	8.8b
Viable male fetuses (%)	49	49	48	59	50	55
Resorptions (mean)	0.6	1.5	4.1	11.9b	15.3b	7.5b
Resorptions (mean %)	11.8	9.0	27.3	82.3b	98.8b	44.9b
Dams with resorptions (%)	54	92	79	100a	100a	92

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

Of the hematology parameters examined, white blood cell and platelet counts were significantly affected. The 500 mg/kg/day group had a reduction in platelet count. An increase in white blood cell count was noted for the 125 and 500 mg/kg/day groups. A linear relationship (>99% confidence level) was found between dose and blood level for both of these parameters. Adverse effects on serum components were noted at the 125 and 500 mg/kg/day
dose levels. Aberrant serum chemistry values were obtained for urea nitrogen, aspartamine aminotransferase (AST), alkaline phosphatase, cholesterol, triglycerides, total protein, albumin, albumin/globulin ratio, uric acid, sodium, potassium, inorganic phosphorus, calcium, and iron. A linear relationship (>99 %; confidence level) was found between dose and serum level for each of the above mentioned components. When historical serum reference values were taken into consideration, the dose-response curves for all but uric acid, sodium, potassium, and inorganic phosphorus fell outside the normal defined by the 10^{th} and 90^{th} percentiles of the historical data

In general, a significant decrease in mean fetal body weight was observed in fetuses from dams exposed to AE at 125 mg/kg/day or greater. At the time of fetal gross examination, one fetus exposed in utero to 125 mg/kg/day
was edematous. Five fetuses (from four litters) exposed in utero to 1000 mg/kg/day on GD 10-12 exhibited various anomalies; two of the fetuses were edematous and the other three fetuses exhibited various anomalies including shortened limbs, shortened and missing digits, shortened trunk, cleft palate, and kinked tail. The incidence of each observation alone was not significant; however, the total number of affected fetuses observed in this group was significantly greater than the control group.

Fetal Endpoints – Weight and Gross Examination (Groups 1-6)

Dose (mg/kg/day)	0	8	30	125	500 (GD 1-16)	1000 (GD 10-12)
Fetal weights (g)	3.5	3.5	3.3	3.0b	2.9	2.7b
Litters evaluated	12	13	14	8	1	13
Fetuses - live	181	190	162	27	2	114
Fetuses – dead	0	0	0	0	0	0
Gross Exam (fetal incidence; %)	0; 0.0	0; 0.0	0; 0.0	1; 3.7	0; 0.0	5; 4.4b
Gross Exam (litter incidence; %)	0; 0.0	0; 0.0	0; 0.0	1; 13	0; 0.0	4; 31

a)Statistically different from control ($p<0.05$)
b)Statistically different from control ($p<0.01$)

A significant increase in rib malformations (costal cartilage misshapen) was observed for the 1000 mg/kg (GD10-12) group. Other malformations observed in the study appeared randomly and at a low frequency throughout the groups. Several fetal variations were noted more frequently in AE exposed fetuses than control fetuses, however, in most instances, the incidence was highest in the 1000 mg/kg group. It should be noted that the control group had a very high incidence of variations.

Fetal Endpoints – Skeletal Malformations and Skeletal Variations (Groups 1-6)

Dose (mg/kg/day)	0	8	30	125	500 (GD 1-16)	1000 (GD 10-12)
Litters evaluated	12	13	14	8	1	13
Fetuses - live	94	99	85	15	1	60
Fetuses – dead	0	0	0	0	0	0
Total skeletal anomalies (fetal incidence; %)	92; 98	89; 90a	82; 96	15; 100	1; 100	6; 100*
Total skeletal anomalies litter incidence; %)	12; 100	13; 100	15; 100	8; 100	1; 100	13; 100

*Significant increase in rib malformations was observed, but did not affect total.

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

Isolated incidences (in seven fetuses) of malformations were noted throughout the AE-exposed groups. These findings were not statistically significant from the control group.

Fetal Endpoints – Soft Tissue Anomalies (Groups 1-6)

Dose (mg/kg/day)	**0**	**8**	**30**	**125**	**500 (GD 1-16)**	**1000 (GD 10-12)**
Litters evaluated	12	13	14	6	1	11
Fetuses - live	87	91	77	12	1	54
Fetuses – dead	0	0	0	0	0	0
Total fetal soft tissue (fetal incidence; %)*	6; 6.9	8; 8.8	6; 7.8	2; 17	0; 0.0	7; 13
Total fetal soft tissue (litter incidence; %)*	5; 42	5; 38	5; 36	2; 33	0.; 0.0	5; 45

a)Statistically different from control (p<0.05)
b)Statistically different from control (p<0.01)

For the postnatal group, no treatment-related findings were noted at the time of maternal necropsy. Although the absolute and relative liver weights of the postnatal group animals were less than the weights of prenatal control group, this finding was not unexpected since the size of the liver increases during pregnancy and then decreases after parturition. The thymus and liver weights of these animals did not differ significantly from the weights of the control animals.

Three female in the AE exposed group were not pregnant; five females resorbed their entire litters and one dam had only two pups which she subsequently cannibalized. Since there was only one viable litter in this group, a meaningful evaluation of post partum effects was not performed for this study.

Summary of Pup Data (Groups 8 & 9)

Dose (mg/kg/day)	**0**	**125**
Pups delivered – total	147	9
Implantation sites (mean)	16.0	15.3c
Litter size- mean (total pups/litter)	14.7	4.5

Liveborn	14.7	7
Stillborn	0	2
Pup weights (gr) – Day 0	*	*
Pup weights (gr) – Day 4	*	*
Live pups/litter Day 0	14.7	7.0
Live pups/litter Day 1	14.4	7.0
Live pups/litter Day 2	14.4	7.0
Live pups/litter Day 3	14.3	7.0
Live pups/litter Day 4	14.3	7.0
Pups surviving 4 days (%)	*	*

*See text above for explanation
a)Statistically different from matched control ($p<0.05$)
b) Statisically different from matched control ($p<0.01$)
c) Includes females who did not deliver due to resorption of entire litter.

Bioavailability/Bioaccumulation Analyses
Dermal absorption of 14-C-carbazole occurred more extensive y than did 3-H-BaP over a 72 hour period. In spite of the dermal bioavailability of 14C-carbazole and 3H- BaP in the dam, the amount of radiolabeled material found in the embryo was very low. These findings indicate that although the radioactive-labeled components can penetrate the skin of the dam, the placenta acts as an effective barrier against carbazole and BaP transport to the embryo. There was no evidence indicating that either of these components accumulates selectively in the embryo.

Conclusion:

Determined by the reviewer:
The maternal NOAEL for dermal exposure to AE for GD 1-19 was determined to be <8 mg/kg/day (LOAEL= 8 mg/kg/day based on increased red vaginal discharge)
The developmental NOAEL for dermal exposure for GD 1-19 was determined to be 8 mg/kg/day (LOAEL = 30 mg/kg/day, based on increased resorptions).

RELIABILITY/DATA QUALITY

Reliability: Valid Without Restrictions (KS=1)

Reliability Remarks: Comparable to guideline study with sufficient detail

Key Study Sponsor Indicator: Key

REFERENCE

Reference:

API. 2008. PAC Analysis Task Group, “The relationship between the aromatic ring class content and selected endpoints of repeat-dose and developmental toxicity of high-boiling petroleum substances.” http://www.petroleumhpv.org/pages/pac.html, accessed 31 Dec 2009

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