HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

WAXES AND RELATED MATERIALS CATEGORY

Submitted to the US EPA

by

The Petroleum HPV Testing Group

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Plain Language Summary

This test plan addresses the refinery streams and finished products involved in the production of petroleum waxes and related materials. The materials in this category are complex petroleum mixtures composed of hydrocarbons with carbon numbers ranging from C12 to C85, with the majority exceeding C20. All the materials in the category are solid or semi-solid at room temperature. The physical state (semi-solid to solid) and the number of carbon atoms in the petroleum waxes severely limit their bioavailability and their environmental distribution, breakdown, or conversion.

Based on the degree of processing and level of residual oil and impurities, the streams and products within this category can be divided into three subcategories:

- unfinished wax (slack wax),
- refined/finished wax (paraffin and/or microcrystalline wax), and
- petrolatum (petroleum jelly).

In addition to being the precursors to refined/finished waxes and petrolatum, slack waxes are generally limited to industrial applications such as lubricants and specialty cleaning/preservative products. Refined/finished waxes and petrolatum (petroleum jelly) have a large and varied number of industrial and consumer product applications. They may be used in lubricants, wire cables, candles, vaseline, cosmetic, and food/drug applications.

Of the three subcategories of waxes, slack wax contains the greatest amount of base oil and impurities as well as having the largest variation of hydrocarbon molecules. Refined/finished waxes and petrolatum are produced from slack waxes by removing these impurities and base oils. Based on the existing data and the physical/chemical nature of these materials, the Testing Group expects the potential toxicity of any of the wax category members will arise primarily from the base oil component of the wax. The base oils will be addressed as a category in the Lubricating Oil Basestocks HPV Test Plan.

After evaluating the extensive toxicology database for the three sub-categories of waxes and using read-across information within the waxes sub-categories and the Lubricating Oil Basestocks HPV Test Plan, the Testing Group is proposing to perform selected toxicity testing on the least refined stream (slack wax) in order to fill any existing data gaps for the Waxes category. The Testing Group thinks the toxicity testing of slack wax will address the hazards of all three subcategories of waxes found in this category. This is because slack wax, as the least processed of the materials, contains the broadest spectrum of chemical components and highest concentration of bioavailable/biologically active components of all the materials addressed in this Test Plan. The use of slack wax as a test sample therefore maximizes any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken. The studies on slack wax will include:

1) Mammalian Toxicity – Repeated Dose Reproductive/Developmental Screening study (OECD 422)
2) Genetic Toxicity – *In Vitro* Bacterial Reverse Mutation (OECD 471) and *In Vivo* Mammalian Erythrocyte Micronucleus test (OECD 474)

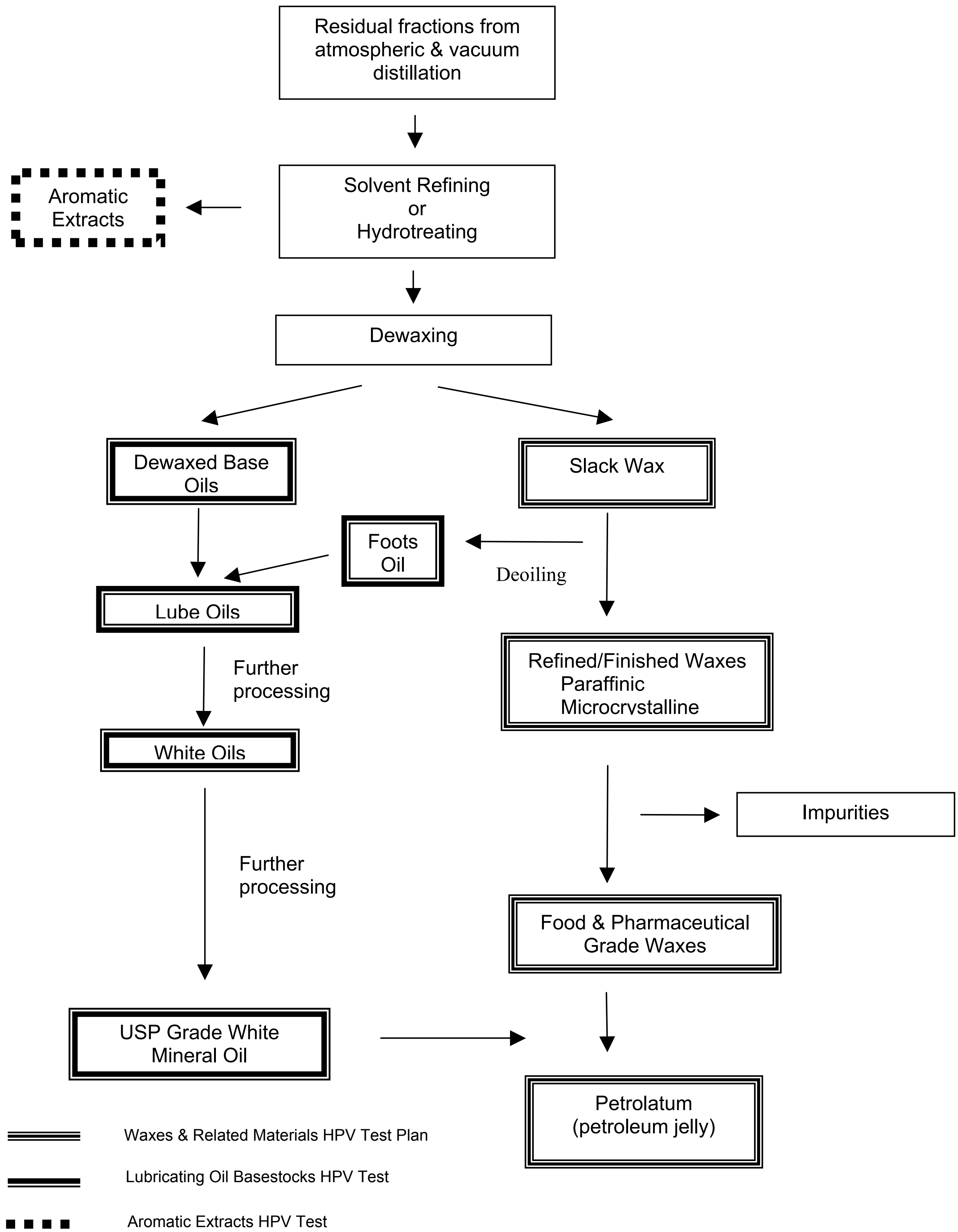
For all three sub-categories of waxes (slack waxes, finished/refined waxes and petrolatum), when physicochemical data did not exist or was impractical to obtain, calculated physicochemical and environmental data for selected constituents of waxes has been developed using the EPIWIN© computer model**.**

The Testing Group thinks the existing ecotoxicity data on waxes adequately describes their potential toxicity. Therefore the Testing Group is proposing no additional ecotoxicity testing on any of the waxes or related category members.

Description of the Waxes and Related Materials Category

The Waxes and Related Materials category includes both refinery streams and finished products. The materials in this category are complex petroleum mixtures composed of predominantly saturated hydrocarbons with carbon numbers ranging from C12 to C85, with the majority exceeding C20. Because they are complex mixtures, the petroleum waxes and related materials are typically defined by process history and product use specifications, not by detailed compositional information that identifies every specific individual molecular compound. All the materials in the category are solid or semi-solid at room temperature with very low volatility and water solubility values. As shown in the Figure 1, the materials included in the category are produced by a series of processing steps that separate the wax and oil portions of selected refinery streams. If present, the biologically available/active impurities such as PAC/PNA (polycyclic aromatic compounds/polynuclear aromatics) and unsaturated chains (olefins) or rings (aromatics) are found in the oil component. At each process step, the oil and impurities content of the wax(es) is lowered. Materials similar to the oil component of the waxes are included in the Lubricating Oil Basestocks HPV Test Plan. While waxes are composed primarily of linear alkane molecules, the compounds in the Lubricating Basestocks category contain primarily of branched-chain alkanes and naphthenics.

Figure 1. Schematic of the Production Process for Waxes and Related Materials



A detailed description of petroleum refining, including the production of the waxes and related materials can be found in the OSHA Technical Manual (OSHA, 1999).

Based on the degree of processing they receive (and the corresponding reduction in oil content and impurities) and their physical properties, the eight substances in the "waxes and related materials" category can be divided into three sub-categories, as shown in Table 1.:

Table 1. Category Members

Sub-category	CAS #[1]	Substance
Slack Waxes	64742-61-6	Slack wax (Petroleum)
Refined/finished Waxes		
(Paraffin)	8002-74-2	Paraffin Waxes and Hydrocarbon waxes
	64742-43-4	Paraffin waxes (petroleum), clay treated
	64742-51-4	Paraffin waxes (petroleum), hydrotreated
(Microcrystalline)	63231-60-7	Paraffin waxes and hydrocarbon waxes microcrystalline
	64742-42-3	Hydrocarbon waxes (petroleum), clay-treated microcrystalline
	64742-60-5	Hydrocarbon waxes (petroleum), hydrotreated microcrystalline
Petrolatum	8009-03-8	Petrolatum (Petroleum Jelly)

[1]Appendix A contains complete CAS descriptions

Slack waxes are generally limited to industrial applications such as lubricants and specialty cleaning/preservative products. Consequently, human exposures are primarily through the dermal route. As can be seen from the process diagram, slack waxes may undergo additional processing to produce finished/refined waxes and petrolatum. Slack waxes derived from low viscosity oils contain predominantly normal paraffins. Heavier oil fractions yield slack waxes with increasing proportions of isoparaffins, cycloparaffins and alkylated aromatics in addition to the normal paraffins. Commonly, slack waxes are derived from solvent-refined vacuum distillates, in which case they contain a very low content of alkylated aromatic hydrocarbons.

The Testing Group has been unable to locate detailed compositional information on slack waxes, particularly information on their PNA content. This is not surprising, since as noted earlier, the petroleum waxes and related materials are typically defined by process history and product use specifications, not by detailed compositional information. However, API has collected compositional information on vacuum residuum, the refinery stream from which slack wax is derived (as represented by the top box in Figure 1). Information received from 8 companies and analysis of 2 toxicity test samples showed aromatic contents ranging from 34.7 to 65.0 wt % (API, 1983; API, 1987). As part of the process of converting vacuum residuum to slack wax, the aromatic content of the refinery stream is reduced via an extraction step. Thus, the aromatic content of vacuum residuum represents the "worst case" with regard to aromatic content of slack wax.

Refined/finished waxes have a large and varied number of industrial, commercial, and consumer product applications. They may be used in lubricants, wire cables, candles, petroleum jellies, cosmetics, and food/drug applications. Thus, human exposures may be via both the dermal and oral routes. Refined/finished waxes that are intended for food, food contact, cosmetic, pharmaceutical and related applications have to meet stringent purity requirements as described in the respective national and international legislations. These generally specify melting ranges, color, polycyclic aromatic hydrocarbon content and other impurity limits. For instance, in regard to the polycyclic aromatic hydrocarbon content, the U.S. FDA has established ultraviolet absorbance limits that set an upper limit of 0.5 mg/kg for the total concentration of all extractable PAH-compounds in the wax sample tested (U.S. FDA, 2001; CONCAWE 1984). The refined waxes may be divided into two subgroups based on melting point, the paraffinic waxes (lower melting paraffin waxes) and the microcrystalline waxes (or high melt waxes). The former are obtained from processing light lube distillate while the latter are obtained from processing vacuum residue, or heavier lube distillate. Paraffin waxes consist mainly of normal alkanes, varying amounts of isoalkanes, cycloalkanes and a very low concentration of alkylated aromatic hydrocarbons. Microcrystalline waxes consist of substantial amounts of iso- and cycloalkanes, usually with a lesser amount of normal alkanes and trace amounts of alkylated aromatic hydrocarbons.

Food-grade petrolatum (petroleum jelly) is a mixture of highly refined higher-melting paraffin wax and typically, greater than 10% USP-grade white mineral oil (evaluated in the Lubricating Oil Basestocks HPV Test Program). Petrolatum consists mainly of branched and straight chain alkanes. Petrolatum has a large and varied number of industrial, commercial, and consumer product applications. Like refined/finished waxes, it may be used in lubricants, wire cables, candles, petroleum jellies, cosmetics, and food/drug applications. And, as with refined/finished waxes, human exposures may occur by both dermal and oral routes. When used in food, food contact, cosmetic, pharmaceutical and related applications, petrolatum has to meet stringent purity requirements as described in the respective national Pharmacopoeia and international legislations. These regulations generally specify melting ranges, color, polycyclic aromatic hydrocarbon content and other impurity limits. As with food-grade refined/finished waxes, in regard to the polycyclic aromatic hydrocarbon content, the U.S. FDA has established ultraviolet absorbance limits that set an upper limit of 0.5 mg/kg for the total concentration of all extractable PAH-compounds in the wax sample tested (U.S. FDA, 2001; CONCAWE, 1984).

The physical and chemical properties that characterize the three subcategories of waxes are summarized in Table 2 (Bennet, 1975; Kauffman et al., 1993; EWF, 1990).

Table 2. Physicochemical Properties of the Three Subcategories of Waxes

	Oil content (%m/m)	**Carbon number range***	**Melting point (°C)**	**Kinematic viscosity at 100°C (mm^2/s)****
Slack wax	2 - 30	C-12 - C-85	43 - 63	3 – 30
Refined/finished waxes				
Paraffin Wax	<2.5	C-18 – C-75	43 - 74	3 – 10
Microcrystalline Wax	< 5	C-23 – C-85	60 - 95	10 – 30
Petrolatum	> 10***	C-12 – C-85	36 - 60	3 – 30

* includes the range of any oil components
** Kinematic viscosity is also expressed in Centistokes (cSt). $1 mm^2/s$ = 1 cSt
*** USP-grade white mineral oil

<u>Category Rationale and Test Material Description</u>

The Testing Group has used the following assumptions when analyzing the existing data, proposing testing and selecting a test material:

1. The materials included in the Waxes and Related Materials category are similar from both process and physical/chemical perspectives
2. Materials in the category are composed of varying ratios of two major components:
   - waxes
   - oil
3. The physical state (semi-solid to solid) and the number of carbon atoms of the wax component severely limit its bioavailability and its environmental distribution, breakdown, or conversion. Toxicity data on refined/finished waxes and petrolatum can be used to characterize the wax component of all members of this category.
4. The potential toxicity of the materials included in the Waxes and Related Materials category is associated with the biologically available/active impurities such as PAC/PNA (polycyclic aromatic compounds/polynuclear aromatics) and unsaturated chains (olefins) or rings (aromatics).
5. These biologically available/active impurities are found in the oil component of the materials in the category, not in the wax component. Materials similar to the oil component of the materials in this Test Plan are included in the Lubricating Oil Basestocks HPV Test Plan. Because of this similarity, information from the Lubricating Oil Basestocks HPV Test Plan may be used by the Testing Group to augment information contained in the Waxes HPV Test Plan.
6. In the process of converting slack waxes to refined/finished waxes and then to food-grade waxes/petrolatum, oil containing potentially biologically available/active impurities is removed from the material. Consequently, the potential toxicity of the materials decreases as they are processed from slack wax to refined/finished waxes to food-grade waxes/petrolatum.

To supplement existing data on the various sub-categories of waxes, the Testing Group will conduct selected toxicity tests on a representative sample of slack wax. The Testing Group thinks the toxicity testing of slack wax will address the HPV data needs of all three subcategories of waxes found in this category. Of the three subcategories of waxes, slack wax is the least refined stream. It therefore contains the broadest spectrum of chemical components of all the categories of materials addressed in this category. The use of slack wax as a test sample will maximize any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken.

The Testing Group is planning to conduct the slack wax mammalian toxicity testing via the dermal route because:

1. the physical/chemical nature of slack wax (semi solid),
2. the primary route of human exposure to this material is dermal, and
3. administration via the dermal route minimizes the potential "first pass" metabolism by the liver of the biologically available/active impurities.

The Testing Group thinks the test results obtained on slack wax via the dermal route of exposure can be "read across" to both dermal and oral routes of exposure for the refined/finished waxes and petrolatum because:

1. historic data shows absorption of PNAs via both dermal and oral routes of exposure in the rat,
2. as noted above, administration via the dermal route minimizes the potential "first pass" metabolism by the liver of the biologically available/active impurities

Specific analytical data on the slack wax test sample will be available when the sample is obtained. The test sample will have physicochemical properties similar to those shown in Table 2. The Testing Group will attempt to select a test sample with a high oil and PNA/impurities content, thereby maximizing the sample's potential biological activity.

Coordination with Other Test Programs and Plans

To avoid duplication of effort and unnecessary use of animals, the Petroleum HPV Testing Group is coordinating its efforts with two of its other test plans. To this end, the Testing Group is relying on the

Lubricating Oil Basestocks and Aromatic Extracts Test Plans to provide supplementary data for the oil and aromatic components of the materials in the waxes category.

Evaluation of Existing Health Effects Data and Proposed Testing

General Evaluation

The Test Plan addresses the health effects endpoints of the category by:

- evaluating the extensive toxicology database for the three sub-categories of waxes (slack waxes, finished/refined waxes and petrolatum),
- using read-across information whenever possible among and between the sub-categories,
- maximizes any efforts to detect adverse effects by proposing to perform selected toxicity testing on slack wax, the category member that contains the broadest spectrum of and highest concentration of potentially bioavailable/biologically active components, and
- anticipating the availability of "read-across" information from the Lubricating Oil Basestocks HPV Test Plan to further characterize the oil component of the category members.

Toxicological data on waxes have been reviewed by an expert panel of the Cosmetics, Toiletries and Fragrances Association (CTFA, 1981) and the review was published in the Journal of the American College of Toxicology (Elder, 1984). The EU Scientific Committee for Food (SCF) and the World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) also assessed the safety of waxes for use as food additives and as food contact materials. The outcome of their reviews and the oral allowable daily intakes (ADIs) that were established has been published (SCF, 1995; JECFA 1996).

Acute Toxicity

Slack waxes

There are no acute toxicity data available for the slack waxes. However, acute toxicity data does exist for both the more highly refined waxes and the base oils that contain the potentially biologically available/active portion of these materials. (Materials similar to the base oils are included in the Lubricating Oil Basestocks HPV Test Plan). Since these represent the two components of the slack waxes, the Testing Group thinks the existing information can be extrapolated to the slack waxes.

Refined/finished waxes

Oral LD_{50} values greater than 5000 mg/kg have been found for samples of both paraffin and microcrystalline waxes (IBR, 1976 a & b). A 50/50% solution of petrolatum and a paraffin wax resulted in a dermal LD_{50} > 4000 mg/kg and slight eye irritation. Paraffin and microcrystalline waxes have been reported to be non- and slightly irritating to the skin, respectively. The information on the non-oral endpoints is taken from a published safety review conducted by an expert panel (Elder, 1984). While few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified, the Testing Group thinks the information is of sufficient quality to allow it to be used to fulfill the data needs for the acute toxicity endpoint.

Petrolatum

No acute oral toxicity data are available. The acute toxicity potential can be estimated from information on refined/finished waxes and the highly refined base oils used to produce these materials (included in the Lubricating Oil Basestocks HPV Test Plan).

A 50/50% solution of petrolatum and a paraffin wax resulted in a dermal LD_{50} > 4000 mg/kg and slight eye irritation. The information is taken from a published safety review conducted by an expert panel (Elder, 1984). Whereas few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified, the Testing Group thinks the information is of sufficient quality to allow it to be used to fulfill the data needs for the acute toxicity endpoint.

Because of their food grade application, a significant amount of acute, subchronic, chronic, reproductive, and sensitization testing has been done on white mineral oils, the oil component of petrolatum. These data are discussed in the HPV Lubricating Oil Basestocks HPV Test Plan.

Summary: No additional testing is planned. Multiple acute toxicity studies have been reported on the waxes and the base oil streams from which they are derived. Ancillary data is also available on the consumer products in which the refined/finished waxes and petrolatum are used, i.e. cosmetics. All these studies have consistently found these materials to have low acute toxicities. The Testing Group thinks the existing data is sufficient to characterize the acute toxicities of this category of materials.

Repeated Dose Toxicity

Slack waxes

Three skin carcinogenicity studies have been reported on slack wax. The quality of these three carcinogenicity studies on slack wax cannot be verified since the reports do not provide a complete set of experimental details. Only one of the studies tested a wax produced by solvent extraction, the current method of preparation. The other two reports are on waxes produced by the older process of "pressing" unfinished or poorly finished materials. When compared to the waxes currently produced, the older “pressing” process resulted in waxes with higher levels of biologically available/active impurities such as PAC/PNA (polycyclic aromatic compounds/polynuclear aromatics) and unsaturated chains (olefins) or rings (aromatics).

The two slack waxes that were produced by the solvent extraction process currently used in refineries were not tumorigenic after application of 25 mg to the backs of male mice 2 days/week for 80 weeks (Kane et al., 1984).

Eight slack waxes (produced by the older process of “pressing”) and the corresponding aromatic extract[1] fractions were tested in a lifetime skin painting study (Smith et al., 1951). Approximately 15 mg of test material were applied 3 days/week to the backs of male albino mice (groups of n=30). At 250 days, the authors reported the slack waxes showed a low order of carcinogenicity, benign skin tumors were observed in 6 of the 8 groups and malignant skin tumors in 2 groups. At 450 days, benign skin tumors had developed in all groups, but malignant tumors had developed in only 5 of the 8 groups. The aromatic extracts exhibited a greater potency. At 250 days all but one sample had produced papillomas and 5 samples had produced cancers. At 450 days all had elicited benign tumors and all but one sample had elicited cancers. The authors concluded that the slack waxes were weakly carcinogenic and that carcinogenic activity was caused by the aromatic compounds found in the oils component, rather than the paraffinic waxes component. Similar results were produced by an additional study from the same laboratory on 11 slack waxes (again, produced by the older process of “pressing”) (Dietz et al., 1952).

Included in the Testing Group’s weight of evidence analysis was the fact that all three studies were reported in the peer review literature and had results consistent with not only each other, but numerous other dermal carcinogenicity studies done on petroleum hydrocarbons. The Testing Group thinks the weight of evidence provided by the aggregate results of these studies are adequate for the carcinogenic endpoints evaluated. These studies also demonstrate the dermal absorption of the biologically active compounds.

However, because the reports of these studies lack experimental details, the Testing Group has concluded the studies do not provide an adequate database for assessing the potential non-carcinogenic repeated-dose toxicity of slack wax.

Refined/finished waxes

A series of 90-day rodent feeding studies have been conducted on various white mineral oils (possible components of petrolatum) and food-grade low and high melting point refined/finished waxes (BIBRA, 1992; 1993; 1999). The waxes were incorporated in the animals’ diets at concentrations up to 2.0% by

[1] Aromatic extracts are products derived from the aromatic components extracted from the residual fractions of atmospheric & vacuum distillation the fractions are processed into slack waxes.

weight. Animals dosed with either low viscosity oils or a low melting point paraffin wax had evidence of inflammatory changes in the liver, spleen, and lymph nodes and macrophage. Higher molecular weight oils and microcrystalline waxes were without effect). In addition, low molecular weight (low-melting point) waxes unexpectedly produced heart mitral valve accumulation of waxy/crystal material in a single strain of rat (Fischer 344), an effect not observed with any other test materials.

Recent, multiple lifetime chronic/cancer studies of medium and high molecular weight white oils confirmed the earlier wax and oil studies (These studies will be assessed in the Lubricating Oil Basestocks HPV Test Plan).

In an earlier study reported by Shubik et al. (1962) the toxicity of a variety of refined/finished waxes were investigated via the oral, dermal and subcutaneous routes of exposure. Five refined/finished wax samples were fed to male and female rats at a dietary concentration of 10% (approximately 5000 mg/kg/day) for a period of 2 years. There were neither treatment-related changes in survival or growth, nor any abnormal effects at necropsy or upon histological examination. Tumor incidence was unaffected by treatment. The chronic dermal exposure studies were conducted in both mice and rabbits. Five refined/finished waxes (15% concentration in benzene) were applied 3 times per week throughout the animals' lifetimes. Treated groups showed mild irritation (limited to desquamation and depilation) that persisted throughout the study. However, there was no evidence of treatment-related tumor incidence or other effects. This study was not reported thoroughly, nor had it been completed at the time the paper was published. Due to the lack of experimental detail in the published report and the fact the study was done before the GLP guidelines were established, the Testing Group is unable to assess the reliability of the rabbit portion of the study.

Finally, Shubik et al. (1962) implanted subcutaneously into groups of male and female mice disks made of 5 different refined/finished waxes. The Testing Group does not consider this to be a relevant route of exposure for human exposure. Consequently, the results from these studies are not discussed in this Test Plan.

Petrolatum
In an oral carcinogenicity study, 3 blends of pharmaceutical and food-grade petrolatums were fed to male and female rats at dietary concentrations of 5% for 2 years (Oser et al., 1965). No treatment-related changes were observed during treatment (body weight, blood chemistry, and hematological endpoints), at necropsy (weights of liver, spleen, kidney, heart, adrenals, thyroid, and pituitary), or through histological examination (a range of tissues). None of the three petrolatum blends produced an increase in tumor incidence.

The 1965 report by Oser et al. also included the results of a chronic study of petrolatum administered subcutaneously. The Testing Group does not consider this to be a relevant route of exposure for human exposure. Consequently, the results from these studies are not discussed in this Test Plan.

There are reports of two dermal carcinogenicity studies on petrolatum. In the first study, no tumors developed following application of two samples of petrolatum (25 mg twice weekly for 80 weeks) to male mice (Kane et al., 1984). The quality of this work cannot be verified since the report that includes these results is a summary of an extensive program of studies and does not include all the experimental details.

In the second study, 60 µl of a 15% solution of amber petrolatum (petrolatum NF grade) in iso-octane was applied twice weekly to the skin of male and female mice for 2 years. It was concluded that amber petrolatum was not carcinogenic (Lijinsky et al., 1966).

Summary: Slack wax will be tested via the dermal route using a 28-day combined, repeated dose and reproductive/developmental toxicity screening protocol (OECD Test Guideline 422). The Testing Group thinks the existing chronic toxicity studies on slack waxes do not adequately address non-carcinogenic endpoints.

Reproductive Toxicity

No studies have been reported on the reproductive toxicity of petroleum waxes and related materials.

Summary: Slack wax will be tested via the dermal route using a 28-day combined, repeated dose and reproductive/developmental toxicity screening protocol (OECD Test Guideline 422). The Testing Group thinks the testing of slack wax will address the hazards of all three sub-categories of waxes found in this category. This is because slack wax, as the least processed of the materials, contains the broadest spectrum of chemical components of all the categories of materials addressed in this program. The use of slack wax as a test sample therefore maximizes any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken.

Genotoxicity

No studies have been reported on the genotoxicity of petroleum waxes and related materials.

The Testing Group thinks the genotoxicity testing of slack wax will screen all three sub-categories of waxes found in this category. This is because slack wax, as the least processed of the materials, contains the broadest spectrum of chemical components of all the categories of materials addressed in this program. The use of slack wax as a test sample therefore maximizes any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken.

While the Testing Group shares the desire to limit animal testing by using *in vitro* methodologies when possible, it decided to conduct the *in vivo* micronucleus test for the following reasons:

1. the physical/chemical nature of the test material precludes testing the intact material *in vitro*,
2. it could be performed using animals that were already included in the repeat dose 28-day study, and
3. it eliminates the need to perform an additional study solely for the purpose of studying *in vivo* genotoxicity.

Summary: Slack wax will be tested in the *in vitro* bacterial reverse mutation assay (OECD 471) and the *in vivo* mammalian erythrocyte micronucleus test (OECD 474). The *in vivo* micronucleus test will be included in the 28 day repeat dose study on slack wax (see "Repeated Dose Toxicity" section).

Human Experience

In addition to the animal studies summarized above, clinical studies and human experience with the use of materials in this category are also available. The information is consistent with the published animal toxicology studies and the program of testing recommended in this test plan.

Slack wax

There have been several reports of cancer in wax pressmen exposed to unfinished/poorly finished oil during the preparation of paraffin wax (Hendricks et al., 1959; Lione and Denholm, 1959). Due to the refining processes at the time, these oils contained high concentrations of polycyclic aromatic hydrocarbons. However, current refining processes are much more stringent and consequently the oils contain much lower levels of polycyclic aromatic hydrocarbons in the oil. .

Refined/finished wax

Clinical studies with two undiluted paraffin waxes and formulated products containing various concentrations of paraffinic (5-16%) and microcrystalline (4.35-15%) waxes were reviewed (Elder, 1984). These studies included a range of acute and repeat application tests in groups of humans to observe skin irritation and skin sensitization potentials. All products produced, at most, slight erythema, and none caused skin sensitization.

The widespread use of paraffin wax in cosmetics and in cosmetic surgery over many years demonstrates the low toxicity of finished waxes and the many guidelines for their safe use (Hjorth, 1987). There have been few reports of adverse effects, such as subcutaneous deposits following injection under the skin (often referred to as paraffinoma), but these are not normally associated with progressive changes (Ho et al., 2001). Another report described an outbreak of skin rashes attributed to occupational exposure to wax fumes (Halton and Piersol, 1994).

Petrolatum

Despite the widespread use of petrolatum for many years as a vehicle in human skin patch testing, only isolated cases of allergy to petrolatum have been reported (Frankel, 1965; Dooms-Goosens and Degreef, 1983; Ayadi and Martin, 1987; Fisher, 1981; Conti et al., 1995).

Evaluation of Existing Physicochemical and Environmental Fate Data and Proposed Testing

General Evaluation
Based on the measured and predicted behavior of the constituent hydrocarbons these substances are nonvolatile materials, do not contain any oxidizing constituents, and are almost totally insoluble in water. Therefore the hydrocarbon components of these substances will have little or no tendency to partition to air, are not susceptible to hydrolysis or direct photolysis under environmental conditions, and will partition primarily to soil and sediment.

Physicochemical Data
Measured data for specific physicochemical properties of the representative substances in the waxes category that can be used in the HPV chemicals program were not available. There are estimation structure-activity relationships for the physicochemical endpoints in the computer program EPIWIN (Estimation Program Interface for Windows) and EPA has suggested that subroutines in this program would be acceptable to develop data for these endpoints. Because of the diversity of compounds encompassing waxes, it is not feasible to model the physicochemical endpoints for each potential compound. Rather, modeling efforts were directed towards those compounds of the waxes that would most likely be dispersed to various environmental media. Since molecular weight and structural conformation determine in large part the solubility and vapor pressure characteristics of the hydrocarbons, modeling focused on paraffinic, napthenic, and aromatic compounds containing thirteen carbon atoms. The C13 compounds are among the shorter chain-length molecules present in waxes and comprise only a small fraction of most waxes.

Melting Point
Adequate data exist.

Boiling Point
Adequate data exist.

Vapor Pressure
Adequate data exist.

Partition Coefficient (Log K_{ow})
The percent distribution of the hydrocarbon groups (i.e., paraffins, naphthenes, and aromatics) and the carbon chain lengths determines in part the partitioning characteristics of the mixture. Generally, hydrocarbon chains with fewer carbon atoms tend to have lower partition coefficients than those with higher carbon numbers (CONCAWE, 2001). However, due to their complex composition, unequivocal determination of the log K_{ow} cannot be made. Rather, partition coefficients of selected C13 chain length hydrocarbon structures representing paraffinic, naphthenic, and aromatic constituents in base oil lubricants were modeled using the EPIWIN©, WSKOW V1.40 computer model (U.S. EPA, 2000). Results showed typical log K_{ow} values from 4.9 and higher, which was consistent with values of >4 for lubricating oil base-stocks reported by CONCAWE (1997).

Summary: No additional modeling is proposed. Partition coefficients (K_{ow}) have been calculated for representative components of C13 hydrocarbon structures.

Water Solubility
As noted for partition coefficient, the water solubility of waxes cannot be determined due to their complex mixture characteristics. Therefore, the water solubilities of individual C13 hydrocarbons representing the different groups making up waxes (i.e., linear and branched paraffins, naphthenes, and aromatics) were modeled using WSKOW V1.40. Based on water solubility modeling of those groups (typically much less than 1 ppm), waxes should be considered almost totally insoluble in water.

Summary: No additional modeling is proposed. Water solubility values have been calculated for representative wax components.

Environmental Fate Data

The typical battery of tests used to measure the environmental fate of a material is not easily performed on the materials of this category because of their physical and chemical properties.

Photodegradation

Chemicals having potential to photolyze have UV/visible absorption maxima in the range of 290 to 800 nm. The hydrocarbon constituents in this category are not expected to photolyze since they do not show absorbance within the 290-800 nm range.

Although wax components typically have low vapor pressures some lower molecular weight components (e.g., C13 branched paraffins and naphthenes) may volatilize, thus creating the potential for atmospheric oxidation. However, these lower molecular weight compounds exhibit atmospheric oxidation half-lives of less than one day (12 hours). Therefore, those compounds that may partition to the atmosphere will be removed through indirect photochemical degradation. Calculation of atmospheric oxidation potential (AOP) was applied to specific hydrocarbons in this category in order to estimate the range of vapor-phase reactivity. The AOP was determined using the EPIWIN© model, AopWIN V1.90 (U.S. EPA, 2000).

Summary: No additional modeling is proposed. Atmospheric half-lives have been calculated for various C13 paraffins, naphthenes and aromatics.

Stability in Water

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 because they lack functional groups that hydrolyze.

Summary: **Computer modeling will not be conducted for materials in the waxes category because they do not undergo hydrolysis.**

Chemical Transport and Distribution in the Environment (Fugacity Modeling)

The US EPA has agreed that computer-modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model. The EQC model is a Level 1 (i.e., steady state, equilibrium, closed system and no degradation) model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment. EPA cites the use of this model in its document titled, "Determining the Adequacy of Existing Data, which was prepared as guidance for the HPV chemicals program." The model was used to estimate the percent distribution in environmental media of various C13 to C29 compounds representing the different classes of hydrocarbons found in waxes. The default model assumptions were used when performing the fugacity estimates.

Summary: **No further modeling is proposed**. Fugacity modeling has been done to provide an estimate of the percent distribution in environmental media of various C13 to C29 compounds representing selected hydrocarbon classes found in waxes.

Biodegradation

The assimilation of paraffin waxes has been described for a diverse range of microorganisms in laboratory studies. A wide range of bacteria, yeasts and fungi grows on paraffin wax as a sole carbon source under aerobic conditions (Rahn, 1906; Sohngen, 1913; Fuhs, 1961; Miyamoto, 1968). Initial decomposition of wax is similar to the degradation of short chain alkanes (Miyamoto, 1968). Assimilation of a highly finished paraffin wax containing 91% normal paraffins from C-25 to C-37 (mainly C28 to C32) occurs in several species of bacteria and yeast (Yamada and Yogo, 1970). Also, yeast cultures grown on paraffin wax results in typical cell yields of 70%, although the paraffin wax is not completely utilized (Miller and Johnson, 1966).

Inherent biodegradability studies have been performed on samples of paraffin wax (CAS No 8002-74-2), and microcrystalline wax (CAS No 63231-60-7) using a shake-flask procedure (referred to by the authors as OECD 301B Modified Sturm test) in which the materials were exposed on glass fiber filters (Hanstveit, 1990). In these tests, the paraffin wax (CAS No. 8002-74-2) degraded by 80% after 28 days and by 87% after 84 days and the microcrystalline wax (CAS No. 63231-60-7) degraded by 21% after 28 days and by 25% after 84 days. API data (API, undated) on paraffin and microcrystalline waxes in a shake-flask test using un-acclimated inocula from sewage sludge and soil generally corroborate the mineralization data from the Hanstveit (1990) study. In the API tests (see Section 3.5 of the Robust Summary for details), the paraffin wax mineralized by 55% in 31 days (comparable to 28-day data) and by 98.5% in 137 days (API, undated). Comparison of the results of the Hanstveit (1990) and API studies for paraffin waxes indicates both that microbial communities may well become enriched in capable species during an acclimation period, based on the day 28 inherent versus day 31 ready biodegradation results (80% versus 55%), and that the hydrocarbons remaining after the standard incubation period continue to be mineralized, based on the day 84 and day 137 results. Conversely, the microcrystalline wax results indicate that acclimation does not tend to enhance the degree of biodegradation of these more-complex materials. In particular, the presence of the higher molecular weight hydrocarbons seems to be a limiting factor, in that they are not biologically available for metabolism. The extended incubation results obtained in the company studies of microcrystalline wax (27% mineralization after 31 days, and 67% after 137 days) indicate some potential for increased degradation over longer periods, contrary to the other tests using this material. This may arise as a result of different enzymatic capabilities of the inocula, or as an artifact of the extended incubation time.

An environmental study was conducted on the decomposition of paraffin and microcrystalline waxed paper samples in a woodland leaf litter layer (Hanstveit, 1991). During a six-month winter period, degradation of paraffin waxed paper samples occurred almost completely in 5 mm mesh bags with a half-life of approximately two months. Lower decomposition rates occurred in the summer period and for the microcrystalline waxed paper sample. Both soil micro- and macro-organisms contributed to the decomposition under field conditions.

Evidence of the ready biodegradability of slack wax was provided by a study conducted on a hydrotreated slack wax (CAS No. 92062-09-4) (Exxon Biomedical Sciences, 1995). 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), which is a member of this category. When the material was used in a manometric respirometry test (OECD Guideline 301F), the mean biodegradation after 28 days was 40%, indicating that slack wax, while not recalcitrant to biodegradation, is not considered readily biodegradable. These results are supported by ready biodegradability data compiled and reviewed by CONCAWE (2001) on three samples of hydrotreated slack wax of differing viscosities. Those data showed 28-day biodegradabilities of 26%, 41%, and 48% using a modified Sturm procedure (OECD Guideline 301B). As with the previous evidence, these results demonstrate that slack waxes have a propensity to biodegrade, but cannot be considered readily biodegradable.

No known biodegradability studies on petrolatum exists, although information on slack and refined waxes and the characteristics of the differing components of petrolatum provide a substantial body of evidence to assess the biodegradability of this product. As described earlier in this test plan, refined/finished waxes are further refined into food and pharmaceutical grade waxes. These in turn are combined with USP Grade white mineral oil to produce petrolatum. Two ready biodegradability tests (OECD 301B) of a white mineral oil (detailed in the Lubricating Oil Basestocks test plan) showed 0% and 24% degradation after 28 days. Therefore, the white mineral oil component of petrolatum is not considered readily biodegradable. The remaining wax fraction consists of the C18 to C85 alkanes characteristic of the refined/finished wax subcategory. Given that neither fraction of petrolatum showed a propensity to be readily biodegradable, the total product is not expected to give incongruous results. For this reason the biodegradable character of the components of petrolatum provides adequate evidence to indicate that the product, petrolatum, would not be readily biodegradable.

It may be concluded that paraffin waxes are inherently biodegradable, while microcrystalline waxes and petrolatum contain hydrocarbons that are more resistant to biodegradation.

Summary: No additional testing is proposed. Sufficient data exists to characterize the biodegradability of these waxes and related materials.

Evaluation of Existing Ecotoxicity Data and Proposed Testing

There is no published information on the aquatic toxicity of petroleum waxes. However, work by Adema and van den Bos Bakker (1986) on the ecotoxicity of alkanes to *Daphnia magna*, *Chaetogamarus marinus* and *Mysidopsis bahia* shows that alkanes of carbon number greater than C-10 are not acutely toxic to these species at their limit of solubility in water. Because paraffin waxes are largely composed of alkanes of carbon number greater than C-20, they are not expected to cause acute or chronic toxicity to aquatic invertebrates. The results of acute and chronic aquatic toxicity studies with lubricant base oils (CONCAWE 1997) supports this expectation. Lubricant base oils contain similar hydrocarbon ranges and structures common to materials in the Waxes and Related Materials category; hence, results of aquatic toxicity testing of base oils provides a representation of what may be expected for waxes. Table 3 provides the results of selected acute and chronic aquatic toxicity studies of lubricant base oils reviewed by Concawe (1997).

Table 3. Acute and Chronic Aquatic Toxicity of Lubricant Base Oils

Base Oil	**Test Species**	**Exposure Method [1]**	**Endpoint [2]**	**Value[3] (mg/L)**
Acute Aquatic Toxicity				
Solvent-refined, light paraffinic distillate	Fish: *Oncorhynchus mykiss*	OWD	96-H LL50	>1,000
Solvent refined, heavy paraffinic distillate	Fish: *Oncorhynchus mykiss*	OWD	96-H LL50	>1,000
Solvent-refined, residual oil	Fish: *Oncorhynchus mykiss*	OWD	96-H LL50	>1,000
Solvent refined, light naphthenic distillate	Invertebrate: *Daphnia magna*	WAF	48-H EL50	>10,000
Solvent-refined, light naphthenic distillate	Invertebrate: *Gammarus pulex*	WAF	96-H EL50	>10,000
Solvent-refined light paraffinic distillate	Alga: *Scenedesmus subspicatus*	WAF	96-H IrL50 96-H IbL50	>1,000 >1,000
Solvent refined, heavy paraffinic distillate	Alga: *Scenedesmus subspicatus*	WAF	96-H i_rL50 96-H i_vL50	>1,000 >1,000
Solvent refined residual oil	Alga: *Scenedesmus subspicatus*	WAF	96-H i_rL50 96-H i_vL50	>1,000 >1,000

Table 3. Acute and Chronic Aquatic Toxicity of Lubricant Base Oils (continued**)**

Base Oil	Test Species	Exposure Method [1]	Endpoint [2]	Value[3] (mg/L)
Chronic Aquatic Toxicity[3]				
Solvent refined, heavy paraffinic distillate	Invertebrate: *Daphnia magna*	WAF	Reprod/ survival	1,000
Hydrotreated, light naphthenic distillate	Invertebrate: *Daphnia magna*	WAF	Reprod/ survival	1
Solvent refined residual oil	Invertebrate: *Daphnia magna*	WAF	Reprod/ survival	1,000

Data taken from CONCAWE (1997)

[1] OWD = Oil-Water dispersion; WAF = Water Accommodated Fraction
[2] LL50 = Lethal loading rate required to kill 50% of test organisms; EL50 = Effective loading rate required to immobilize 50% of the test organisms; i_rL50 = Inhibitory loading rate required to reduce algal growth rate by 50%; i_bL50 = Inhibitory loading rate required to reduce area under growth curve (biomass) by 50%
[3] Chronic aquatic toxicity values represent the no observable effect levels (NOEL)

As shown, no acute toxicity to fish, invertebrates or algae was apparent when tested at the maximum exposure level reported (1,000 to 10,000 mg/L). When tested for chronic toxicity, fish were not affected at concentrations of 1,000 and 5,000 mg/L, while invertebrates showed no chronic effects below 1 mg/L.

Some bioaccumulation of lower molecular weight components from the oils/waxes would be expected to occur to a limited degree based on animal and environmental studies (CONCAWE, 1997). However, work by Parkerton et. al. (2001) has shown that those hydrocarbon substances with calculated octanol-water partition coefficient (log P) values $\geq$ 3 indicating a high potential to bioaccumulate, were readily metabolized based on fish tissue analysis from dietary bioaccumulation studies. Higher molecular weight hydrocarbons (C12 and up) with structural linearity showed faster depuration rates than the branched isomers. Therefore, although log P values may indicate potential to partition to fat/tissue compartments in aquatic organisms, bioaccumulation does not occur in all instances due to metabolism. Similarly, by inference from the results of chronic ecotoxicity studies conducted on lubricating oil basestocks (CONCAWE, 1997), wax and petrolatum are not expected to cause chronic effects in aquatic organisms.

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 beaches in the Pacific Rim National Park. 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 (ExxonMobil Biomedical Sciences Inc., 2001).

Summary: No additional tests are proposed.

Matrix of Available Data and Proposed Testing

Table 4: Waxes and Related Materials: Matrix of Available Data and Proposed Testing

Test	Slack Wax	Petrolatum	Refined Wax
Physical/Chemical Properties			
Melting Point	Adequate	Adequate	Adequate
Boiling Point	Adequate	Adequate	Adequate
Vapor Pressure	Adequate	Adequate	Adequate
Water Solubility	Adequate	Adequate	Adequate
Partition coefficient (log Kow)	Adequate	Adequate	Adequate
Ecotoxicity			
Algae Growth Inhibition	Adequate	Adequate	Adequate
Acute Freshwater Invertebrate	Adequate	Adequate	Adequate
Acute Freshwater Fish	Adequate	Adequate	Adequate
Environmental Fate			
Biodegradation	Adequate	Read Across[1]	Adequate
Stability in Water	Adequate	Adequate	Adequate
Photodegradation (estimate)	Adequate	Adequate	Adequate
Transport and Distribution	Adequate	Adequate	Adequate
Mammalian Toxicity			
Acute	Read-Across[2]	Adequate	Adequate
Repeat Dose	TEST	Adequate	Adequate
Repro/Develop	TEST	Read-Across[3]	Read-Across[3]
Genotoxicity, *in vitro* & *in vivo*	TEST	Read-Across[3]	Read-Across[3]

[1] Data will be read across from the slack wax & refined waxes subcategories; and the Lubricating Oil Basestocks Test Plan
[2] Data will be read across from the petrolatum & refined waxes subcategories; and the Lubricating Oil Basestocks Test Plan
[3] Data will be read across from the slack wax subcategory

REFERENCES

ACGIH (1998) Threshold limit values (TLVs) for chemical substances and physical agents and biological exposure indices (BEIs) - 1998. Cincinnati OH: American Conference of Governmental Industrial Hygienists

Adema, D.M.M. and van den Bos Bakker, G.H. (1986) Aquatic toxicity of compounds that may be carried by ships (Marpol 1973, Annex 11). Progress report for 1986 from TNO to the Dutch Ministry of Housing, Physical Planning and the Environment. Report No. 86/326a. Delft: TNO

API (1983) Compositional Data on Potential Toxicology Test Samples. Unpublished data. Washington DC: American Petroleum Institute

API (1987) Comprehensive Analytical Analysis of API Generic Refinery Streams. Unpublished report. Washington DC: American Petroleum Institute

API (1999) A subchronic 90-day dietary toxicity study of a low melting point paraffin wax in two rat strains. Study No. 95-2394 conducted by Huntingdon Life Sciences. Unpublished report. Washington DC: American Petroleum Institute

Ayadi, M. and Martin, P. (1987) Contact allergy to petrolatum. *Contact Dermatitis 16,1,51*

Battersby, N.S. et al (1992) A correlation between the biodegradability of oil products in the CEC L-33-T-82 and modified Sturm tests. *Chemosphere 24, 12, 1989-2000*

Battersby, N.S. (1993) Personal communication to CONCAWE, 3rd September 1993

Bennet, H. (1975) Industrial waxes. Volume 1: Natural & synthetic waxes. New York: Chemical Publishing Company Inc.

BIBRA (1992) A 90-day feeding study in the rat with six different mineral oils (N15 (H), N70 (H), N70 (A), P15 (H), NIO (A) and P100 (H), three different waxes (a low melting point wax, a high melting point wax and a high sulphur wax) and coconut oil. BIBRA Project no. 3.1010

BIBRA (1993) A 90-day feeding study in the rat with two mineral waxes identified as paraffin wax 64 (OFH-064) and micro/paraffin wax mixture. BIBRA Project no. 3.1205

BIBRA (1999) A subchronic 90-day dietary toxicity study of a low melting point paraffin wax in two rat strains Study No. 95-2394, API study No. HES1516-L-00880-Oral

Boitnott, J.K. and Margolis, S. (1970) Saturated Hydrocarbons in Human Tissues III. Oil Droplets in the Liver and Spleen. *Johns Hopkins Medical Journal*, Vol 127: 65-78.

CONCAWE (1980) Disposal techniques for spilt oil. Report No. 9/80. Brussels: CONCAWE

CONCAWE (1981) A field guide to coastal oil spill control and clean-up techniques. Report No. 9/81. Brussels: CONCAWE

CONCAWE (1983) A field guide to inland oil spill and clean-up techniques. Report No. 10/83. Brussels: CONCAWE

CONCAWE (1984) Assessment and comparison of the composition of food-grade white oils and waxes manufactured form petroleum by catalytic hydrogenation versus conventional treatment. Report No. 84/60. Brussels: CONCAWE

CONCAWE (1988) A field guide to the application of dispersants to oil spills. Report No. 2/88. Brussels: CONCAWE

CONCAWE (1993) White oils and waxes - summary of 90-day studies. Report No. 93/56. Brussels: CONCAWE

CONCAWE (1997) Lubricating oil basestocks. Product Dossier No. 97/108. Brussels: CONCAWE

CONCAWE (2001) Environmental classification of petroleum substances - Summary data and rationale. Report 01/54 Brussels: CONCAWE

Conti, A. et al (1995) Sensitization to white petrolatum used as a vehicle for patch testing. *Contact Dermatitis 33 201-202*

CTFA (1981) Waxes: summary of unpublished safety data. Prepared for Cosmetic Ingredient Review by the Cosmetic, Toiletry and Fragrance Assoc. Inc. Washington DC

Cunninghame, M., Brantom, P., Scotter, M., and Castle, L. (2001) Identification of the causative agent(s) for mineral hydrocarbon toxicity. TNO-BIBRA International Ltd. and Central Science Library Report FD 00/51

Dietz, W.A. et al (1952) Properties of high boiling petroleum products: carcinogenicity studies. *Ind Eng Chem 44, 8, 1818-1827*

Dooms-Goossens, A. and Degreef, H. (1983) Contact allergy to petrolatums. 1. Sensitizing capacity of different brands of yellow and white petrolatums. *Contact Dermatitis 9, 175-185*

Elder, R. (1984) Final report on the safety assessment of fossil and synthetic waxes. *J Am College Toxicol 3, 3, 43-99*

EWF (European Wax Federation) (1990) Specifications for petroleum derived hydrocarbon waxes - food grade. Brussels.

Exxon Biomedical Sciences, Inc. (1995) Ready Biodegradability, Manometric Respirometry. Study #102094A.

ExxonMobil Biomedical Sciences Inc. (2001) C. Lee personal communication to S. Fraser, Environment Canada, 01EMBSI.748; 2001EMBSI.ZZJNK; and 1EMBSI.749; 2001EMBSI.ZZJNK

Fisher, A.A. (1981) Cutaneous reactions to petrolatum. *Cutis 28, 23*

Fleming, K.A. et al (1998) Granulomas in the livers of humans and Fischer rats associated with the ingestion of mineral hydrocarbons: a comparison. *Regul Toxicol and Pharmacol 27,* 75-81

Frankel, E.B. (1985) Acne secondary to white petrolatum use. *Arch Dermatol 121, 589-590*

Freeman, J.J. et al. (2001) Lifetime feeding studies of Paraffinic 70 and Paraffinic 100 white mineral oil in F-344 rats. Laboratory Reports, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ.

Fuhs, G.W. (1961) Der mikrobielle Abbau von Kohlenwasserstoffen. *Archiv für Mikrobiologie 39, 374-422*

Haines, J.R. and Alexander, M. (1974) Microbial degradation of high-molecular-weight alkanes. *Applied Microbiol 28, 6,* 1084-1085

Halton, D.M. and Piersol, P. (1994) Investigation into an outbreak of rashes in a wax coating treatment process. *Appl Occup Environ Hyg 9, 12, 941-944*

Hanstveit, A.0. (1990) Inherent biodegradability of waxes. Report No. R 90/198b. Delft: TNO

Hanstveit, A.0. (1991) A study of the fate of waxed paper materials in a woodland litter layer. Report No. R 90/243a. Delft: TNO

Hendricks, N.V. et al (1959) Cancer of the scrotum in wax pressmen. I. Epidemiology. *AMA Arch Ind Health 19 524-529*

Hjorth, N. (1987) Diagnostic patch testing. In: Marzulli, F.N. and Maibach, H.I. (Eds). Dermatotoxicology (3rd ed.). Chapter 13, p. 307-317. Washington DC: Hemisphere Publishing Corp.

Ho WS, Chan AC, Law BK. (2001) Management of paraffinoma of the breast: 10 years' experience. *Br J Plast Surg* Apr; 54(3): 232-4

IBR (1976a) Akute Toxizitätsprüfung von 'R 9107' nach oraler Applikation an der Ratte. Hannover: International Bio-Research

IBR (1976b) Akute Toxizitätsprüfung von 'R 9269' nach oraler Applikation an der Ratte. Hannover: International Bio-Research

JECFA (1996) Toxicological evaluation of certain food additives and contaminants. Prepared by the 44th meeting of the Joint FAO/WHO expert committee on food additives (JECFA). WHO Food Additives Series 35. Geneva: World Health Organization

Kane, M.L. et al (1984) Toxicological characteristics of refinery streams used to manufacture lubricating oils. *Am J Ind Med 5, 183-200*

Kaufman, J.J. and Weisberger, G.A. (1993) Petroleum waxes, including petrolatums. ASTM Manual on significance of tests for petroleum products (6th ed). Chapter 10.

Klemish, H.J., Andreae, M. and Tillman, U. (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Reg Toxicol and Pharmacol 25, 1-5*

Lijinsky, W. et al (1966) Skin tumourigenesis by an extract of amber petrolatum. Toxicol Applied *Pharmacol 8, 113-117*

Lione, J.G. and Denholm, J.S. (1959) Cancer of the scrotum in wax pressmen. II. Clinical observations. AMA Arch *Ind Health 19, 530-539*

Miller, T.L. and Johnson, M.J. (1966) Utilization of gas oil by a yeast culture. *Biotechnology and Bioengineering VIII, 567-560*

Miyamoto, Y. (1968) On cerophility of microbes. Physiology *and Ecology.L5, 55-66*

OSHA (Occupational Safety and Health Administration) (1999) Technical Manual, TED 1-0.15A, Section IV. Chapter 2, http://www.osha-slc.gov/dts/osta/otm/otm_toc.html

Oppenheimer, E.T. et al (1961) Observations on the effects of powdered polymer in the carcinogenic process. *Cancer Research 21, 132-134*

Oser, B.L. et al (1965) Toxicologic studies of petrolatum in mice and rats. Toxicol Applied *Pharmacol* Z, *382-401*

Parkerton, T. et al (2001) A Practical Testing Approach For Assessing The Bioaccumulation Potential Of Poorly Water Soluble Organic Chemicals, Presentation At The SETAC Europe Annual Meeting, Madrid, Spain, Manuscript Number 00.7014. New Jersey: Exxon Mobil Biomedical Sciences Inc.

Rahn, 0. (1906) Ein Paraffin zersetzender Schimmelpilz. *Centralblatt f Bakt* Abt *II 16, 382-384*

Rose, H.G. and Lieber, A.F. (1966) Accumulation of saturated hydrocarbons in human Spleens. *J. Lab. Clin. Med.*, 68:474-476.

SCF (1995) Opinion on mineral and synthetic hydrocarbons (expressed on 22 September 1995). CS/ADD/MsAd/132-FINAL. Brussels: European Commission

Schmähl, D. and Reiter, A. (1953) Versuche zur Krebserzeugung mit flüssigern Paraffin, gelber Vaseline und Wollfett. *Arzneimittel-Forsch 3, 403-406*

Sequira, A. (1994) Lubricant base oil and wax processing. New York: Marcel Dekker Inc.

Shoda, T., Toyoda, K., Uneyama, C., Takada, K., and Takahashi, M. (1997) Lack of carcinogenicity of medium-viscosity liquid paraffin given in the diet of F344 rats. *Food and Chem Toxicol* 35: 1181-1190.

Shubik, P. et al (1962) Studies on the toxicity of petroleum waxes. Toxicol Applied *Pharmacol 4, Suppl, 1-61*

Smith, W.E. et al (1951) Experimental analysis of the carcinogenic activity of certain petroleum products. *Arch Ind Hyg Occ Med 4, 299-314*

Söhngen, N.L. (1913) Oxidation of petroleum, paraffin, paraffin oil and benzine by microbes. *Proc Royal Acad Amsterdam XV*, *1145-1151*

Twerdok, L.E. et al (1998) Comparison of immunopathological changes following subchronic feeding of low melt point paraffin wax in two strains of rats. API Report in press. Washington DC: American Petroleum Institute

UK HSE (1999) Occupational exposure limits 1999. HSE Guidance Note EH40/99. London: Health and Safety Executive

U.S. Environmental Protection Agency (2000) EPI Suite Estimation Program Interface. http://www.epa.gov/opptintr/exposure/docs/episuite.htm.

U.S. Food and Drug Administration (2001) Food Additives Permitted for Direct Addition to Food for Human Consumption *Code of Federal Regulation Title 21, Vol 3 Part 172, Subpart I, Section 172.886.*

Yamada, K. and Yogo, M. (1970) Studies on the utilization of hydrocarbons by microorganisms. Part XV. Yeast and bacterial cell production from paraffin wax. *Agr Biol Chem* 34, 2, 296-301

ZoBell, C.E. (1963) The occurrence, effects, and fate of oil polluting the sea. *Int J Air Wat Pofi Z, 173-198*

APPENDIX A.

CAS Descriptions of Category Members

The CAS descriptions for refinery streams, including the petroleum waxes, were intentionally written to be qualitative in nature. Section 8(b) of the Toxic Substances Control Act required identification and registration with the Environmental Protection Agency before July 1979 of each “chemical substance” being manufactured, processed, imported or distributed in commerce. Due to analytical limitations, identification of every specific individual molecular compound in every refinery process stream under all processing conditions was impossible. In addition, there is known variability in stream composition due to things such as the crude oil used and small changes in process conditions. Even with reference to TSCA’s Candidate Inventory List, members of the industry would have reported refinery streams using a wide variety of names, with each company’s differing from all others. Perhaps 3000 or more different names for refinery streams would have been submitted to the EPA by the petroleum industry. Recognizing these problems, in 1977 API initiated an effort to compile a list of terms consistent with industry operations and with nomenclature included in API’s Thesaurus of petroleum industry technical terms.

As a result of this effort, API recommended to the EPA a list of generic names for refinery streams covering all known processes used by refiners. A definition of each stream was included, giving typical carbon number distribution, boiling or viscosity range, or general composition. Along with CAS numbers, this information was published by EPA as “Addendum I, Generic Terms Covering Petroleum Refinery Process Streams.”

As can be seen from the listing below, the descriptions accompanying the CAS number of each petroleum wax are written in broad, general terms. The descriptions often contain ranges of values, with little if any quantitative analytical information or concern for possible compositional overlaps. In these descriptions, process history, specifically the final process step, and not chemical composition, was one of the primary criteria to differentiate streams and assign CAS numbers. As a result, streams with the same or substantially similar compositions may have different CAS numbers if they originate in different process units.

CAS Number[1]

64742-61-6
Slack wax, petroleum

A complex combination of hydrocarbons obtained from a petroleum fraction by solvent crystallization (solvent dewaxing) or as a distillation fraction from a very waxy crude. It consists predominantly of saturated straight and branched chain hydrocarbons having carbon numbers predominantly greater than C20.

8002-74-2
Paraffin waxes and Hydrocarbon waxes

A complex combination of hydrocarbons obtained from petroleum fractions by solvent crystallization (solvent deoiling) or by the sweating process. It consists predominantly of straight chain hydrocarbons having carbon numbers predominantly greater than C20.

64742-43-4
Paraffin waxes, petroleum, clay-treated

A complex combination of hydrocarbons obtained by treatment of a petroleum wax fraction with natural or modified clay in either a contacting or percolation process to remove the trace amounts of polar compounds and impurities present. It consists predominantly of straight chain saturated hydrocarbons having carbon numbers in the range of C20 through C50.

64742-51-4
Paraffin waxes, petroleum, hydrotreated

A complex combination of hydrocarbons obtained by treating a petroleum wax with hydrogen in the presence of a catalyst. It consists predominantly of straight chain paraffinic hydrocarbons having carbon numbers predominantly in the range of about C20 through C50.

63231-60-7
Paraffin waxes and Hydrocarbon waxes, microcryst.

A complex combination of long, branched chain hydrocarbons obtained from residual oils by solvent crystallization. It consists predominantly of saturated straight and branched chain hydrocarbons predominantly greater than C35.

64742-42-3
Hydrocarbon waxes, petroleum, clay-treated microcryst.

A complex combination of hydrocarbons obtained by treatment of a petroleum microcrystalline wax fraction with natural or modified clay in either a contacting or percolation process to remove the trace amounts of polar compounds and impurities present. It consists predominantly of long branched chain hydrocarbons having carbon numbers predominantly in the range of C25 through C50.

64742-60-5
Hydrocarbon waxes, petroleum, hydrotreated microcryst.

A complex combination of hydrocarbons obtained by treating a petroleum microcrystalline wax with hydrogen in the presence of a catalyst. It consists predominantly of long, branched chain hydrocarbons having carbon numbers predominantly in the range of C25 through C50.

8009-03-8
Petrolatum

A complex combination of hydrocarbons obtained as a semi-solid from dewaxing paraffinic residual oil. It consists predominantly of saturated crystalline and liquid hydrocarbons having carbon numbers predominantly greater than C25.

[1] Note from Testing Group: The carbon numbers included in these CAS descriptions include the carbon numbers of any oil components that might be present.

APPENDIX B.

Robust Summary (Separate Document)