HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

Waxes and Related Materials

CATEGORY ANALYSIS AND HAZARD CHARACTERIZATION

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by

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CONTENTS

List of Tables, Figures, and Appendices

EXECUTIVE SUMMARY

This document addresses the potential mammalian and environmental hazards of refined/finished waxes, slack wax and petrolatum, hereafter referred to as **waxes and related materials (WRM)**. The members of this category are complex petroleum substances containing hydrocarbons with carbon number ranges predominantly from C_{20} to C_{85} but may contain a small fraction of smaller hydrocarbons. Because they are complex substances, members of the WRM category are defined by process history and product use specifications, not by detailed compositional information that identifies individual molecular compounds. Waxes are predominantly saturated paraffins which are solid or semisolid at ambient temperature. Refined or finished waxes are typically used in candles or, if adequately refined, as food-grade waxes. Slack waxes are usually the precursors to refined/finished waxes. Slack waxes are frequently produced by de-waxing lubricant base oils during production of the latter so both are derived from the same vacuum distillates. Slack waxes may be just an intermediate stream for production of refined waxes or slack waxes may be used in products such as fire logs and particle board. Petrolatum (also called petroleum jelly) usually refers to a combination of highly-refined mineral oil and refined microcrystalline wax. Petrolatum, however, can also refer to a cruder product which is similar to a slack wax with high oil content.

Physical-Chemical Properties

Substances in the WRM category have melting points from 36 to 95 °C and they have boiling points > 350 °C. They have very low vapor pressures and negligible solubility in water.

Environmental Fate

The environmental fate of WRM depends on the individual hydrocarbons present within the specific product. Because of their physical and chemical properties, WRM will tend to agglomerate rather than disperse if released to the environment. Although category members have very low vapor pressures, individual hydrocarbon constituents at the lower molecular weight range (e.g. $\leq C_{20}$) may volatilize during weathering. Individual hydrocarbon constituents that evaporate would be expected to undergo rapid indirect photodegradation. WRM are not expected to partition to water because the water solubility of their constituent hydrocarbons is extremely low. Modeled partition coefficients (log Kow) of the low molecular weight hydrocarbons (e.g. C_{13} and C_{20} compounds) typically exceed 4.7 with higher molecular weight hydrocarbons having partition coefficients > 6. Environmental distribution modeling predicts that with the exception of the smaller mineral oil components, individual hydrocarbon constituents would generally partition to soil. The mineral oil fraction of slack waxes consists of straight and branched-chain alkanes that will partition to air. Once released to the environment, WRM are not likely to undergo rapid biodegradation, but hydrocarbons in general are considered inherently biodegradable.

Ecotoxicity

WRM are related to lubricating oil basestocks as both are derived from vacuum distillates and contain similar saturated hydrocarbons with similar carbon numbers. Lubricating oil basestocks consist primarily of branched-chain alkanes and naphthenic hydrocarbons (cycloparaffins; compounds with one or more saturated rings). Waxes are composed primarily of linear alkanes but may contain some branched-chain and cycloparaffins. The aquatic toxicity of both

lubricating oil basestocks and WRM is severely limited by their low solubility and low bioavailability. Multiple acute studies of lubricating oil basestocks have not produced any adverse toxicological effects in the fish, invertebrates, and algae exposed to water accommodated fractions prepared at loading rates ≥ 1000 mg/L. In tests for chronic toxicity in aquatic invertebrates with lubricating oil basestocks similar in molecular size to the WRM, neither survival nor reproduction was impaired in the adult generation. Read-across from the studies with lubricating oil basestocks indicates that the members of the WRM category should have low toxicity to aquatic organisms.

Human Health Effects

WRM have low acute, repeat dose and chronic toxicity in mammals. This is primarily due to the large size of their constituent hydrocarbons which limits absorption or penetration into the body, tissues or cells after oral or dermal exposures. Exposure by inhalation is not expected due to the high molecular weights and low vapor pressures of wax constituents. The saturated hydrocarbons in WRM are also relatively inert to biological systems. Refined/finished waxes and petrolatums are referred to as mineral hydrocarbons. Since many refined/finished waxes and petrolatums are used in food-grade and pharmaceutical applications, more extensive repeated dose studies have been conducted with these materials. Repeat dose studies of lower melting point waxes and lower viscosity mineral oils resulted in inflammatory changes (primarily in the liver and mesenteric lymph nodes) in one strain of rat. In contrast, minimal or no effects were observed in other rat strains and other species. Evidence from several studies indicates this response to dietary mineral hydrocarbons is due to greater retention of ingested hydrocarbons and an enhanced inflammatory response in this sensitive strain of rat versus other laboratory animals. This strain-specific effect has not been observed in humans. Because of their low oral and dermal toxicity, refined/finished waxes and petrolatum have been safely used for decades in food, cosmetic and pharmaceutical applications. The overall evidence from many subchronic and chronic feeding studies indicates that WRM have low repeat dose and chronic toxicity with no-observed-adverse-effect-levels (NOAELs) from ≥100 to 1000 mg/kg/day. Human dietary exposure from the use of food-grade waxes and petrolatum in the U.S. is estimated to be 0.044 and 0.404 mg/kg/day, respectively.

Since the substances in the WRM category are derived from petroleum vacuum distillates, aromatic molecules including polycyclic aromatic compounds (PACs[*]) could potentially carry through into the wax products. Production methods prior to the 1960s resulted in some slack waxes containing significant levels of PACs. Later production has applied more severe processing methods to the vacuum distillates prior to the separation of slack waxes and subsequent wax products. Slack wax and crude petrolatum are the least refined of the category members as they contain 5% to 20% oil. The oil in some slack waxes could contain PACs depending on the source of the material. For the HPV program, samples of slack wax, refined/finished waxes and petrolatum from U.S. facilities were analyzed specifically for PACs and none were detected. Imported slack waxes produced with less refining could contain significant levels of PACs, but the potential for such substances entering commerce in the US is

[*] "Polycyclic aromatic hydrocarbons" (PAH) refers to compounds of two or more fused-aromatic rings consisting of carbon and hydrogen only. Polycyclic aromatic compounds (PAC) is a more comprehensive term that includes PAHs and molecules in which one or more atoms of nitrogen, oxygen or sulfur replaces one of the carbon atoms in the ring system.

unknown. The removal of impurities in the production of refined/finished waxes and petrolatum from slack wax also removes aromatics and PACs.

PACs in petroleum vacuum distillate streams have been associated with some repeat dose and developmental toxicity in laboratory studies. The developmental and reproductive toxicity potential of the substances in the WRM category is negligible if the substance has been sufficiently refined. The developmental and reproductive toxicity of WRM was also assessed using read-across from data with lubricating oil basestocks. The saturated hydrocarbons in WRM are similar to those in lubricant base oils and are derived from the same streams. No effects on reproduction or fetal development were observed in reproduction and developmental toxicity studies with lubricating oil basestocks including a screening study in rats conducted with oral doses of approximately 1000 mg/kg/day.

1. DESCRIPTION OF WAXES AND RELATED MATERIALS

1.1. Composition and Structure

Waxes and related materials (WRM) include both refinery streams and finished products. These materials are complex petroleum substances containing mostly saturated hydrocarbons with carbon numbers predominantly ranging from C_{20} to C_{85}. A small fraction of saturated hydrocarbons down to C_{13} may be present. Because they are complex substances, WRM are typically defined by process history and product use specifications, not by detailed compositional information that identifies specific molecular components. All the materials in this category are solid or semi-solid at ambient temperature with very low volatility and water solubility. Waxes are graded according to their melting point and oil content.

As shown in Figure 1, the substances 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, potentially biologically active impurities such as polycyclic aromatic compounds (PAC[*]) are found in the oil component of the stream. At each process step in the production of waxes, the oil and impurities content is lowered. Substances similar to the oil component of the waxes are covered within the HPV program under the Lubricating Oil Basestocks. While waxes are composed primarily of linear alkanes, the similar saturated compounds in the Lubricating Oil Basestocks category consist primarily of branched-chain alkanes and naphthenics (cycloparaffins; compounds with one or more saturated rings). Higher melting point waxes may also contain branched-chain alkanes and cycloparaffins.

A detailed description of petroleum refining, including the production of WRM can be found in the OSHA Technical Manual (OSHA, 1999). Whole crude oils are first fractionated by distillation at atmospheric pressure to remove lighter substances such as gasoline and other fuel components leaving a residue (residuum) that contains the saturated hydrocarbons that can become lubricating oil basestocks or waxes. This atmospheric residuum is then distilled under vacuum yielding a range of fractions (unrefined lubricating oil basestocks) containing the waxes and a vacuum residuum.

Waxes are separated from the lubricating oil basestocks by chilling or solvent extraction. The wax molecules may also be catalytically converted to isoparaffin structures by a process called catalytic dewaxing. In typical de-waxing using solvent extraction, the lubricating oil basestock is mixed with solvents to increase the fluidity of the oil at low temperatures and to precipitate the wax fraction. Toluene and methylethylketone (MEK) are solvents commonly used for this purpose. The mixture is chilled to precipitate the waxes which are removed by filtration.

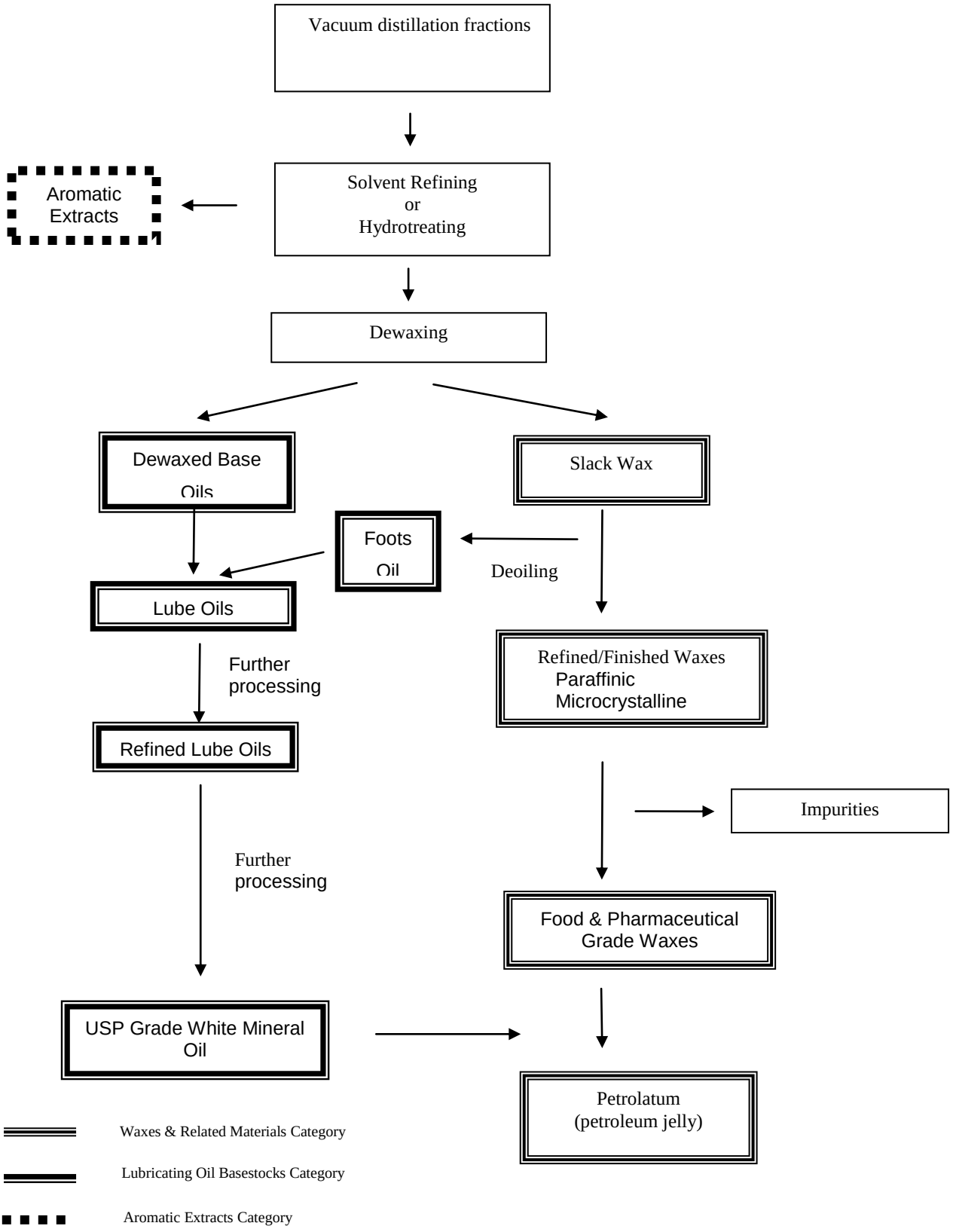
The initial filtered material is **slack wax** containing predominantly straight and branched hydrocarbons. Slack wax contains 5% to 20% oil (Case, 2003). The composition of this oil varies depending on the other processes that have been employed in prior refining steps.

[*] "Polycyclic aromatic hydrocarbons" (PAH) refers to compounds of two or more fused-aromatic rings consisting of carbon and hydrogen only. Polycyclic aromatic compounds (PAC) is a more comprehensive term that includes PAHs and molecules in which one or more atoms of nitrogen, oxygen or sulfur replaces one of the carbon atoms in the ring system.

Slack wax can be further de-oiled by repeated cold precipitation, "sweating" (heating to selectively mobilize the oil), or membrane filtration until the oil content is reduced. Waxes of about 1% to 5% oil are classified as scale waxes. Waxes that have oil content below 1% (typically 0.5%) and meet FDA criteria are classified as fully refined waxes (Case, 2003). **Paraffin waxes** are obtained from vacuum distillate streams and **microcrystalline waxes** come from vacuum residuum streams. If the resulting product is a semi-solid it may be known as **petrolatum** but it is important to recognize that petrolatum is also a name given to highly purified waxes dissolved in white oil to make food-grade products.

Crude petrolatums, which are essentially slack waxes, may be derived from less refined feedstock streams or with less processing and may contain PACs or other contaminants. Petrolatum which is produced from feedstocks that have undergone extraction or hydrotreatment steps to thoroughly remove PACs and other impurities are referred to as severely refined petrolatum, food or pharmaceutical grade petrolatum, or petroleum jelly. Highly refined petrolatum is used for food, cosmetic and pharmaceutical applications. The soft consistency of petrolatum is due to a gel-like dispersion of mineral oil in a paraffin or microcrystalline wax base. It is important to note that the CAS number for petrolatum (8009-03-8) may refer to either crude petrolatum or food-grade petrolatum.

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



1.2. Typical Compositional Properties

Based on the extent of processing they undergo and their physical properties, the eight substances in the WRM category are divided into three groups (Table 1).

Unrefined **slack waxes** and **crude petrolatums** are used as products but the uses are generally limited to industrial applications such as fillers, binders, lubricants, release agents or made into fuel sources such as firelogs (ExxonMobil, 2009). Consequently, human exposures are primarily through the dermal route. As can be seen from the process diagram (figure 1), slack waxes may undergo additional processing to produce **finished/refined waxes** and refined **petrolatum**. Slack waxes derived from low viscosity oils contain predominantly normal paraffins. Heavier oil fractions yield slack waxes with increasing proportions of isoparaffins and cycloparaffins in addition to the normal paraffins (Case, 2003).

Refined/finished waxes have a large and varied number of industrial, commercial and consumer product uses. They may be used in lubricants, wire cables, candles, petroleum jellies, cosmetics and food/drug applications. Human exposures may be via both the dermal and oral routes. Refined/finished waxes and petrolatum that are intended for food, food contact, cosmetic, pharmaceutical and related applications must meet stringent purity requirements as described in the respective national and international regulations. These generally specify melting ranges, color, polycyclic aromatic hydrocarbon (PAH) content and other impurity limits. For instance, in regard to the PAH content, the U.S. Food and Drug Administration has established ultraviolet light absorbance limits that set an upper limit of 0.5 mg/kg for the total concentration of all extractable PAH-compounds in the wax (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 melting waxes). The former are obtained from processing light vacuum distillate while the latter are obtained from processing vacuum residuum or heavier vacuum distillate. **Paraffin waxes** consist mainly of normal alkanes, varying amounts of isoalkanes, cycloalkanes and trace amounts of alkylated aromatic hydrocarbons. **Microcrystalline waxes** consist of substantial amounts of iso- and cycloalkanes usually with a lesser amount of normal alkanes and, depending on the severity of refining, trace amounts of alkylated aromatic compounds (CONCAWE, 1999). It should be emphasized that there is no sharp delineation between classes of wax and that commercial waxes represent a continuous spectrum from paraffin wax to microcrystalline wax.

Table 1. Waxes and Related Materials (WRM) Category Members

Groups	CAS Number[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	Crude or refined petrolatum (Petroleum Jelly)

[1]Complete CAS descriptions are in Table 4

Food-grade **petrolatum** (petroleum jelly) is a mixture of highly refined, higher-melting point paraffin wax and (typically) greater than 10% food-grade white mineral oil. White mineral oil is discussed in the lubricating oil basestocks category assessment document. Petrolatum consists mainly of branched and straight chain alkanes. Like refined/finished waxes, petrolatum may be used in lubricants, wire cables, candles, cosmetics and food/drug applications. Human exposure may occur by both the oral and dermal routes. When used in food, cosmetic, pharmaceutical and related applications, petrolatum must meet stringent purity requirements as described in the respective national Pharmacopoeia and international regulations (U.S. FDA, 2001; CONCAWE, 1984). These regulations generally specify melting ranges, color, PAH content and other impurity limits. As with food-grade waxes and PAH 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 PAHs in the petrolatum.

The physical and chemical properties of the three groups of products in the WRM category are summarized in Table 2 (Bennet, 1975; Kauffman *et al*., 1993; EWF, 1990).

Table 2. Physical/Chemical Properties of the Waxes and Related Materials

Subcategory	Typical Oil content (wt %)	Carbon number range*	Melting Point (°C)	Kinematic viscosity at 100°C (mm^2/sec)**
Slack wax	2 – 30	$C_{12} – C_{85}$	43 – 63	3 – 30
Refined/finished waxes				
Paraffin	< 2.5	$C_{18} – C_{75}$	43 – 68	3 – 10
Microcrystalline	< 5	$C_{23} – C_{85}$	60 – 95	10 – 30
Petrolatum (refined)	> 10***	$C_{12} – C_{85}$	36 – 60	3 – 30

*The low end of the range includes any inadvertent oil constituents
**Kinematic viscosity is also expressed as Centistokes (cSt); mm^2/sec = cSt
***USP-grade white mineral oil
Methods: Melting Point ASTM D127; Carbon number ASTM D2887 or D7169; Viscosity ASTM D445

1.3 Analytical Characterization

As noted earlier, the WRM are typically defined by process history and use specifications and not by detailed compositional information.

One substance in this HPV category which could contain hazardous components is **slack wax** because it contains 5% to 20% oils and these oils may contain PAC. Food-grade waxes and petrolatum are regulated with strict limitations governing the content of impurities including polycyclic aromatics. Information on the molecular composition of slack waxes, particularly information on their PAC profile, was not available when the HPV program started.

Specific data are now available on the PAC profile of samples in the WRM category. Samples of slack wax, paraffin wax, hydrotreated paraffin wax, microcrystalline wax and petrolatum were obtained from Petroleum HPV member companies. A sample of hydrotreated slack wax was also analyzed but is not a member of the WRM category. This substance is similar to slack wax except it has been treated with hydrogen. These samples were analyzed for PACs using the "PAC-2 method" (Roy *et al*., 1985; Roy *et al*., 1988). Using this method, the percent of sample mass is determined for each aromatic hydrocarbon ring class (1 through 7) from PAC-enriched dimethyl sulfoxide (DMSO) extracts of the test material. The analysis is performed by gas chromatography (GC) with flame ionization detection (GC/FID). The results are listed in Table 3. PACs were not detected in any of the eight samples (Port Royal Research, 2009). It should be noted that the samples of slack wax in Table 3 were from U.S. manufacturing sites. Imported slack waxes from other sources could contain significant levels of PAC, depending on the severity of the refining applied to them; however, whether such substances enter commerce in the US is unknown.

Table 3. PAC Profile of Samples in the WRM Category

	DMSO Extractable Wt % in each Aromatic Ring Class (1 to 7 rings)							
	ARC 1	**ARC 2**	**ARC 3**	**ARC 4**	**ARC 5**	**ARC 6**	**ARC 7**	**Total**
Slack Wax Samples								
64742-61-6	0	0	0	0	0	0	0	0
"	0	0	0	0	0	0	0	0
"	0	0	0	0	0	0	0	0
92062-09-4[1]	0	0	0	0	0	0	0	0
Paraffin Wax Samples								
8002-74-2	0	0	0	0	0	0	0	0
64742-51-4	0	0	0	0	0	0	0	0
Microcrystalline Wax								
63231-60-7	0	0	0	0	0	0	0	0
Petrolatum								
8009-03-8	0	0	0	0	0	0	0	0

[1]Hydrotreated slack wax (CAS 92062-09-4) is not included in this HPV category, but as it is a related substance, the data have been provided.

2. CATEGORY DEFINITION AND JUSTIFICATION

The names, CAS numbers and descriptions of the eight substances in this category are listed in Table 4. The eight substances were included in the WRM category based upon the following rationale:

1. The materials included in the category are similar from both process and physical/chemical perspectives.
2. The materials in the category are composed of varying ratios of waxes and oils.
3. The physical state (semi-solid to solid) and the number of carbon atoms of the wax components severely limit its bioavailability and its environmental distribution, breakdown, or conversion to other chemical compounds. Available toxicity data on refined/finished waxes and petrolatum are used to characterize the potential hazards of all members of the category.
4. A source of potential toxicity for this category is associated with PACs. Depending on the severity of refining, PACs are possibly present in the oil component of WRM. Any potential toxicity of WRM decreases as they are refined from slack wax to refined/finished waxes and petrolatum.
5. Hydrocarbons similar to the oil component in WRM are covered in the Lubricating Oil Basestocks Category. Because of their common source and similarity, data on lubricating oil basestocks are used to characterize the hazards for all members of this category when specific wax data are not available (e.g. reproductive and developmental endpoints).

Table 4. Substances in the Waxes and Related Materials Category

CAS Number	Name	Description
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 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 de-oiling) 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 treatment of 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,	A complex combination of long, branched chain hydrocarbons obtained from residual oils by solvent crystallization. It consists

	microcrystalline	predominantly of saturated straight and branched chain hydrocarbons predominantly greater than C35.
64742-42-3	Hydrocarbon waxes, petroleum, clay-treated microcrystalline	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 microcrystalline	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.

API has evaluated the association between the PAC profile of high-boiling petroleum substances and certain repeat-dose, developmental, and in vitro mutagenicity endpoints. API has developed statistical models that can be used to predict the toxicity based on the PAC profile of the untested substance (API, 2008; TERA, 2008). This association and the statistical models are described in detail in Appendices 1 and 2. The PAC profile is generated using an analytical technique referred to as the "PAC-2 Method" (Roy et al., 1985; Roy et al., 1988). In the PAC-2 method, the percent of sample mass is determined for each PAC ring class (1 through 7) contained in DMSO extracts of the test material. The analyses are performed using gas chromatography with flame ionization detection (GC/FID) or mass spectroscopy (GC/MS). The models are applicable only to those categories of petroleum refinery streams that may contain appreciable amounts of PAC. For example, kerosene and naphtha streams would not contain significant amounts of PAC, were not used in developing the models, and are not among the categories covered by the models. The PAC models can be used to predict the repeat-dose toxicity, developmental toxicity, and mutagenic index of untested samples of slack wax, refined/finished waxes and petrolatum if the PAC profile is available (see Appendices 1 and 2).

3. TEST MATERIALS

The original test plan for the WRM category included a proposal to conduct a repeated dose and reproductive/developmental toxicity screening test via the dermal route on a sample of slack wax. Slack wax was the proposed test material because it is the least processed of the substances in this category with the greatest chance of containing bioavailable/biologically active PAC. An *in vitro* bacterial reverse mutation assay and an *in vivo* micronucleus assay (as part of the repeated dose study) were also proposed on slack wax to represent a worst-case assessment.

However, *in vitro* bacterial mutation assays have been conducted with several samples of slack wax and the results are reported in this document. None of the samples tested showed evidence of genotoxic potential. The proposed mammalian studies were not conducted because analyses of slack wax samples provided by Petroleum HPV member companies indicated that none had detectable levels of PAC (Table 3). PACs are the potentially toxic constituents in WRM which, if present in the oils would signal the potential for systemic and developmental effects. However, as these constituents were not found in the tested samples, there seems no justification for more extensive studies. Further, the limited toxic potential of WRM is also shown by the existing data on refined/finished waxes, petrolatum and the lubricating oil basestocks (derived from the same vacuum distillates). In summary, between the available data on related substances and predictions which can be made from analytical information, the limited toxic potential of WRM is sufficiently demonstrated and no further testing seems justified.

4. PHYSICAL-CHEMICAL PROPERTIES

4.1 Physical-Chemical Screening Information Data Set (SIDS)

The physical-chemical endpoints in the HPV chemicals program include the following:

- Melting Point
- Boiling Point
- Vapor Pressure
- Octanol/Water Partition Coefficient
- Water Solubility

Because the substances in this HPV category are complex materials of variable composition, it is not possible to measure or calculate a single numerical value for most of the physico/chemical properties. Based on the measured and predicted behavior of the constituent hydrocarbons these substances do not contain any oxidizing constituents and are almost totally insoluble in water. Only the lowest molecular weight fractions of slack waxes, which may contain up to 20% mineral oil, show any propensity to volatilize.

Measured data for many specific physicochemical properties of the representative WRM that can be used in the HPV chemicals program are not available. Therefore, calculated and measured representative data have been identified and included in the robust summaries, where appropriate. The EPI-Suite™ computer model (U.S. EPA 2000), as discussed in the U.S. EPA document entitled "*The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program*" was used to calculate physicochemical properties of representative constituents in the waxes and related materials. Because of the diversity of compounds present in these materials, it was not feasible to model the physicochemical endpoints for each potential compound. Instead, modeling efforts were directed toward the principal isomeric carbon chain lengths comprising typical constituents in the category members. Since molecular weight and structural conformation determine in large part the solubility and vapor pressure of the hydrocarbons, modeling focused on different isomeric structures of representative compounds in these substances. The C_{13} compounds are among the shortest chain length molecules present in waxes and comprise only a small fraction of most WRM. This low end was chosen because slack wax is a category member that may contain 5% to 20% oil (Case, 2003).

4.1.1. Melting Point

Since the materials in this category are solid or semi-solid at room temperature, some melting point data are available (Bennet, 1975; CONCAWE, 1999; EWF, 1990; Kaufman and Weisberger, 1993).

A summary of Melting point data based on ASTM method D127 includes the following:

Slack wax	43 – 63 °C
Paraffin wax	43 – 68 °C
Microcrystalline wax	60 – 95 °C
Petrolatum	36 – 60 °C

4.1.2. Boiling Point:

Boiling points for slack wax and petrolatum are not available because their constituent oil components contain hydrocarbons produced from vacuum distillation. These heavier hydrocarbons tend to decompose when subjected to high temperatures at atmospheric pressure. They will have boiling points above 485 °C. In a survey of the composition of food-grade waxes which do not have high-boiling oil components, the boiling range of paraffin waxes was reported to be 350 – 485 °C (CONCAWE, 1984; CONCAWE 1997).

Conclusions: The boiling point of substances in the WRM category is >350 °C

4.1.3. Vapor Pressure

All the materials in this category are solid or semi-solid at room temperature. Any measurable vapor pressure attributable to these materials would be from the oil component of the material, if any oil is present. As discussed in the lubricating oil basestocks category assessment document, the vapor pressures of these oils are expected to be very low, e.g. the vapor pressure of one sample of lubricating oil was reported as 1.7×10^{-4} Pa (Hazleton UK, 1991).

Conclusions: The vapor pressures of substances in the WRM category are very low ($< 1.7 \times 10^{-4}$ Pa).

4.1.4. Partition Coefficient

The WRM consist of hydrocarbons with carbon numbers in the range C_{12} to C_{85}. The hydrocarbon constituents (i.e., normal paraffins, isoparaffins and cycloparaffins) and the carbon chain lengths determine in part the partitioning characteristics of these complex substances. Due to their complex composition and low water solubility, measurements of the log Kow of these complex hydrocarbon substances typically cannot be made. Modeled partition coefficients (log Kow) of the low molecular weight hydrocarbons (e.g. C_{13} and C_{20} compounds) typically exceed 4.7 with higher molecular weight hydrocarbons having partition coefficients > 6. As size increases above C_{20}, as is the case for the vast majority of the wax constituents, log Kow values >6 are predicted. Modeling focused on selected C_{13} and C_{20} hydrocarbons since waxes contain mostly C_{20} to C_{85} compounds, with some minimal content of C_{12} through C_{20} compounds. Partition coefficients of selected C_{13} and C_{20} hydrocarbons were modeled using EPIWIN©, KOWWIN V1.65 (U.S. EPA, 2001).

Conclusions: The complex compositions, high molecular weights, and low water solubility of these substances precludes the measurement of partition coefficients. Based on modeling of the smaller constituent hydrocarbons that may exist at low levels in WRM, log Kow values are estimated to range from 4.7 to > 6.

4.1.5. Water Solubility

The water solubility of waxes cannot be readily determined because they are complex

substances. Therefore, the water solubility of individual C_{13} to C_{20} hydrocarbons was modeled using WSKOW Version 1.36 (EPIWIN, EPA, 2001). The highest solubility (5.96 mg/l for a C_{13} hydrocarbon) would be expected for only a tiny fraction of the molecules present in waxes. Increasing carbon number results in rapidly decreasing solubility so that the most soluble C_{18} and C_{20} analogues yield model values of 0.01195 and 0.00125 mg/l, respectively. Constituents with higher carbon numbers are even less water soluble.

Conclusions: The high molecular weights of constituent hydrocarbons in the WRM category severely limit the water solubility of these substances. Modeled solubility estimates range from < 0.002 to 5.96 mg/l at 25 °C for the smallest hydrocarbon constituents which may be present (C_{20} to C_{13}).

4.2 Assessment Summary for Physical-Chemical Endpoints

The physical-chemical characteristics of WRM indicate that these substances are solid to semi-solid at ambient temperatures with melting points from 36 – 95 °C and boiling points > 350°C. The vapor pressures of these materials are very low. Modeling of partition coefficients for selected C_{13} and C_{20} hydrocarbons, which are similar to the smaller compounds in the wax materials, indicated log Kow values from 4.7 to > 6. Most hydrocarbons in these materials are larger than C_{20} with log Kow values much greater than 6. Based on solubility estimates and the fact that WRM are semi-solid to solid at ambient temperatures, the water solubilities of these substances are extremely low.

5. ENVIRONMENTAL FATE

5.1 Environmental Fate Endpoints

To assess the environmental fate properties for the HPV program, the U.S. EPA has selected important fate endpoints by which these substances may be characterized. The environmental fate endpoints include the following:

- Photodegradation
- Stability in water [Hydrolysis]
- Transport Between Environmental Compartments [Fugacity/Distribution]
- Biodegradation

5.1.1. Photodegradation

5.1.1.1. Direct

The direct photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation. Only light energy at wavelengths between 290 and 750 nm can potentially result in photochemical transformations but light absorption is not always sufficient for a chemical to undergo photochemical degradation (Harris, 1982). The degree and rate at which these compounds might engage in direct photolytic reactions depend upon penetration of light with sufficient energy to effect a chemical change.

Saturated and one-ring aromatic hydrocarbons do not show absorbance in the 290 to 800 nm range and would not be expected to photodegrade. Polycyclic hydrocarbons have shown absorbance in this range of light energy and could potentially undergo photolysis reactions. Since samples of slack waxes, refined/finished waxes and petrolatum contain no detectable PAC, direct photodegradation is expected to be negligible.

5.1.1.2. Indirect

Components in WRM that do not directly photodegrade (e.g., paraffins, isoparaffins, naphthenic compounds) may be subject to indirect photodegradation. Indirect photodegradation is the reaction with photosensitized oxygen in the atmosphere in the form of hydroxyl radicals (OH^-). The potential to undergo indirect photodegradation can be estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.89) in EPIWIN© (EPA, 2001), which calculates a chemical half-life and an overall OH^- reaction rate constant based on a 12-hour day and a given OH^- concentration. Atmospheric oxidation rates and half-lives were calculated for the lowest molecular weight constituents of various components of wax category members (e.g. C_{13} hydrocarbon structures), since these would have the greatest potential to volatilize to the atmosphere. All modeled components were predicted to have rapid degradation in the atmosphere. The AOP half life estimate for the most volatile component was 0.91 days. All other modeled C_{13} components had both lower volatility and shorter half-lives.

Conclusions: Since WRM are solids or semi-solids at ambient temperatures with very low volatility and water solubility, direct and indirect photodegradation are not likely to be important fate processes. Should conditions exist where some constituents volatilize to the atmosphere, half-lives are estimated to be 0.9 days or less. Therefore, constituents of WRM will not persist in the atmosphere.

5.1.2. 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 (Harris, 1982). Because members of the WRM category do not contain significant levels of these functional groups, these substances are not subject to hydrolysis.

Conclusions: WRM are stable to hydrolysis.

5.1.3. Transport between Environmental Compartments (Fugacity/Distribution)

Fugacity-based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (e.g., air, water, soil, sediment, suspended sediment and biota). 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 (Trent University, 1999). 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 "Determining the Adequacy of Existing Data" that was prepared as guidance for the HPV chemicals program (U.S. EPA, 1999).

Results of Level 1 models are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition in the environment. Modeling indicated that the majority of high molecular weight hydrocarbons ($C_{>20}$) in waxes would be distributed to soil. Distribution estimates were modeled for different C_{13} to C_{29} isoparaffins as this class of wax hydrocarbons shows a greater potential for distribution to air than constituents with higher carbon numbers. Aromatic compounds with carbon numbers from C_{13} to C_{85} will partition principally to soil. Linear paraffins and naphthenics distribute to both soil and air with increasing partitioning to soil for hydrocarbons greater than C_{20} as vapor pressure decreases. Since most of the hydrocarbons in waxes are normal paraffins larger than C_{20}, with moderate amounts of naphthenics, isoparaffins and trace amounts of aromatics, volatility is not a significant environmental fate for these substances. This is due to their negligible vapor pressures.

Modeling results (Table 5) show that wax constituents released to the environment would be distributed to soil with only negligible amounts dissolving in water. As the substance is exposed to weathering elements (e.g., processes related to the physical and chemical actions of air, water and organisms on the material after a release) some of the lowest molecular weight paraffins,

isoparaffins and naphthenics may partition to the air over time. Based on the calculated half-lives for indirect photodegradation, the very small fraction of WRM that partitions to the atmosphere will not persist.

Table 5. Environmental Distribution of Representative Isoparaffins in Waxes and Related Materials (WRM) Determined by the EQC Level 1 Fugacity Model

Isoparaffin	C-Number	Percent Distribution in Environmental Compartment					
		Air	Water	Soil	Sediment	Suspended Sediment	Biota
Iso-tridecane	13	92	<0.1	8	0.2	<0.1	<0.1
Iso-octadecane	18	11	<0.1	87	2	<0.1	<0.1
Iso-icosane	20	4	<0.1	94	2	<0.1	<0.1
Iso-tetraicosane	24	0.2	<0.1	98	2	<0.1	<0.1
Iso-nonaicosane	29	0.1	<0.1	98	2	<0.1	<0.1

Conclusions: WRM are complex substances comprised of hydrocarbon compounds having high molecular weights and low water solubilities. When released to the environment, individual hydrocarbons will partition in accordance with their specific physical-chemical attributes. Since most constituent hydrocarbons in WRM are larger than C_{20}, fugacity modeling shows that the vast majority of hydrocarbons in these substances will partition to soil.

5.1.4. Biodegradation

Laboratory studies indicate that paraffin waxes can be metabolized by a variety of microorganisms under aerobic conditions (Rahn, 1906; Sohngen, 1913; Fuhs, 1961; Miyamoto, 1968). Studies of biodegradability 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 an OECD 301B Modified Sturm Test) in which the waxes were placed on glass fiber filters for exposure to the medium (Hanstveit, 1991). The paraffin wax degraded by 80% after 28 days and by 87% after 84 days. The microcrystalline wax degraded by 21% after 28 days and by 25% by 87 days. API data on paraffin and microcrystalline waxes from a shake-flask study using unacclimated organisms from sewage sludge and soil are in general agreement – the paraffin wax mineralized by 55% in 31 days and by 98% in 137 days. These studies indicate that microbes may become adapted to the paraffin hydrocarbons and those compounds remaining after the standard incubation period will continue to be mineralized. The results with microcrystalline waxes indicate that acclimation does not enhance the degree of biodegradation of these more complex materials. This may be due to the higher content of branched versus normal paraffins in microcrystalline waxes.

The biodegradation of a hydrotreated slack wax (CAS No. 92062-09-4) was studied using a manometric respirometry test (OECD Guideline 301F; Exxon Biomedical Sciences, 1995). While this substance is not among those included in this HPV category, the hydrotreating

procedure does not substantially change the composition of the saturated hydrocarbons of the source slack wax which is in this category. The mean biodegradation after 28 days was 40% indicating that this substance was inherently biodegradable although it was not readily degradable. Similar results on three samples of hydrotreated slack wax of different viscosities were compiled and reviewed by CONCAWE (2001). Those data showed biodegradation of 26%, 41% and 48% at 28 days using the modified Sturm procedure (OECD Guideline 301B).

Biodegradation studies with petrolatum are not available but information on slack waxes and refined/finished waxes provide substantial data for assessing its potential biodegradation. Petrolatum is a mixture of food-grade wax and white mineral oil. Two tests (OECD Guideline 301B) of white mineral oil (covered under the Lubricating Base Oil HPV category) showed 0% and 24% biodegradation after 28 days. Therefore, the mineral oil component of petrolatum is not readily biodegradable. The remaining wax component of petrolatum may be somewhat more biodegradable but the evidence indicates that the total substance is not readily biodegradable.

Conclusions: Some paraffin waxes are readily biodegradable but slack waxes, microcrystalline waxes and petrolatum are not readily biodegradable. These substances, however, will undergo some biodegradation over time and may be considered inherently biodegradable.

5.2 Assessment Summary for Environmental Fate

WRM are complex substances consisting almost entirely of saturated hydrocarbons. These substances are non-volatile, although some of the individual hydrocarbons smaller than C_{20} are volatile enough to partition to air. Most of the constituent hydrocarbons are larger than C_{20} and most partitioning will be to soil. Any partitioning to the atmosphere will result in indirect photodegradation of the hydrocarbons. The hydrocarbons in WRM are not water soluble and are resistant to hydrolysis. Releases to the environment will result in little mobility, but eventually these substances will be incorporated into soils followed by slow biodegradation.

6. ENVIRONMENTAL EFFECTS

6.1. Aquatic Endpoints - Acute Toxicity

The HPV chemicals program includes acute toxicity to a freshwater fish, an invertebrate (*Daphnia magna*) and an alga. The substances in the WRM category contain predominantly normal alkanes, isoalkanes and cycloalkanes (naphthenics). There are no published data on the aquatic toxicity of WRM but an evaluation of their potential toxicity to aquatic organisms can be made based on:

- The size, structure and partition coefficients of the constituent compounds.
- Read-across from the aquatic toxicity of smaller n-alkanes.
- Read-across from the aquatic toxicity of lubricating oil basestocks which contain hydrocarbons similar in structure and size to the hydrocarbons in WRM.

Because the substances in this category are composed primarily of alkanes with carbon numbers greater then C_{20}, they will not cause acute or chronic toxicity to aquatic organisms. This conclusion is based, in part, on the results of Adema and van den Bos Bakker (1986) who studied the toxicity to three different aquatic invertebrates of various paraffin compounds up to n-tetradecane, a C_{14} alkane. Their results indicate that alkanes larger than C_{10} are not acutely toxic at the limit of their solubility. The smallest hydrocarbons in WRM are C_{12} in size and most constituents are larger than C_{20}. Therefore, the substances in WRM will not be acutely toxic to aquatic invertebrates.

The results of acute and chronic aquatic toxicity studies with lubricating oil basestocks are directly applicable to WRM because the base oils are derived from the same vacuum distillates and vacuum residues and contain hydrocarbons similar in size and structure. The aquatic toxicity of the base oils is discussed in the lubricating oil basestocks HPV category assessment document. Table 6 lists the results of several acute and chronic aquatic toxicity studies of lubricant base oils reviewed by CONCAWE (1997).

As with most hydrocarbons having very low solubility in water, aquatic tests are conducted by thoroughly mixing the hydrocarbon substance with water in a manner which maximizes the content of the hydrocarbon constituents in the water in which the test organisms are placed. These methods include the preparation of oil-water dispersions and water accommodated fractions (WAFs). The latter are prepared by prolonged mixing at the indicated loading level followed by removal of the resulting WAF from the mixing vessel.

The partition coefficients of hydrocarbons (expressed as log Kow) increase with increasing carbon number. Quantitative structure activity relationships, relating log Kow values of single hydrocarbons to toxicity, show that water solubility decreases more rapidly with increasing log

Table 6. Aquatic Toxicity of Lubricating Oil Basestocks (CONCAWE, 1997)

Base oil	Test Species	Exposure Method	Toxicity Endpoint	Result (mg/L)
Acute Toxicity				
Solvent-refined, light paraffinic distillate	Fish: *Oncorhyncus mykiss*	OWD[1]	96-Hr LL_{50}[3]	>1,000
Solvent-refined, heavy paraffinic distillate	Fish: *Oncorhyncus mykiss*	OWD	96-Hr LL_{50}	>1,000
Solvent-refined residual oil	Fish: *Oncorhyncus mykiss*	OWD	96-Hr LL_{50}	>1,000
Solvent-refined, light naphthenic distillate	Invertebrate: *Daphnia magna*	WAF[2]	48-Hr EL_{50}[4]	>10,000
Solvent-refined, light naphthenic distillate	Invertebrate: *Gammarus pulex*	WAF	96-Hr EL_{50}	>10,000
Solvent-refined, light paraffinic distillate	Alga: *Scenedesmus subspicatus*	WAF[7]	96-Hr IrL_{50}[5] 96-Hr IbL_{50}[6]	>50% WAF >50% WAF
Solvent-refined, heavy paraffinic distillate	Alga: *Scenedesmus subspicatus*	WAF[7]	96-Hr IrL_{50} 96-Hr IbL_{50}	>50% WAF >50% WAF
Solvent-refined residual oil	Alga: *Scenedesmus subspicatus*	WAF[7]	96-Hr IrL_{50} 96-Hr IbL_{50}	>50% WAF >50% WAF
Chronic Toxicity				
Solvent-refined, heavy paraffinic distillate	Invertebrate: *Daphnia magna*	WAF	Reproduction/ Survival effects	> 1,000
Solvent-refined residual oil	Invertebrate: *Daphnia magna*	WAF	Reproduction/ Survival effects	> 1,000

[1]Oil-water dispersion [2]Water accommodated fraction [3]Lethal loading rate required to kill 50% of the organisms
[4]Effective loading rate required to immobilize 50% of the organisms
[5]Inhibitory loading rate required to reduce algal growth rate by 50%
[6]Inhibitory loading rate required to reduce the area under the growth curve (biomass) by 50%
[7]Tests were run at a maximum exposure of a 50% WAF prepared at a 1000 mg/L loading rate

Kow than does the concentration causing effects. Therefore, 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 paraffinic hydrocarbons larger than C_{10} (log Kow > 5) show no acute toxicity and that alkylbenzenes larger than C_{14} (log Kow > 5) also show no acute toxicity (Adema and van den Bos Bakker, 1986). These demonstrated solubility "cutoffs" for acute toxicity of hydrocarbon compounds relate to their physico-chemical properties. Since the vast majority of hydrocarbons in the WRM category have partition coefficients > 5, the materials in this HPV category are not expected to be acutely toxic.

6.1.1 Acute Toxicity to Aquatic Vertebrates

As seen in Table 6 no acute toxicity to the test organism *S. subspicatus* was observed for lubricating oil basestocks tested at the maximum exposure of a 50% WAF created at a 1,000 mg/L loading level (CONCAWE, 1997). Therefore, slack wax, refined/finished waxes and petrolatum, which are derived from the same vacuum distillates as the base oils and contain similar hydrocarbons, are not expected to be acutely toxic.

Conclusions: Hydrocarbon constituents in the WRM category are high molecular weight and low water solubility compounds. These characteristics limit the bioavailability and toxicity of these substances to aquatic vertebrates. These solubility limits on the hydrocarbons and read-across to the data on lubricating oil basestocks indicate that WRM are not be acutely toxic to aquatic vertebrates.

6.1.2 Acute Toxicity to Aquatic Invertebrates

As seen in Table 6, no acute toxicity to two aquatic invertebrates was observed for lubricating oil basestocks tested at the maximum exposure loading level of 10,000 mg/L (CONCAWE, 1997). Therefore, it can be concluded that WRM (which are derived from the same vacuum distillates as base oils and contain similar hydrocarbons) are also not acutely toxic.

Conclusions: Hydrocarbon constituents in WRM are high molecular weight and low water solubility compounds. These characteristics limit the bioavailability and toxicity of these substances to aquatic invertebrates. These solubility limits on the hydrocarbons and read-across to the data on lubricating oil basestocks indicate that WRM are not acutely toxic to aquatic invertebrates.

6.1.3 Toxicity to Aquatic Plants (Algae)

As seen in Table 6 no acute toxicity to the test organism *S. subspicatus* was observed for lubricating oil basestocks tested at the maximum exposure loading level of 1,000 mg/L (CONCAWE, 1997). Therefore, it can be concluded that WRM (which are derived from the same vacuum distillates as the base oils and contain similar hydrocarbons) are also not acutely toxic.

Conclusions: Hydrocarbon constituents in WRM are high molecular weight and low water solubility compounds. These characteristics limit the bioavailability and toxicity of these substances to aquatic plants. These solubility limits on the hydrocarbons and read-across to the data on lubricant base oils indicate that WRM are not acutely toxic to aquatic plants.

6.2 Aquatic Endpoints - Chronic Toxicity to Aquatic Invertebrates

Seven different lubricating oil basestocks were tested for chronic aquatic toxicity to *Daphnia magna* (OECD Guideline 202, part 2) and reported by CONCAWE (1997). Two streams, one distillate and one residual, are reported in Table 6 as surrogates for the streams encompassing the WRM category. For the heavy paraffinic distillate and the residual oil, no toxicity was observed at the maximum exposure loading level of 1000 mg/L (Table 6). These results are typical for the other lubricating oil basestocks reported by CONCAWE (1997). Light base oils may contain a substantial fraction of hydrocarbons smaller than C_{20}. In contrast, most hydrocarbons in slack wax, refined/finished waxes and petrolatum are larger than C_{20}. The hydrocarbons in WRM are most appropriately matched to the hydrocarbons present in the heavy paraffinic distillate (predominantly C_{20} to C_{50}) and the residual oil (predominantly $C_{>25}$) in Table 6. Read-across from test results with these lubricant base oils indicates WRM will also have no chronic toxicity at a loading level of 1,000 mg/L.

Conclusions: Hydrocarbon constituents in WRM are high molecular weight and low water solubility compounds. These characteristics limit the bioavailability and toxicity of these substances to aquatic organisms. These solubility limits on the hydrocarbons and read-across to the data on lubricating oil basestocks indicate that WRM will have no significant chronic toxicity to aquatic invertebrates at levels which exceed their limits of water solubility.

6.3. Assessment Summary for Environmental Effects

The hydrocarbons comprising WRM are mostly high molecular weight saturated alkanes that exist in a solid to semi-solid state at environmental temperatures. Their extremely low water solubilities limit their bioavailability. The substances in the WRM category contain hydrocarbons similar in structure and size to the lubricating oil basestocks which are derived from the same vacuum distillates. Read-across from studies with lubricant base oils indicates WRM have low acute toxicity to fish, invertebrates and algae with loading levels causing no effects at ≥ 1000 mg/L. Read-across from chronic studies with larger molecular weight lubricating oil basestocks indicates that WRM have low chronic toxicity to aquatic invertebrates with no toxicity expected at their limits of water solubility.

7. HUMAN HEALTH ENDPOINTS

7.1 Acute Toxicity

The oral LD_{50}s of waxes, both paraffin wax and microcrystalline wax, are ≥ 5 g/kg in the rat (IBR, 1976a & b). The dermal LD_{50} of a 50% solution of paraffin wax and petrolatum in the rabbit was greater than the 3.6 g/kg dose which was tested (Elder, 1984).

Acute toxicity data are not specifically available for slack wax but data are available on the acute toxicity of the raw vacuum distillate from which both lubricating oil basestocks and slack waxes are derived (API, 1986). Acute toxicity data are also available on the lubricating oil basestocks that contain the same types of saturated hydrocarbons with similar carbon numbers found in slack wax and are derived from the same vacuum distillates. Materials similar to the oil portion of slack wax are covered under the lubricating oil basestocks HPV category. Both the raw vacuum distillate precursor stream and the lubricating base oils have oral LD_{50}s in the rat > 5 g/kg (CONCAWE, 1997). Therefore, both the waxy and oil portions of slack wax have oral LD_{50}s > 5 g/kg.

There are no acute toxicity data specifically on undiluted, refined petrolatum (petroleum jelly). However, a sample of petrolatum mixed with a paraffin wax had an acute dermal LD_{50} > 3.6 g/kg (see above). In addition, petrolatum is widely used in cosmetic applications. Petrolatum is a mixture of refined wax and white mineral oil. Because of its food and cosmetic applications, the acute, repeat dose, chronic, reproductive toxicity of white mineral oil is well characterized. White mineral oil has an oral LD_{50} > 5 g/kg in the rat (Hulse et al, 1992). Because the wax portion of petrolatum is also expected to have a very high oral LD_{50}, the acute oral toxicity of petrolatum in the rat is estimated to be > 5 g/kg.

Inhalation toxicity studies of slack wax, refined/finished waxes and petrolatum have not been conducted due to their physical characteristics (low vapor pressure) and low potential for inhalation exposure. Under circumstances where waxes are heated to a high temperature, wax fumes may be generated and these are reported to be irritating to the respiratory tract (ACGIH, 2001).

The skin irritation potential of paraffin wax and microcrystalline wax was reviewed by an expert panel on cosmetic ingredients (CTFA, 1981). When tested undiluted under a single closed patch in the rabbit the paraffin wax was non-irritating after 24 hours and the microcrystalline wax was slightly irritating. The same expert panel reported on the eye irritation of four samples of 50% paraffin wax in petrolatum. These samples were tested in the rabbit with observations for three days. Two samples caused mild irritation in one animal on day one and the other samples were not irritating.

Conclusions:
Based on available studies, slack wax, refined/finished waxes and petrolatum (petroleum jelly) have very low acute systemic toxicity. In the rat, the oral LD_{50}s of these materials is > 5 g/kg, the dermal LD_{50}s are > 3.6 g/kg and the eye and skin irritation potentials are low.

7.2 Repeated Dose Toxicity

The results of several repeat dose and chronic studies with materials from this HPV category are discussed in this section of the document and in Section 7.6.1. These studies are listed in Table 7.

Table 7. Repeated Dose Studies on Waxes and Related Substances

Study	Test Materials	Duration	Exposure	Species/ Strain
Shubik *et al*., 1962	5 Refined waxes	2 yrs	Dietary	Rat/S-D[1]
Oser *et al*., 1965	3 Petrolatums	2 yrs	Dietary	Rats/FDRL
BIBRA 1992; 1993 Smith, *et al*., 1996	Low melt paraffin wax (LMPW) and 4 other waxes (Table 8) Several white mineral oils	90 days	Dietary	Rat/F344[2]
Firriolo *et al*., 1995	Low viscosity white mineral oil	≤ 92 days	Dietary	Rats/ S-D and F344
Shoda *et al*., 1997	Med viscosity white mineral oil	2 yrs	Dietary	Rat/F344
Hoglen, *et al*., 1998	Low melt paraffin wax (LMPW)	60 days	Dietary	Rats/ S-D and F344
Scotter, *et al*., 2003	Low melt paraffin wax (LMPW) C80 synthetic wax 3 White mineral oils	28 or 90 days	Dietary	Rats/F344
Griffis *et al*., 2010	Low melt paraffin wax (LMPW)	≤ 90 days	Dietary	Rats/ S-D and F344
Smith *et al*., 1951 Dietz *et al*., 1952	Slack waxes produced using "sweating" process	Lifetime	Dermal	Mice/albino
Shubik *et al*., 1962	Refined waxes	Lifetime	Dermal	Rabbit
Lijinsky *et al*., 1966	Petrolatum	Lifetime	Dermal	Mice/Swiss
Kane *et al*., 1984	Slack wax Petrolatum	80 wks	Dermal	Mice/C3H

[1]Sprague-Dawley derived [2]Fischer-344

7.2.1 Slack Wax

The one substance in the WRM category which could possibly contain biologically significant concentrations of PAC is slack wax. Current processing in the U.S. generally results in slack waxes with very low PACs since they are derived after solvent dewaxing and/or hydrotreatment which are used to reduce or eliminate PAC and other impurities. Slack waxes potentially imported into the USA, may contain oil with significant levels of PAC which carries through from the original vacuum distillates from which they are derived. However, it is not known whether slack waxes from unrefined distillates are imported into the US.

Samples of slack wax analyzed for the HPV Challenge program contained no detectable PAC (Table 3) and are therefore predicted to be non-hazardous (e.g., NOAEL in rats is predicted to be ≥ 1000 mg/kg/day). If slack waxes which contain measurable levels of PAC are identified, a significant dermal hazard may exist for repeated dose toxicity (Appendix 1). Such an assessment would have to be based on the PAC profile of that sample or inferred from knowledge of process history.

An assessment of the repeated dose toxicity of slack waxes can also be based on toxicity data on petroleum waxes which are derived from slack waxes and the toxicity data on lubricating oil basestocks which are similar to the oil component in slack wax. Slack waxes comprised of lower melting point wax and lower viscosity oils will have repeat dose toxicity similar to that observed with low melting point paraffin wax (LMPW) and low viscosity mineral oils. Slack waxes comprised of higher melting point wax and higher viscosity oils will have even less potential repeat dose toxicity. The repeated dose studies on refined/finished waxes and oils, and the no observed adverse effect levels, are discussed at length below.

7.2.2 Refined/finished waxes

The chronic toxicity of five refined/finished waxes was investigated in feeding studies (Shubik *et al*., 1962). The waxes were fed to male and female Sprague-Dawley rats at a dietary concentration of 10% (approximately 5,000 mg/kg/day) for two years. All grossly abnormal tissues were examined histologically. After the dietary exposures there were no treatment-related changes in survival or growth, and no treatment-related changes were observed at necropsy or during histopathological examination of tissues. Thus, the no observed adverse effect levels (NOAELs) for all 5 waxes assessed in this lifetime study were approximately 5,000 mg/kg/day.

Several 90-day feeding studies were conducted in rats on various white mineral oils and food-grade waxes ranging from low melting point paraffin wax to microcrystalline wax (BIBRA, 1992; 1993). These studies are described in the publication of Smith *et al.* (1996). The results with the mineral oils are discussed below.

These studies were conducted according to Good Laboratory Practices and thoroughly reported. In these studies, five different waxes (Table 8) were tested ranging from a low melting point paraffin wax (LMPW) to a clay-treated microcrystalline wax containing 0.2% sulfur (called high sulfur wax, HSW) and a hydrotreated high melting point microcrystalline wax (HMPW). The waxes were fed to groups of male and female Fischer-344 (F344) rats in diets containing 0, 0.002, 0.02, 0.2 or 2.0% for 90 days. Additional groups were treated at the high dose for 90 days and then fed control diet for a 28-day recovery period.

Growth rates, food intakes and clinical condition of the rats fed the two microcrystalline waxes were not affected. The only hematological change noted in the groups fed the HSW or HMPW was a slight (2%) increase in hemoglobin concentration in the males at the high dose. Serum glucose was slightly elevated (8-13%) in all the groups fed HMPW and in all but the highest dose group fed HSW. There were no treatment-related increases in tissue weights in the groups fed HSW or HMPW. No treatment-related histopathological changes were observed in the groups fed the microcrystalline waxes (HSW or HMPW). The NOAEL was considered to be 2.0% or about 1,000 mg/kg/day.

Table 8. Paraffin and Microcrystalline Waxes Used in Subchronic Dietary Studies in Rats (BIBRA 1992, 1993; Smith *et al*., 1996)

Wax	Refining Method	Viscosity @ 100°C	Average Molecular Wt.	Carbon Number Range
Low melting point wax (LMPW)	Hydrogenation	3.3 cSt	375	19-42
LMPW + HMPW (1:1) (Mixed Paraffins or MP)	Hydrogenation	7.2 cSt	305	19-74
Intermediate melting point wax (IMPW)	Hydrogenation	6.3 cSt	450	21-49
High sulfur wax (HSW)[1]	Percolation	13.7 cSt	600	20-74
High melting point wax (HMPW)[1]	Hydrogenation	15.4 cSt	630	22-80

[1]The HSW and HMPW were microcrystalline waxes

The groups of F344 rats fed the lower melting point waxes (LMPW, IMPW or MP) in the BIBRA study showed increased liver and spleen weights at the 0.2% and 2% doses with some, but not complete reversal after the recovery period. The weight of mesenteric lymph nodes (MLN) was only available in the 2% dose group. The MLN weight was increased in the LMPW-fed rats and this was only partly reversed during the recovery period. Several hematological parameters were affected in the rats fed LMPW (hemoglobin and white blood cell counts), and IMPW or MP (white blood cell counts). Some serum enzymes were elevated in females at the two highest doses of LMPW and MP or at the highest dose of IMPW. Males fed the highest dose of LMPW also had increases in some serum enzymes.

Histopathological evaluation revealed inflammatory changes in the liver, MLN, ileum and jejunum of rats fed LMPW, IMPW or MP. Liver effects included hepatocellular vacuolation and granulomas. Histiocytosis and microgranulomas were observed in the MLN and there was an increased incidence of macrophage accumulation in the Peyer's patches in the ileum and jejunum. The MLN changes were noted at all dose levels although at a reduced severity at the low doses. Focal inflammatory lesions (cellular infiltrates) were observed in the heart at the base of the mitral valve in female rats fed 0.2% or 2% LMPW and in rats fed 2% MP or IMPW. The inflammatory changes in the liver and MLN were reduced but still present after the recovery periods. The most important findings were the liver and LMN inflammatory changes and, overall, female F344 rats were more sensitive than males. The microcrystalline waxes (HMPW and HSW) were without significant effect. The mineral hydrocarbon (MHC) concentrations in target tissues from rats were determined using chemical analysis. No MHCs were detected in tissues from rats fed the microcrystalline waxes (HMPW, HSW) but measurable amounts were found in the liver and MLN of F344 rats fed LMPW, IMPW or MP.

The inflammatory changes in the F344 rats treated with dietary LMPW, IMPW or MP described above were also observed in the same study in the rats fed highly-refined, lower viscosity food-grade white oils except the mitral valve changes were not observed with the white oils. When tested in additional 90-day feeding studies in the F344 rat, higher molecular weight materials such as microcrystalline waxes and higher viscosity white oils were without biological effects

while paraffin waxes and low to medium viscosity white oils produced inflammatory effects in the liver and MLN that were inversely related to molecular weight, viscosity and melting point (Smith *et al*., 1996). The type of crude oil from which the wax or white oil was derived, and the refining method, did not appear to affect the response.

Scotter *et al*. (2003) reported on 28- and 90-day feeding studies in which groups of 12 female F344 rats were administered 0 or 2% LMPW. All animals were monitored for weight gain, food intake and clinical condition throughout the study. Tissues taken at necropsy were examined for histopathological changes, and samples of tissues were extracted and analyzed for mineral hydrocarbons. There were no overall effects on body weight during either the 28- or 90-day studies. At the end of the 28-day study, a significant increase in the weights of MLNs was observed in the rats fed LMPW. At the end of the 90-day study, significant increases were seen in liver, spleen and MLN weights from rats fed LMPW. After the 28- and 90-day studies, histiocytosis was seen in the MLNs of LMPW-treated rats but histopathological changes in the liver were only observed after the 90-day study. The liver changes were focal collections of macrophages surrounded by inflammatory cells and were classified as granulomas. In the mitral valve of the heart, focal inflammation characterized as an infiltration of inflammatory cells within the cusps and a loss of fibrous core was observed in a number of rats fed LMPW for 90 days. MHCs were detected in the tissues of rats fed LMPW and detailed analyses showed that the carbon number range of accumulated hydrocarbons was approximately C_{20} to C_{35} for both n-alkanes and isoalkanes. A NOEL was not established in these studies.

The feeding studies of mineral hydrocarbons (including lower molecular weight waxes and white oils in dogs and various rat strains) conducted prior to the BIBRA studies described above showed no significant toxicity (Hulse *et al*., 1992; Smith *et al*., 1951; Shubik *et al*., 1962). The Shubik *et al*. study involved feeding very high levels (10%) of different waxes to rats with no significant effects after a lifetime exposure. After the unusual response of the F344 rat to mineral hydrocarbons was confirmed, several studies were conducted to evaluate any potential differences in the responses of different strains of rat to ingestion of mineral hydrocarbons.

In a direct comparison study in which F344 rats and a common Sprague-Dawley (S-D) derived rat strain were both fed a low-viscosity white mineral oil at 0.2% or 2% in the diet for up to 92 days, the S-D rats showed only a slight increase in inflammatory cells in the liver at the highest dose with no formation of microgranulomas. In contrast, the F344 rats had hematological and clinical chemistry changes, increased liver, MLN and spleen weights, and microgranulomas in the liver and MLN (Firriolo *et al*., 1995). The MHC concentrations determined by chemical analysis in the MLN from both rat strains were similar but the F344 rats had a 3 to 4- fold greater liver burden than the S-D rats at the same doses. Although the levels of hydrocarbons in the MLN of the S-D rats were similar to those seen in the F344 (1.4 mg/g vs. 1.5 mg/g, respectively), no treatment-related histiocytosis or microgranulomas were observed in the MLN of the treated S-D rats. The results of this study indicate that the F344 rat strain accumulated more MHCs in the liver and lymph nodes than did the SD rats and had a much greater inflammatory response to the ingested white oil. The NOAEL in the S-D rats appeared to be 2% in the diet (no liver microgranulomas) or about 1600 mg/kg/day.

Hoglen *et al*. (1998) examined differential effects of dietary exposure to 2% LMPW for 60 days on female F344 and Sprague-Dawley (S-D) rats (9-10 animals/group). LMPW was chosen as the test material because it appeared to elicit a greater response than other waxes or white oils in the previous studies and females were studied because they appeared to have a somewhat greater response than males in the previous studies. At necropsy, a portion of the liver was collected for determination of MHC concentration. Kupffer cells were also isolated from the livers for morphological and functional evaluations because these cells can be involved in the formation of granulomas.

Mean body weights were not affected in either strain of rat after 60 days of LMPW exposure. A number of changes were observed within the livers of F344 rats fed LMPW but not in livers from treated S-D rats. Histopathological evaluation revealed the presence of microgranulomas in the livers of all LMPW-treated F344 rats but only in the liver of one treated S-D rat. Lymphoid cell aggregates were found only in livers from treated F344 rats. ALAT, ASAT and GGT activities were greater in the serum of LMPW-treated F344 rats compared to treated S-D rats and controls. Total white blood cell and neutrophil counts were significantly elevated in the blood of treated F344 rats. Electron microscopy of Kupffer cells from the livers of F344 rats fed LMPW revealed the presence of large, irregularly shaped, membrane-associated vacuoles in over half the cells. These vacuoles were not observed in Kupffer cells from control rats and were rarely detected (< 5%) in Kupffer cells from treated S-D rats. In addition, indices of Kupffer cell function including phagocytosis and nitric oxide and superoxide anion production were significantly increased in Kupffer cells from LMPW-treated F344 rats. Lipopolysaccharide-stimulated production of tumor necrosis factor-α and leukotriene B4 were significantly decreased in Kupffer cells from F344 rats fed LMPW. No significant changes in these functional endpoints were observed in the Kupffer cells from the S-D rats fed LMPW. The observed differences in effects on Kupffer cells may account, in part, for the strain differences in response to LMPW and other MHC.

In another comparative repeat dose study, female F344 and S-D rats were evaluated for general toxicity as well as specific immunological effects after 90 days dietary treatment with 0.2% or 2% LMPW (Griffis *et al*., 2010). Effects in the F344 rats were significantly greater than in the S-D rats. Increased liver and spleen weights and inflammatory changes (e.g. liver microgranulomas) in these tissues were observed only in the F344 rats. Microgranulomas were observed in the MLN of both strains but the effects were substantially greater in the F344 rats. Cellular markers of inflammation were examined in a subset of rats from each group using immunohistochemical staining. An increase in staining for CD3 (T-cells), CD8a (suppressor/cytotoxic T-cells) and CD4 (helper T-cells) correlated with an increase in lymphoid cells in the livers of treated F344 rats. The majority of macrophages in the liver microgranulomas of treated F344 rats were negative for the ED2 marker, indicating a likely origin from nonresident macrophages. Electron microscopy showed liver macrophage hypertrophy and hyperplasia in treated F344 rats. Lysozyme staining (indicating activation of macrophages), however, decreased with increasing granuloma size. Non-ED2 expressing cells may have been recruited to the liver but were not sufficiently activated to be lysozyme positive. Inflammatory changes in the cardiac mitral valves noted in previous studies were also seen in the F344 rats in this study but not in the S-D rats. Chemical analysis showed that MHC accumulated in the livers from treated F344 but not S-D rats and the concentration was more than 2-fold greater in the

MLN from the F344 rats than from the S-D rats. Since only minimal changes were observed in the MLNs of S-D rats at 2.0%, this treatment level (about 1600 mg/kg/day) may be considered the NOAEL.

There is strong evidence that the response observed in the F344 rat to ingestion of lower molecular weight waxes and white oils is also due, in part, to species- and strain-specific differences in absorption and metabolism of these mineral hydrocarbons. Limited absorption (2 to 20%) of ingested mineral hydrocarbons has been demonstrated for a number of mammalian species, including humans (Barrowman *et al*., 1989; Lester, 1979). In rats, studies have shown that absorption of aliphatic hydrocarbons is inversely related to carbon number, ranging from 60% for C_{14} to 5% for C_{28} compounds with essentially no absorption observed above C_{32} (Albro and Fishbein, 1970; Boitnott and Margolis, 1970; Low *et al*., 1992). Studies of low viscosity white oil showed large differences in absorption and elimination between F344 and S-D rats with the F344 rat retaining more hydrocarbons (Halladay *et al*., 2002). Other studies have shown that the uptake of mineral hydrocarbons by the liver of F344 rats is significantly greater than for other rat strains (S-D, Long-Evans) and another species (beagle dogs) (Firriolo *et al*., 1995; Smith *et al*., 1995). Most of the hydrocarbons detected in the livers of F344 rats fed waxes or white oils were approximately C_{20} to C_{35} in size which is evidence that mineral hydrocarbons greater than about C_{35} are not significantly absorbed while those smaller than C_{20} are absorbed and then readily metabolized and excreted (CONCAWE, 1993; Scotter *et al*., 2003; Griffis *et al*., 2010).

In addition to differences in pharmacokinetics, differences in immune sensitivity probably play a role in the greater response of F344 rats to waxes and mineral oils. Micrograniulomas form spontaneously in F344 rats, particularly in females, but only rarely in other rat strains (Boorman *et al*., 1990; Dixon *et al*., 1995). As discussed above, Kupffer cells from the livers of F344 rats fed 2% LMPW for 60 days were morphologically different and markedly activated compared to Kupffer cells from the livers of treated S-D rats (Hoglen *et al*., 1998).

In humans, lipogranulomas in the liver, lymph nodes and spleen appear to result from ingestion of mineral oil. These lipogranulomas occur in a high percentage of people but they do not progress and are not associated with clinical abnormalities (Fleming *et al*., 1998). In contrast to the inflammatory character of the micrograniulomas observed in F344 rats, the human lipogranulomas have been described as small histiocytic collections with minimal signs of inflammation (Fleming *et al*., 1998; Carlton *et al*., 2001). The human lipogranulomas have not been observed to progress to clinically significant lesions and a collective review of mineral hydrocarbon-related histological changes by several human and veterinary pathologists concluded that the changes induced by waxes and white oils in the F344 rat are different from those seen in human tissues and the latter are considered incidental and inconsequential (Carlton *et al*., 2001). The evidence from several different studies indicates that the toxicological response to dietary waxes and white oils seen in the F344 rat is different from the response in other rat strains and other species and has no clinical significance to humans.

In addition to the studies discussed above on refined/finished waxes and white mineral oils, there are several repeat dose dermal studies on lubricating oil basestocks. The results of some of these studies are summarized in Table 9. The oils tested in these studies ranged from mildly refined to highly refined although most of the lubricating oil basestocks are not refined enough to meet the

purity standards of the white mineral oils discussed previously (Table 7). The lubricating oil basestocks are closely related to the oils in slack waxes.

Table 9. Summary of Selected Subchronic Dermal Toxicity Studies of Lubricating Oil Basestocks.

CAS Number	Lubricating Oil Basestock	Study Summary	Systemic NOAEL (mg/kg/day)
	Untreated or Mildly Refined		
64741-50-0	Distillates (petroleum), light paraffinic	200, 1000 and 2000 mg/kg of API 84-01 was given 3 days/wk for 28 days to New Zealand white rabbits. Body weight decreased marginally at 2000 mg/kg (CONCAWE, 1997).	1000
	Highly Refined		
64741-96-4	Distillates (petroleum), solvent refined heavy naphthenic	5000 mg/kg of API 79-1 was given 4 hr/day, 3 days/wk for 21 days to New Zealand white rabbits (CONCAWE, 1997).	5000
64741-97-5	Distillates (petroleum), solvent refined light naphthenic	5000 mg/kg of API 78-5 was given 4 hr/day, 3 days/wk for 21 days to New Zealand white rabbits (CONCAWE, 1997).	5000
64742-54-7	Distillates (petroleum), hydrotreated heavy paraffinic	800 and 2000 mg/kg of Ssangyong 150N were given non-occluded 5 days/wk for 13 weeks to S-D rats. Slight dermal irritation was seen. Body weights were lower in treated males at both doses. Liver weights were increased in both males and females but histological changes were minimal. Thymus weight was decreased in males and adrenal weight was increased in males. No histological changes were observed in either organ and the weight differences were not considered an adverse effect (Mobil, 1988).	2000
64742-65-0	Distillates (petroleum), solvent -dewaxed heavy paraffinic	5000 mg/kg of API 79-3 was given 4 hr/day, 3 days/wk for 21 days to New Zealand white rabbits (CONCAWE, 1997).	5000

The only consistent effect observed in the dermal studies of lubricating oil basestocks was skin irritation with many of the tested samples, ranging from none detected to moderate. Slightly increased liver weights were observed in some of the dermal studies but there were no significant histopathological changes. The NOAEL for systemic toxicity in these studies ranged from 1000 to 5000 mg/kg/day.

7.2.3 Petrolatum

In a two year study with three different petrolatums, groups of 50 male and 50 female rats were fed diets containing 50,000 ppm petrolatum (Oser *et al*., 1965). All tissues identified as abnormal at necropsy plus all the tissues sampled from 10 animals in each group were examined for histopathology. Growth rates and survival were unaffected by petrolatum exposure. There were no exposure-related effects on hematological and clinical chemical endpoints. There were no treatment-related histopathological effects or increases in tumor incidences.

Repeated dose test data on the higher viscosity white mineral oils are applicable to the toxicological evaluation of petrolatum because the oil component of petrolatum is equivalent to white mineral oil. Two-year feeding studies in F344 rats have been conducted with 70 cSt and 100 cSt paraffinic white mineral oils (P70 and P100, respectively) at doses up to 1200 mg/kg/day with no chronic toxicity or carcinogenicity observed (Trimmer *et al*., 2004). Shoda *et al.* (1997) conducted a two-year feeding study in F344 rats on a white oil mixture with a viscosity of about 65 cSt (at 40°C) and they also reported no chronic toxicity or carcinogenicity after dietary doses of 1250 and 2500 mg/kg/day.

Since petrolatum consists of refined wax plus higher viscosity white mineral oil, the repeated dose toxicity of petrolatum has been assessed from studies with equivalent waxes and mineral oils (discussed above) in addition to the chronic study of Oser *et al*. (1965). These studies indicate that the repeated dose toxicity of petrolatum is very low with a NOAEL ≥ 1000 mg/kg/day.

Conclusions:
The samples of WRM solicited from Petroleum HPV member companies had no detectable PAC. Therefore their predicted toxicity is negligible (NOAEL ≥ 1000 mg/kg/day). Slack waxes which are not as well refined may contain significant levels of PAC and, therefore, may have potential health hazards (see Appendix 1).

The repeated dose toxicity of WRM category members is also characterized by studies on petroleum waxes and petrolatums themselves plus studies on lubricating oil basestocks and white mineral oils. Lower melting point waxes and lower viscosity white mineral oils caused some inflammatory changes in one strain of rat but only minimal changes in other rat strains and species. Comparative toxicity, pharmacokinetic and pathology studies indicate that the response seen in the F344 rat is not applicable to human health assessment. Based on repeat dose and chronic studies of refined waxes, petrolatums and mineral oils in other animal models, plus decades of safe use in food and pharmaceutical applications, the repeated dose toxicity of these materials is low. Based on only minimal changes observed in S-D rats fed waxes or white oil at 2% in the diet, the NOAELs of slack wax, refined/finished waxes and petrolatum are ≥ 1000 mg/kg/day.

7.3 Genetic Toxicity

7.3.1 *In Vitro*

Slack wax represents the worst-case substance relative to potential mutagenic activity in this category since finished/refined waxes are made from slack wax by removing the remaining oil from the slack wax and the oil portion of slack wax would contain any PAC which may be present. Since slack wax was the substance in the WRM category which could have contained significant amounts of PAC, an optimized Ames test (see Appendix 2) was conducted on four samples received from a Petroleum HPV member company.

Four different samples of slack wax of varying molecular weight were tested as described in the optimized Ames assay for lubricating base oils (ASTM Standard Method E 1687-98; Blackburn *et al*., 1986). Data for all four slack wax samples indicated a lack of mutagenic activity (Table 9).

Table 10. Results[1] of Optimized Ames Test with Four Samples of Slack Wax

Sample	Mutagenic Index	Fold Increase in Revertants
100 Slack Wax	0	1.12
200 Slack Wax	0	0.98
350 Slack Wax	0	0.90
650 Slack Wax	0	0.83

[1](Petrolabs, 2004)

This result is consistent with the lack of detectable PACs in the four samples of slack wax as determined by the PAC-2 chemical analysis procedure (Table 3). The statistical model developed to predict the mutagenic index from PAC profiles of high-boiling petroleum substances was not necessary to assess these samples (see Appendix 2).

Refined/finished waxes and refined petrolatum have not been assessed for mutagenic potential. Studies have been conducted, however, on synthetic wax comprised of normal alkanes which make up a significant portion of all waxes. A DMSO extract of a Fischer-Tropsch synthetic paraffin wax was tested in a bacterial reverse mutation assay according to OECD guideline 471 (TNO, 2005a). *S. typhimurium* test strains TA1535, TA1537, TA98, TA100 and *E. coli* strain WP2 uvrA were tested in the absence and presence of a metabolic activation system. The extract of synthetic paraffin wax was not mutagenic.

A mouse lymphoma assay was also conducted on a DMSO extract of a synthetic Fischer-Tropsch paraffin wax according to OECD guideline 476 (TNO, 2005b). Mouse lymphoma L5178Y cells were treated with 0.018 to 10 mmol/l of the extract for 4 hours with and without a metabolic activation system. The extract of synthetic wax was not cytotoxic and no relevant increase in mutation frequency at the TK-locus was observed with or without metabolic activation. The negative and positive controls responded as expected. The extract of synthetic paraffin wax was not mutagenic to mouse lymphoma cells.

An extract of a synthetic Fischer-Tropsch paraffin wax tested for the potential to induce structural chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without a metabolic activation system (TNO, 2005c). The extract of synthetic wax and its serial dilutions were not cytotoxic and did not induce structural chromosomal aberrations. The negative and positive controls responded as expected. The extract of synthetic paraffin wax did not cause chromosomal aberrations in CHO cells in this assay.

7.3.2 *In Vivo*

In vivo genetic toxicity studies have not been reported for WRM but such studies have been reported for lubricating oil basestocks. Lubricant base oils are derived from the same vacuum distillates as WRM and these data will be used for an assessment of the waxes. A bone marrow cytogenetics assay was conducted in the rat on five paraffinic and two naphthenic lubricant base

oils (Conaway *et al.,* 1984). Rats were dosed once a day orally for five days with the paraffinic base oils at doses up to 2000 mg/kg and with the naphthenic base oils at doses up to 5000 mg/kg. Controls received corn oil or saline for the paraffinic or naphthenic oil treatment groups, respectively. Positive controls responded as expected but none of the base oils caused an increase in the percentage of aberrant cells in the bone marrow of treated rats.

Also, solvent extracted paraffinic oils were inactive in the micronucleus assay (McKee et al, 1990) and micronucleus tests with petroleum streams that contain higher amounts of PACs have also been negative, leading to the conclusion that PAC-containing petroleum substances do not produce chromosomal effects when tested in SIDS-level assays under *in vivo* conditions (McKee et al, 2010).

Conclusions:
The genetic toxicity testing of slack wax addresses the genetic toxicity hazards of all substances in the WRM category. This is because slack wax, as the least processed of the materials, contains the broadest spectrum of chemical components and highest concentration of potentially toxic components. Samples of slack wax were tested in an *in vitro* assay which is optimized to detect mutagenic PACs and all the samples were negative. *In vitro* tests on synthetic Fischer-Tropsch wax are additional data that indicate the n-alkanes in WRM are not mutagenic. A negative *in vivo* cytogenicity study of several lubricant base oils also supports the assessment that WRM derived from the same vacuum distillates are not genotoxic. Food-grade waxes and petrolatum have very low levels of potentially genotoxic PACs since they must meet stringent standards for total aromatic content. The genetic toxicity hazard potential for WRM is extremely low.

7.4 Developmental Toxicity

Although there are no direct studies of the developmental toxicity of the substances in the WRM category, their potential developmental toxicity can be accessed from the following:

- Developmental toxicity of petroleum streams from vacuum distillates is related to the PAC profile of the substance (API, 2008).
- The PAC profile of the substances in the WRM category (Table 3).
- Read-across from reproductive/developmental toxicity studies conducted on lubricating oil basestocks which are derived from the same vacuum distillates as WRM.

The one substance in the WRM category which could possibly contain biologically significant concentrations of PAC is slack wax. Current processing in the U.S. generally produces slack waxes with very low PACs since they are derived after solvent dewaxing and/or hydrotreatment which are used to reduce or eliminate PAC, other aromatics, olefins and sulfur. With respect to slack waxes which may be imported into the USA, some may contain oil (and significant levels of PAC) which carries through from the original vacuum distillates from which they are derived.

Samples of slack wax analyzed for the HPV Challenge program contained no detectable PAC (Table 3) and are therefore predicted to be non-hazardous (e.g., NOAEL in rats is predicted to be $\geq$ 1000 mg/kg/day). For slack wax samples which contain measurable levels of PAC, a

significant hazard may exist for developmental toxicity but such an assessment would have to be based on the PAC profile of that sample (see Appendix 1).

In addition to an assessment based on their PAC profile, the potential developmental toxicity of the WRM can be evaluated based on data from studies with lubricating oil basestocks because the WRM and oils are derived from the same vacuum distillate refinery streams and their constituent hydrocarbons are of similar size and structure. A developmental toxicity study was conducted on a lubricant base oil (Mobil, 1987). Pregnant S-D rats were dosed dermally at 0, 125, 500 and 2000 mg/kg/day on gestation days 0-19. The lowest dose was maternally toxic due to skin irritation. There was no evidence of developmental abnormalities at the highest dose tested so the developmental NOAEL was >2000 mg/kg/day.

The results of a developmental toxicity study with a highly refined lubricating oil basestock was recently reported (Kuhl et al., 2010). Pregnant SD rats (25/group) were dosed dermally (non-occluded) with the oil once/day at a dose of 0 or 1000 mg/kg on gestation days 0 - 19. Maternal effects were limited to an increase in mean adrenal gland weight and a decrease in mean thymus weight. These effects were not considered adverse. Intrauterine growth and fetal survival were not affected and there were no treatment-related effects on fetal development. The NOAEL for maternal and fetal effects was considered to be >1000 mg/kg/day.

A one generation reproductive/developmental toxicity screening study (OECD guideline 421) was conducted on a lubricant base oil (WIL Research, 1995). The oil was administered by oral gavage to groups of 12 male and 12 female S-D rats for a minimum of 14 days prior to mating until a total dosing period of 30 days had elapsed for males and until day 4 of lactation for females (39 days). The dose was approximately 1000 mg/kg/day and this group of rats served as the controls for a study conducted on a lubricant additive which was diluted in the oil for the study. There were no significant effects on the adults or offspring dosed with the oil. Fertility and mating indices were both 100%. Although the study included no untreated controls for the group dosed with the lubricating base oil, all recorded values were within normal limits and the NOAEL was >1000 mg/kg/day.

Conclusions:
The samples from the WRM category solicited from Petroleum HPV member companies had no detectable PAC. Therefore the predicted toxicity is negligible (e.g., NOAEL ≥ 1000 mg/kg/day). Slack waxes obtained from vacuum distillates which are not as well refined may contain significant levels of PAC and, therefore, may have potential health hazards (see Appendix 1).

The developmental toxicity of the substances in this category can also be assessed by read-across from two developmental toxicity studies and a reproductive/developmental toxicity screening study conducted on lubricating oil basestocks. No developmental effects were observed in these studies and the NOAEL was >1000 mg/kg/day. Based on the lack of PAC in the samples of materials from this category and studies on lubricant base oils, the potential developmental toxicity of substances in the WRM category is low.

7.5 Reproductive Toxicity

Although there are no specific studies of the reproductive toxicity of the substances in this category, their reproductive toxicity can be assessed from the following:

- Reproductive toxicity of petroleum streams from vacuum distillates is related to the content of PAC in the substance (API, 2008).
- The PAC profile of the substances in the waxes HPV category (Table 3).
- Lack of effects on reproductive organs observed in subchronic and chronic studies on waxes and mineral oils.
- Read-across from reproductive/developmental toxicity studies conducted on lubricating oil basestocks which are derived from the same vacuum distillates as WRM.

Reproductive studies of a petroleum stream containing a high level of PAC (clarified slurry oil) indicated that reproductive endpoints (e.g., fertility and sperm production) were unaffected at doses at which fetal survival was severely compromised in a developmental toxicity study that extended to postnatal day 4 (Hoberman *et al*., 1995). Thus, it can be reasonably concluded that reproductive effects, such as fertility and sperm production, are not as sensitive to PAC-containing materials as are developmental toxicity effects. Samples of substances from the WRM category, including slack wax, were analyzed for PAC and none were detected (Section 1.3 and Table 3). Since reproductive endpoints are less sensitive than developmental toxicity endpoints to PAC and PAC were not detected in samples from the WRM category, their potential for reproductive toxicity is low.

Several repeat dose (90-day) and chronic toxicity studies have been conducted with waxes, lubricating oil basestocks, white mineral oils and petrolatum. These studies were summarized in Section 7.2. In the studies described by Smith *et al*. (1996), dietary treatment of F344 rats with a low melting point paraffin wax (LMPW), an intermediate melting point wax and two different microcrystalline waxes for 90 days at treatment levels up to 20,000 ppm caused no effects detected by histopathological examination of reproductive organs (ovaries, uterus, testes, seminal vesicles). In the study reported by Griffis *et al*. (2010) there were no significant effects on the ovaries of F344 and S-D rats fed diets containing 20,000 ppm LMPW for 90 days. In the chronic study by Shoda *et al*. (1997) groups of 50 male and 50 female F344 rats were fed diet containing up to 5% white mineral oil for two years. Histopathological examination revealed no treatment-related effects on reproductive organs. In a two year study with three different petrolatums, groups of 50 male and 50 female rats were fed diets containing 50,000 ppm petrolatum (Oser *et al*., 1965). All tissues identified as abnormal at necropsy plus all the tissues sampled from 10 animals in each group were examined for histopathological effects. There were no treatment-related effects on reproductive organs after these very high doses of petrolatum.

As described in the section 7.4 on Developmental Toxicity, a one generation reproductive/ developmental toxicity screening study (OECD guideline 421) was conducted on a lubricant base oil (WIL Research, 1995). There were no significant effects on the adults or offspring after receiving oral doses of approximately 1000 mg/kg/day. Fertility and mating indices were both 100% and the reproductive NOAEL was approximately 1000 mg/kg/day.

Conclusions:
Reproductive toxicity endpoints are much less sensitive than developmental toxicity endpoints to dermally applied PAC containing petroleum substances (API, 2008). Since samples of WRM contained no detectable PACs, the potential developmental and reproductive toxicity of substances in the WRM category are predicted to be low. In addition, no effects on reproductive organs were observed in several repeat dose and two year studies in which rats were fed very high levels of waxes, white mineral oils or petrolatums (see sections 7.2 and 7.6).

The reproductive toxicity of WRM can also be assessed by read-across from a reproductive/developmental screening study conducted on a lubricant base oil. No toxicity was observed in this study, and the NOAEL via the oral route was approximately 1000 mg/kg/day. Based on the lack of PAC in the WRM samples, the lack of effects on reproductive organs in long-term studies, and the absence of evidence of reproductive effects in a study of a lubricant base oil, the potential reproductive toxicity of the WRM category is low.

7.6 Health Effects Other

7.6.1 Carcinogenicity - Dermal

Carcinogenicity testing is beyond the scope of the HPV program but it should be noted that studies have been performed to evaluate the dermal carcinogenicity of slack waxes, refined/finished waxes and petrolatum. Three skin carcinogenicity studies have been reported on slack waxes (Smith *et al*., 1951; Dietz *et al*., 1952; Kane *et al*, 1984). The quality of these studies could not be verified but the results are summarized below. Only one of these studies (Kane *et al*., 1984) tested a wax produced by solvent extraction, the process used in modern refining. The two older studies tested waxes produced using an older process of "pressing" unrefined or poorly refined vacuum distillates. This older "pressing" process which is no longer used in the United States to manufacture waxes resulted in slack waxes which contained higher levels of toxic impurities such as aromatics and PAC. As discussed in Section 1.3, samples of slack waxes produced using modern refining practices had no detectable PACs.

Eight slack waxes produced with the older "pressing" process 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 (30 per group). At 450 days, benign skin tumors had developed in all groups and malignant skin tumors in five of the eight groups. The authors of this report concluded that the slack waxes were weakly carcinogenic and that the carcinogenic activity was caused by the aromatic content in the oil component of the slack waxes. Another study from the same laboratory tested 11 more slack waxes also produced using the "pressing" process and similar results were reported (Dietz *et al*., 1952).

Kane *et al*. (1984) reported on three skin-painting studies. One was with a slack wax produced from a solvent extracted lubricant base oil, the process currently used in wax refining. After application of 25 mg to the backs of male mice 2 days/week for 80 weeks, no tumorigenic response was produced. A second study tested the carcinogenicity of a petrolatum using the same protocol except the twice-weekly dose was 50 mg. This study was repeated using a dose of 25 mg/application. No skin tumors developed in any of the petrolatum-treated mice in either study.

The carcinogenicity of five refined/finished waxes was tested in a lifetime skin-painting study in which 3 drops of a 15% solution of the waxes dissolved in benzene were applied to rabbits three times per week (Shubik *et al*., 1962). Some skin irritation was noted at the application sites throughout the study. There were no treatment-related increases in the incidence of skin tumors. This study was not thoroughly reported but the results are in agreement with the chronic dermal studies of other refined wax substances in mice.

A lifetime skin painting study of a petrolatum was conducted in Swiss mice (Lijinsky *et al*., 1966). A 15% solution of the petrolatum in isooctane was applied twice weekly, approximately 0.06 ml per dose. Treatment with the petrolatum solution resulted in moderate epidermal hyperplasia at the application site. There was no significant increase in the incidence of skin tumors. The authors also reported that there was no increase in the incidence of internal tumors.

7.6.2 Human Experience

Slack Wax
There are several reports in the older literature of cancer in wax pressmen exposed to unfinished or poorly finished oil during the preparation of paraffin wax (Hendricks *et al*., 1959). Due to the refining processes used at the time, the oils pressed from the wax would have contained high concentrations of PAC. This method of wax production is no longer used in the US. The current processes used in U.S. refineries to produce waxes are more stringent and samples of slack wax in the WRM category did not contain detectable levels of PAC (Section 1.3 and Table 3).

Refined/finished Waxes
Clinical studies with two undiluted paraffin waxes and formulated products containing various concentrations of paraffinic (5-16%) and microcrystalline (4-15%) waxes were reviewed (Elder, 1984). These studies included a range of acute and repeat application tests to observe skin irritation and skin sensitization potentials. The materials produced, at most, slight erythema and none caused skin sensitization.

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

Petrolatum
Only isolated cases of allergy to petrolatum have been reported despite widespread use of petrolatum for many years by dermatologists as a vehicle in human patch testing (Frankel, 1985; Dooms-Goosens and Degreef, 1983; Ayadi and Martin, 1987; Fisher, 1981; Conti *et al*., 1995).

7.7 Assessment Summary for Health Effects

Studies on the substances in this category and read-across from studies with lubricant base oils and white oils indicate that when produced using current US refining practices, slack wax, petroleum (refined/finished) waxes and petrolatum have a low order of acute oral and dermal toxicity. Feeding studies in the rat indicate that higher melting point refined/finished waxes including microcrystalline waxes have low repeat dose toxicity with NOAELs of approximately 1000 mg/kg/day. Several studies indicate that one strain of rat (F344) fed diets containing lower melting point waxes or lower viscosity white mineral oils develops inflammatory changes in the liver and mesenteric lymph nodes (MLN) whereas other rat strains and species show no changes or only minimal MLN changes. Other studies indicate that the unusual sensitivity of the F344 rat to mineral hydrocarbons is due to differences in uptake and retention and an enhanced inflammatory response versus other rat strains and other species. These effects are not believed to be relevant to humans. Subchronic and chronic feeding studies in other animal models indicate the lowest repeat dose NOAEL for the WRM category is ≥ 1000 mg/kg/day.

Studies indicate that toxicity to the developing fetus observed with some vacuum distillates is associated with the PAC in these materials and that reproductive toxicity endpoints are less sensitive to PAC than developmental endpoints. Current refining processes effectively remove aromatic impurities from WRM and no PACs were detected in several samples of the substances from the WRM category including samples of slack wax. Along with the absence of PAC in the WRM samples, the results of dermal and oral studies with refined lubricating oil basestocks indicate that the hydrocarbons in the refined oils and waxes have low potential developmental or reproductive toxicity with a NOAEL of ≥ 1000 mg/kg/day via the oral route.

Samples of slack wax were negative for mutagenicity in an Ames test optimized to detect petroleum PAC. Several oral and dermal studies of slack wax, refined/finished waxes and petrolatum indicate that members of the WRM category manufactured by current US refining practices are not carcinogenic. The long history of safe use of refined/finished waxes and petrolatums in cosmetic and pharmaceutical applications confirms their low toxicity.

8. HUMAN EXPOSURE SUMMARY

Exposure to waxes is generally limited to dermal exposures during the manufacture and use of products containing them with the exception of food-grade waxes. When waxes are heated to facilitate their application to surfaces in the molten state, some inhalation exposure to wax fumes is possible. Potential exposures to wax fumes are limited to industrial settings where waxes are heated well above their melting point. The workplace threshold limit value (TLV) for wax fume is 2 mg/m^3 and is based on human experience of upper respiratory tract irritation at airborne concentrations well above this value (ACGIH, 2001).

The cosmetic and pharmaceutical uses of refined waxes and petrolatum result in some dermal and oral exposures from the intended applications. Food-grade waxes and petrolatum are approved for uses resulting in direct or indirect food contact. These uses are regulated under various national and international laws which set strict limits for the content of impurities, particularly extractable aromatic compounds which include PAHs. The U.S. FDA regulations covering paraffin waxes and petrolatum are found in 21CFR 172.

Dietary exposures to food-grade mineral hydrocarbons (MHC) which include waxes and petrolatum were estimated for the U.S. population in an extensive study by Heimbach *et al*. (2002). This study covered both direct-additive uses in which the MHC is intentionally applied to the food and indirect uses in which the MHC become components of the food due to migration from food-contact surfaces. Exposures were primarily based on a food consumption approach in which MHC concentrations in foods were multiplied by the amount of these foods consumed. This resulted in conservative estimates because it assumed that all foods that might contain MHC in fact do so at maximum estimated concentrations. Exposures from food packaging uses were estimated using the FDA's food-factor approach which takes into account the volumes and kinds of food packaged with specific types of materials. A conservative estimate of mean total exposure to all MHC types was 0.875 mg/kg/day. Half of this, 0.427 mg/kg/day, was white mineral oils used as pan-release lubricants in baking, for de-dusting of stored grain, in confectionaries and in fruit and vegetable coatings. Nearly all the remainder, 0.404 mg/kg/day, was petrolatum, primarily from its use in bakeries. Exposure to paraffin and microcrystalline waxes combined was estimated at 0.044 mg/kg/day.

9. MATRIX OF AVAILABLE DATA

Endpoint	Slack Wax	Refined/Finished Waxes	Microcrystalline Waxes	Petrolatum
	CAS# 64742-61-6	CAS# 8002-74-2 64742-43-4 64742-51-4	CAS# 63231-60-7 64742-42-3 64742-60-5	CAS# 8009-03-8
	Physical/Chemical Properties			
Melting Point Range	43-63 °C	43-68 °C	60-95 °C	36-60 °C
Boiling Point	>350°C			
Vapor Pressure	< 0.0002 Pa			
Partition Coefficient	Log Kow = 4.7 to > 6 (estimated using EPIWIN KOWWIN)			
Water Solubility	Based on individual hydrocarbon solubility estimates using EPIWIN WSKOW, the low molecular weight components (C_{13} to C_{20} hydrocarbons) in the wax materials have water solubility values ranging from <0.002 to 6 mg/L. As molecular weight increases, solubility decreases for these hydrocarbons. Solubility of the complex mixtures would be negligible as these substances exist in a solid to semi-solid state at environmental temperatures.			
	Environmental Fate			
Photodegradation	The low vapor pressures and the physical state of the wax materials indicate that volatilization to the atmosphere will not be a significant fate process. However, the individual hydrocarbon constituents in these materials have the capability to undergo photodegradation through interaction with atmospheric hydroxyl radicals. Should conditions exist where some constituents volatilize to the atmosphere, half-lives are estimated to be 0.9 days or less.			
Stability in Water	The majority of chemical constituents in the wax materials are saturated hydrocarbons which lack the chemical linkages known to undergo hydrolysis. Therefore, they are stable in water.			
Environmental Transport	Fugacity modeling of individual C_{13} and C_{29} hydrocarbon constituents indicates that these substances will tend to become incorporated into the soils/sediments.. Although some of the lowest molecular weight constituents in these wax materials show a potential to partition to the atmosphere, the physical state of these substances indicates that such partitioning would occur at a slow rate.			
Biodegradation	Some paraffin waxes are readily biodegradable. The rest of the wax materials are not readily biodegradable (based on read-across from hydrotreated slack wax, refined waxes and white mineral oil).			
	Environmental Effects			
Acute Fish 96-hr LL50	> 1000 mg/L (Based on read-across from studies with lubricating oil basestocks)			
Acute Invertebrate 48-hr EL50	> 10,000 mg/L (Based on read-across from studies with lubricating oil basestocks)			
Algae 72-hr EbL50 72-hr ErL50	> 1000 mg/L > 1000 mg/L (Based on read-across from studies with lubricating oil basestocks)			
Endpoint	**Slack Wax**	**Refined/finished Waxes**	**Microcrystalline Waxes**	**Petrolatum**
Chronic Daphnia 21-d survival EL50 21-d repro EL50	> 1000 mg/L > 1000 mg/L (Based on read-across from studies with lubricating oil basestocks: solvent-refined heavy paraffinic distillate and solvent-refined residual oil)			
	Health Effects			
Acute Oral (Rat)	LD50 > 5 g/kg (Based on data on wax, petrolatum and read-across from lubricating oil basestocks)			

Repeated Dose Toxicity (Feeding)	NOAEL 1000 mg/kg/day (oral) (Based on no detected PAC in category samples and on subchronic and chronic studies with waxes, petrolatum and white mineral oils)	
Genotoxicity, *in vitro*	Negative in modified Ames test with metabolic activation	Negative (synthetic wax in Ames, mouse lymphoma and CHO assays.) (Also based on read-across from slack wax)
Genotoxicity, *in vivo*	Negative (Based on read-across from negative bone marrow cytogenetics assays on lubricating oil basestocks)	
Developmental Toxicity (Rat)	NOAEL approximately 1000 mg/kg/day (oral) (Based on no detected PAC in category samples and read-across from studies on refined lubricating oil basestocks)	
Reproductive Toxicity	NOAEL approximately 1000 mg/kg/day (oral) (Based on no detected PAC in category samples, no effects on reproductive tissues in repeated dose studies of waxes and petrolatum, and read-across from studies on refined lubricating oil basestocks)	

10. CATEGORY ANALYSIS CONCLUSIONS

Slack wax, refined/finished waxes and petrolatum are solid or semi-solid substances at ambient temperatures with a melting point of 36 to 95°C. They contain primarily saturated alkanes with carbon numbers predominately > C_{20} and have very low vapor pressures. Water solubility is expected to be negligible for these materials and their extremely low water solubility limits their bioavailability. Releases to the environment will result in little mobility, but eventually these substances will be incorporated into soils followed by slow biodegradation. The wax substances are closely related to lubricating base oils as both are derived from vacuum distillates and contain similar hydrocarbons of a similar size range. Multiple acute studies of lubricant base oils with fish, invertebrates, and algae have not produced any adverse toxicological effects in the test organisms exposed to WAF preparations at a loading rates ≥ 1000 mg/L. In tests for chronic toxicity in aquatic invertebrates with base oils similar in molecular size to the wax substances, neither survival nor reproduction was impaired in the adult generation. Read-across from the studies with base oils indicate that the wax substances have low toxicity to aquatic organisms.

Since the members of the WRM category are derived from vacuum distillates, aromatics including PAC could potentially carry through into the wax products. Slack wax is the least refined of the category members and contains 5% to 20% oil. The oil portion of slack wax could contain some PAC. The removal of impurities in the refining of wax products also removes aromatics and PAC. Samples of slack wax, refined/finished waxes and petrolatum were analyzed specifically for PAC and none were detected. Samples of slack wax were also negative in an *in vitro* mutagenicity assay specifically designed to detect petroleum PAC.

The wax materials have low acute systemic toxicity in mammals. Because of their low oral and dermal toxicity, refined waxes and petrolatum have been used for decades in cosmetic and pharmaceutical applications. Microcrystalline waxes have low repeat dose toxicity in mammals. Dietary exposure in repeat dose studies to lower melt point waxes and lower viscosity mineral oils causes some inflammatory changes in one strain of rat versus minimal or no effects in other rat strains and other species. Evidence from several studies indicates this response to dietary mineral hydrocarbons is due to greater retention of ingested mineral hydrocarbons and an enhanced inflammatory response in the sensitive strain versus other laboratory animals. Further, evidence indicates these effects have no clinical relevance to humans. The overall evidence from many subchronic and chronic feeding studies indicates that slack wax, refined/finished waxes and petrolatum have a low repeat dose toxicity with a NOAEL ≥ 1000 mg/kg/day.

Since the wax substances are closely related to lubricating base oils, the developmental and reproductive toxicity of these materials was assessed using read-across from data with lubricant base oil. No effects on reproduction or fetal development were observed in a rat reproduction/developmental toxicity screening study conducted with dermal doses of base oil at approximately 1000 mg/kg/day.

The estimated dietary exposure to food-grade waxes and petrolatum in the U.S. using conservative assumptions is 0.044 and 0.404 mg/kg/day, respectively.

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12. LIST OF ABBREVIATIONS AND ACRONYMS

API – American Petroleum Institute
ASTM – Formerly "American Society for Testing and Materials", now ASTM International
CAS RN/CAS #/CAS No. - Chemical Abstract Service Registry Number
CONCAWE – Conservation of Clean Air and Water in Europe
cSt - centiStokes
d – day
DMSO – dimethyl sulfoxide
EL_{50} – effective loading rate lethal to 50% of the test population
E_bL_{50} – effective loading rate that causes 50% reduction in algal cell biomass
E_rL_{50} – effective loading rate that causes 50% reduction in algal growth rate
EPA/US EPA – United States Environmental Protection Agency
FDA – Food and Drug Administration
h - hour
HPV – High Production Volume
HSW – high sulfur wax
Pa - pascal
LC_{50} – lethal concentration for 50% of the test population
LD_{50}– lethal dose level for 50% of the test population
LL_{50} – lethal loading rate for 50% of the test population
LMPW – low melting point wax
Loading Rate – total amount of test substance added to dilution water to prepare water accommodated fractions (WAFs) for ecotoxicity testing
LOAEL – lowest observable adverse effect level
MHC – mineral hydrocarbon
MLN – mesenteric lymph node (or nodes)
nm - nanometer
NOAEL – no observable adverse effect level
OECD – Organization for Economic Cooperation and Development
PAC - Polycyclic aromatic compound
PAH – polycyclic aromatic hydrocarbon
PNA – polynuclear aromatic
ppm – part per million
SIDS – Screening Information Data Set
US EPA – United States Environmental Protection Agency
USP – United States Pharmacopoeia
WAF – water accommodated fraction

13. GLOSSARY

NOTE: The following terms are used in this document. To the extent possible, definitions were taken from relevant authoritative sources such as EPA, OECD, ASTM and IUPAC.

Bioavailability: The state of being capable of being absorbed and available to interact with the metabolic processes of an organism. Typically a function of chemical properties, physical state of the material to which an organism is exposed, and the ability of the individual organism to physiologically take up the chemical. Also, the term used for the fraction of the total chemical in the environmental which is available for uptake by organisms. (AIHA 2000)
Category Member: The individual chemical or substance entities that constitute a chemical category.
Category: A chemical category, for the purposes of the HPV Challenge Program, is a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. These structural similarities may create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and environmental effects, and/or human health effects. (US EPA 2007b)
Dose: The amount of a substance available for interactions with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism. The **potential dose** is the amount ingested, inhaled, or applied to the skin. The **applied dose** is the amount presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism). The **absorbed dose** is the amount crossing a specific absorption barrier (e.g., the exchange boundaries of the skin, lung, and digestive tract) through uptake processes. **Internal dose** is a more general term denoting the amount absorbed without respect to specific absorption barriers or exchange boundaries. The amount of the chemical available for interaction by an particular organ or cell is termed the delivered or **biologically effective dose** for that organ or cell (US EPA 2002).
Dose-Response Relationship: The relationship between a quantified exposure (dose) and the proportion of subjects demonstrating specific biological changes in incidence or in degree of change (response) (US EPA 2002).
Ecological Effects – all endpoints (OECD definitions)
Fish, Acute Toxicity Test: In a four-day exposure, acute toxicity is defined by the LC_{50}, the concentration of test substance in water which kills 50% of the test population of fish. Test methodology is described in OECD Guideline 203, in OECD Guidelines for the Testing of Chemicals.
***Daphnia* sp., Acute Immobilization Test:** In a one or two-day exposure, acute toxicity is defined by the EC_{50}, the concentration of test substance in water which causes immobilization to 50% of the test population of invertebrates. Test methodology is described in OECD Guideline 202, Part 1, in OECD Guidelines for the Testing of Chemicals.
Alga, Growth Inhibition Test: In a three-day exposure, growth inhibition is defined by the EC_{50}, the concentration of test substance in growth medium which results in a 50% reduction in either alga cell growth or growth rate relative to a control group. Test methodology is described in OECD Guideline 201, in OECD Guidelines for the Testing of Chemicals.
Endpoint: In the context of the EPA High Production Volume Challenge Program, an endpoint is a physical-chemical, environmental fate, ecotoxicity, and human health attribute measurable by following an approved test methodology (e.g., OECD Guidelines for Testing of Chemicals).

Melting point, biodegradation, fish acute toxicity, and genetic toxicity are examples of endpoints that are measured by an approved test method**.** (US EPA 1999)
Environmental Fate Effects – all endpoints (OECD definitions)
Photodegradation: The photochemical transformation of a molecule into lower molecular weight fragments, usually in an oxidation process. This process may be measured by Draft OECD Guideline, "*Phototransformation of Chemicals in Water – Direct and Indirect Photolysis*". This process also may be estimated using a variety of computer models.
Stability in Water: This environmental fate endpoint is achieved by measuring the hydrolysis of the test substance. Hydrolysis is defined as a reaction of a chemical RX with water, with the net exchange of the group X with OH at the reaction center. Test methodology for hydrolysis is described in OECD Guideline 111, in OECD Guidelines for the Testing of Chemicals.
Transport Between Environmental Compartments: This endpoint describes the distribution of a chemical between environmental compartments using fugacity-based computer models. The results of the model algorithms provide an estimate of the amount of the chemical within a specific compartment. The environmental compartments included in many models are air, water, soil, sediment, suspended sediment, and aquatic biota.
Biodegradation: Breakdown of a substance catalyzed by enzymes *in vitro* or *in vivo*. As an endpoint in EPA's HPV program, biodegradation is measured by one of six methodologies described in OECD Guidelines 301A-F, in OECD Guidelines for the Testing of Chemicals.
Exposure: Contact made between a chemical, physical, or biological agent and the outer boundary of an organism. Exposure is quantified as the amount of an agent available at the exchange boundaries of the organism (e.g., skin, lungs, gut). (US EPA 2002)
Feedstock: A refinery product that is used as the raw material for another process; the term is also generally applied to raw materials used in other industrial processes. (Speight, 2007)
Female Mating Index: Number of females with confirmed mating (sperm and/or vaginal plug)/number of females placed with males. (OECD)
Hazard Assessment: The process of determining whether exposure to an agent can cause an increase in the incidence of a particular adverse health effect (e.g., cancer, birth defect) and whether the adverse health effect is likely to occur in humans (US EPA 2002).
Hazard Characterization: A description of the potential adverse health effects attributable to a specific environmental agent, the mechanisms by which agents exert their toxic effects, and the associated dose, route, duration, and timing of exposure (US EPA 2002).
Hazard: A potential source of harm (US EPA 2002).
Health Effects – all endpoints (OECD definitions, unless otherwise specified)
Acute Toxicity: The adverse effects occurring within a short time-frame of administration of a single dose of a substance, multiple doses given within 24 hours, or uninterrupted exposure over a period of 24 hours or less. Exposure may be via oral, dermal or inhalation routes as described in OECD Guidelines 401, 402, 403, and 420 in OECD Guidelines for the Testing of Chemicals.
Developmental Toxicity: Adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally until the time of sexual maturation. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth, and functional deficiency. (US NLM 2007)
Genetic Toxicity *in vivo* (Chromosomal Aberrations): The assessment of the potential of a chemical to exert adverse effects through interaction with the genetic material of cells in the

whole animal. Genotoxicity may be studies in the whole animal using methods described in OECD Guideline 475, in OECD Guidelines for the Testing of Chemicals.

Genetic Toxicity *in vitro* (Gene Mutations): The assessment of the potential of a chemical to exert adverse effects through interaction with the genetic material of cells in cultured mammalian cells. Genotoxicity may be studies in cultured cells using methods described in OECD Guideline 476, in OECD Guidelines for the Testing of Chemicals.

Repeated Dose Toxicity: The adverse effects occurring due to repeated doses that may not produce immediate toxic effects, but due to accumulation of the chemical in tissues or other mechanisms, produces delayed effects. Repeated dose toxicity may be studied following methods described in OECD Guidelines 407, 410, or 412 in OECD Guidelines for the Testing of Chemicals.

Reproductive Toxicity: The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents. The toxicity may be expressed as alterations to the female or male reproductive organs, the related endocrine system, or pregnancy outcomes. The manifestation of such toxicity may include, but not be limited to, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, gestation, parturition, lactation, developmental toxicity, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems. (US EPA 1996f)

Lowest-Observed-Adverse-Effect Level (LOAEL): The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group (US EPA 2002).

No-Observed-Adverse-Effect Level (NOAEL): The highest exposure level at which there are no biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group; some effects may be produced at this level, but they are not considered adverse or precursors to adverse effects (US EPA 2002).

Portal-of- Entry Effect: A local effect produced at the tissue or organ of first contact between the biological system and the toxicant (US EPA 1994).

Read Across: Read-across can be regarded as using data available for some members of a category to estimate values (qualitatively or quantitatively) for category members for which no such data exist. (OECD 2007)

Systemic Effects or Systemic Toxicity: Toxic effects as a result of absorption and distribution of a toxicant to a site distant from its entry point (US EPA 2002).

Target Organ: The biological organ(s) most adversely affected by exposure to a chemical or physical agent (US EPA 2002).

APPENDIX 1. Correlation between PAC Profile and Selected Endpoints of Mammalian Toxicity

As explained in Section 2 on category definition and justification, the mammalian toxicity of the substances found in the Waxes and Related Materials category is expected to be related to their PAC profile; particularly the toxicity measured in repeat-dose, developmental, and *in vitro* mutagenicity studies. The PAC[1] profile is the weight percent of DMSO-extractable, aromatic compounds contained in each of seven separate ring classes.

The initial indication that PAC content could be used to predict the toxicity of untested substances in some categories of petroleum substances was based on the publication by Feuston *et al*. (1994). Their research, based on thirteen refinery streams, examined the correlations between the weight percentage of several chemical classes of compounds and the magnitude of various effects produced in rats treated dermally with these substances in repeat-dose and developmental toxicity studies. In general, Feuston *et al*. found that the toxicity of the streams was correlated with the concentrations of the 3 to 7 ring PACs. The analyses were based on the ranks of several measures of toxicity and the individual PAC concentrations.

In 2004, the API Testing Group recognized the need to further evaluate the observations made by Feuston *et al*. (1994) and commissioned a Task Group (PAC Analysis Task Group, or TG) comprised of experts in the fields of petroleum chemistry, toxicology, and biostatistics. The TG issued a report describing the relationships between PAC profile and the repeat-dose, developmental toxicities of high-boiling petroleum substances, i.e. those with initial boiling points greater than approximately 300 °F (API, 2008). Predictive models for 7 selected repeat-dose and developmental dermal toxicity endpoints in the rat were developed and discussed (API, 2008). The report was reviewed in a peer consultation process and the report and results are publicly available (TERA, 2008). Reports are in preparation on the relationship between PACs and reproductive and genetic toxicities of high-boiling petroleum substances.

Four potential sources of information were reviewed for the project: the publication by Feuston *et al.* (1994), other published literature on the toxicity of individual PAH and PAC-containing materials, studies sponsored by the American Petroleum Institute (API), and unpublished company laboratory reports. The unpublished laboratory reports consisted of: (1) reports of repeat-dose toxicity studies, (2) reports of developmental toxicity studies, (3) two reproductive toxicity screening studies, one each with treated males and females, on a single substance containing a high concentration of PAC, (4) an exploratory dose range-finding study in non-pregnant female rats, (5) reports of mutagenesis tests, primarily results of optimized Ames tests, and (6) reports of compositional data on the tested substances. All unpublished company laboratory reports (repeat-dose, developmental toxicity, and analytical) were judged to be either "reliable without restrictions" or "reliable with restrictions, i.e. reliability scores of 1 or 2 (Klimisch, *et al*. 1997).

[1] Note that "polycyclic aromatic hydrocarbons" (PAH) refers to compounds of two or more fused-aromatic rings consisting of carbon and hydrogen only. Polycyclic aromatic compounds (PAC) is a more inclusive term than PAH since, in addition to the PAHs, PAC also includes compounds in which one or more atoms of nitrogen, oxygen or sulfur (a heteroatom) replaces one or more of the carbon atoms in a fused ring system and perhaps more importantly includes alkylated (methyl, ethyl, etc.) rings (API, 2008).

The relationship between acute toxicity and PAC was not investigated statistically since the reported oral LD_{50} values for high-boiling petroleum substances are generally greater than the maximum doses tested, typically 5 g/kg and 2 g/kg for oral and dermal exposures, respectively (API, 2008). These data demonstrate that the respective petroleum-derived streams are not toxic, at least within the operational definitions of the regulatory testing guidelines.

To model the outcomes of repeat-dose and developmental studies, sets of matched data of PAC composition and biological effects were selected. Each biological endpoint had an average of about 80 data points. The 7 biological endpoints that were selected for final statistical characterization were 4 repeat-dose measures (thymus weight, liver to body weight ratio, platelet count and, hemoglobin concentration), and 3 developmental measures (fetal weight, live fetal count, and percent resorptions). The endpoints selected for modeling are consistent with effects reported for both individual PACs and PAC-containing substances (SCF, 2002, ATSDR, 1995; IPCS, 1998; IRIS 2007; RAIS, 2007). The endpoints selected are also supported by other studies on PAC-containing petroleum substances prepared and submitted by the Petroleum HPV Testing Group as robust study summaries to satisfy the USEPA HPV Challenge Program requirements for the Aromatic Extracts, Crude Oil, Gas Oils, Heavy Fuel Oils, Lubricating Oil Basestocks, and Waxes and Related Materials).

The PAC compositional data were developed using an analytical technique referred to as the "PAC-2 Method," or 'Mobil Oil PAC Method" or, simply "Method II" (Feuston *et al*., 1994; Roy *et al*., 1985; Roy *et al*., 1988), a variation of the Institute of Petroleum IP 346 method (CONCAWE, 1994). In the PAC-2 Method, the percent of sample mass is determined for each PAC ring class (1 through 7) contained in PAC-concentrated dimethyl sulfoxide (DMSO) extracts of the test material. The analysis was performed by gas chromatography with flame ionization detection (GC/FID) or mass spectrometry (GC/MS).

The dose-response relationships between the "PAC profile" and specific biologic effects were successfully predicted using linear regression models. The correlations between observed and model-predicted data were very high ($r > 0.90$). The predictive ability of the models was rigorously tested and the models were found to be accurate predictors when they were used for interpolated data. A test material that has its PAC profile and dose within the range of the PAC profiles and doses used to develop the model gives rise to an interpolated model prediction. Predictions from samples that do not meet this requirement are considered extrapolated predictions. Extrapolated predictions might not be accurate and are considered unreliable by the Testing Group.

Interpolated model results can be used to estimate the dose that would cause a 10% change in the response relative to the control group (PDR_{10}). The concept is similar to the Benchmark Dose (BMD) for continuous endpoints developed by Crump (Crump, 1984). Comparison of the PDR_{10} and BMD_{10} from a series of samples shows close agreement; this indicates the usefulness of the PDR_{10} when there is no biological endpoint testing data and only the PAC profile is available to assess toxicity.

While similar to the BMD, the PDR_{10} has several advantages:

- The PDR_{10} is based on one validated model, whereas the BMD can be developed from several competing models, making the BMD strongly dependent on the selected model (Gephart et al, 2001).
- The PDR_{10} can be applied to untested materials for which there are compositional data (ie, PAC profiles) but no response data, whereas the BMD cannot be used for untested materials.
- The PDR_{10} is based on the large amount of data accumulated over multiple studies, whereas the BMD is based on a single study, usually with only 3 to 5 data points.

A copy of the full report detailing the development and testing of the predictive models developed by the Testing Group can be obtained through either API or TERA (API, 2008; TERA, 2008).

APPENDIX 2: Optimized Ames Test and Statistical Modeling

The optimized Ames test was developed to improve the performance of the reverse mutation *Salmonella* assay for detecting mutagenic and potentially carcinogenic lubricant base stocks and related refinery streams (ASTM, 2002). The method involves concentration of polycyclic aromatic compounds (PAC) by extraction, employing the most consistently PAC- sensitive strain of *Salmonella* [TA98] and increasing the metabolic activation system to maximize metabolism of the streams being evaluated. These modifications allowed detection of positive bacterial gene mutation response identified as an increase of mutant colonies in treated groups at least 2-fold that of negative controls as in the Standard Ames Assay and allowed prediction of potential dermal carcinogenesis by calculation of a mutagenic index (MI).

The mutagenic index (MI) is the slope of the initial portion of the dose response curve expressed in units of revertants per microliter. The mutagenic index was highly correlated with dermal carcinogenic potential, suggesting that oils with MI values < 1 were unlikely to be dermally carcinogenic, oils with MI values > 1 but < 2 were indeterminate, and oils with MI values > 2 would likely produce skin tumors if tested in mice. The test method was refined to provide the greatest predictive value of gene mutagenicity and potential carcinogenicity for the widest range of high boiling [$>300^0$C] PAC-containing streams and thus provides a more sensitive general *Salmonella* protocol for this class of petroleum substances. In 1995, the optimized Ames test was standardized as an ASTM method [ASTM E1687-95].

Correlation of Mutagenic Activity with PAC Profile

The relationship of the MI with the PAC profile of refinery streams with known dermal carcinogenic potential has been established. The method of quantifying PAC constituents in which the condensed ring aromatics are removed by DMSO extraction and analyzed for 3-7 ring PAC by gas chromatography (GC) with flame ionization detection (FID) was developed by Roy *et al*. (1985; 1988). Having demonstrated a strong correlation between analytical distribution of PAC and mutagenicity in the optimized Ames test for petroleum-derived substances which produce dermal tumors when tested in mice, the utility of this relationship for read-across to untested substances has been expanded by statistical modeling.

Statistical Modeling of Analytical Data with the Optimized Ames Test

A statistical model has been developed to predict MI scores for untested substances encompassing precision in the critical 0-2 range (Nicolich et al., 2010). This model employs the 1-7 ring PAC profile for each sample to predict MI scores. This model separated the data from 193 samples of a range of PAC-rich petroleum streams into those with mutagenic index values equal to or greater than 1.0 and those with MI values less than 1.0. This model was not designed to quantify mutagenic potency but to identify whether or not a substance had an MI value less than 1 or not; this result can be used as an indication of whether the material has the potential to induce gene mutations in the optimized *Salmonella* assay and thus, to potentially be active in dermal carcinogenesis assays as well.

The statistical model is based on a series of three steps each predicting if the test substance was above or below an MI cut-point using a binary logistic general additive model. Step 1 predictes the probability that the substance has an MI of 5 or larger. The second step used only the

substances predicted to have an MI below 5 and tested for a split at an MI of 2 or larger (the samples from the first step that are predicted to be above 5 were set at 5 and were no longer in the model process). The third step uses only the substances predicted to have an MI below 2 and tested for a split at an MI of 1 or larger (again with the substances from the second step that were predicted to be greater than 2 were set to 2 and were no longer in the modeling process). At each step the probability for a decision is based on a value of 0.50. For example, in the first step, if the probability of the substance having an MI less then 5 was greater than 0.50 the substance was assigned a predicted MI of 'less than 5.' The final result was the combination of the results from the 3 steps with each substance predicted as being either < 1 or ≥ 1.

The model predictions agreed with the experimentally determined results 98% of the time, with the majority of the incorrect predictions being at MI values that were close to 1.0. When the model was tested with 49 hold out samples, 94% of the predictions were in agreement with the experimentally determined values.

From this information it is apparent that the outcome of optimized Ames tests can be predicted from compositional information with an accuracy that seems comparable to that associated with variability inherent with either the experimental methods or the methods used to calculate mutagenic index from the experimental data.