GASOLINE BLENDING STREAMS CATEGORY ASSESSMENT DOCUMENT

Submitted to the US EPA

by

The American Petroleum Institute
Petroleum HPV Testing Group

Consortium Registration # 1100997

Contents

EXECUTIVE SUMMARY

The purpose of this Gasoline Blending Streams Category Assessment Document is to present the potential environmental and human health hazards of the numerous refinery streams used in the blending of gasoline. The 81 substances in the Category are volatile liquids at standard temperature and pressure and are referred to as naphthas. Gasoline-blending naphthas are complex petroleum substances consisting of paraffinic hydrocarbons (normal and branched-chain), olefinic hydrocarbons, naphthenic hydrocarbons (cycloparaffins), and aromatic hydrocarbons (mainly alkylbenzenes). By TSCA definition, the hydrocarbons in these substances contain carbon numbers approximately in the range of C4 to C12. These four basic chemical classes generally present in all naphthas - Paraffins, Olefins, Naphthenes and Aromatics- are identified by the acronym PONA. The basic strategy of this category for characterizing the human health, physical chemistry characteristics, environmental fate and ecotoxicity hazards was to use data from naphthas that are higher in one of these four chemical classes to estimate the boundaries of toxicity and to predict the potential hazards of untested substances. Data from gasoline are used as supplemental information to support the overall and endpoint specific hazard characterizations. Detailed analysis of the data from new and existing studies on these and similar streams combined with results of studies on formulated gasoline demonstrated substantial similarities in the HPV hazard endpoint results. This has allowed a simplification of the category assessment to characterize the potential hazard for most human health and environmental endpoints without using the high PONA class separation. Consequently, prediction of values for untested category members consists of the range of the highest and lowest values obtained from key studies on all PONA substances and formulated gasoline.

Physical-chemical properties, environmental fate, environmental effects and human health effects are summarized below and discussed in the body of the category analysis. With the exception of oral acute toxicity, *in vitro* and intraperitoneal *in vivo* genetic toxicology studies, the mammalian health data are derived from dermal and inhalation studies. Inhalation is the most relevant route of human exposure. However most of the substances in this category are blended into formulated gasoline and are not found outside the refinery. Environmental toxicity studies were performed with water accommodated fractions (WAF) of whole samples of representative streams. Environmental endpoints were either measured or modeled using compositional data from detailed hydrocarbon analyses of selected streams.

Physical-Chemical Properties:

Substances in the Gasoline Blending Streams Category are liquids composed of numerous individual hydrocarbons that boil within the general range of 37°C to 200°C at atmospheric pressure. Measured vapor pressures on samples of whole product ranged from 1290 hPa to 9150 hPa, indicating a considerable tendency to volatilize. Partition coefficients and water solubility values of constituent hydrocarbons making up these streams ranged from 1.23 to 4.85 and < 1 mg/l to 2000 mg/l, respectively.

Environmental Fate:

If Gasoline Blending Streams are released to the environment, individual components will disperse and partition according to their individual physical-chemical properties. Their final

dispositions are shaped by both abiotic and biotic processes. Based on modeling individual structures encompassing the different types and molecular weights of hydrocarbons making up gasoline blending streams, volatilization to the atmosphere is an important fate process. Residence times in the atmosphere are relatively short due to indirect photodegradation reactions. In water, hydrolysis is not likely to occur, as the chemical linkages of hydrocarbons do not allow for these reactions. However, biodegradation data show that these substances can exhibit a moderate to rapid rate of biodegradation and are considered at least inherently biodegradable.

Ecotoxicity:

The substances in the Gasoline Blending Streams Category are expected to produce a similar range of toxicity for the aquatic species when studies using similar solution preparation and exposure techniques are compared. The endpoint values reflect the loading rates of the test substance added to exposure solutions. Termed water accommodated fractions, the WAF preparation is recommended as the appropriate procedure for testing complex substances having low water solubility. The range of acute toxicities was similar for the three trophic levels (fish, invertebrates, and algae) based on similar testing methodology using water-accommodated fractions (WAF). The proposed “read-across” ranges of acute toxicity endpoints (expressed as lethal loading rates) that are expected to represent the potential toxicity are:

- Fish 2.09 to 46mg/L
- Invertebrates 0.9 to 32mg/L
- Algae 1.1 to 64mg/L

These ranges are reasonable when compared to other test data that employed similar WAF testing methods. In 21-day reproduction studies, the no-observed-effect-loading rate (NOELR) for gasoline blending naphthas ranged from <0.39 to 2.6 mg/L, while the NOELR for a 14-day exposure to fish ranged from 2.6 to 6.4 mg/L. Since constituents are volatile and biodegradable these substances would not persist due to partitioning and transformation processes.

Human Health Effects:

Inhalation is considered the route of exposure that is most relevant to hazard assessment for humans both in the workplace and the general population. Studies employing the oral or dermal routes are included for completeness. LOAEL and NOAEL designations for repeated dose and developmental/ reproduction studies for read-across to untested category members are derived from inhalation results.

Acute Toxicity: Results of testing various naphthas for acute toxicity demonstrate consistently low toxicity by the oral [Rat LD50 >5g/kg], dermal [Rabbit LD50 >2g/kg] and inhalation [Rat LC50 >5g/m^3] exposure routes, are mild to moderate eye and skin irritants and are not skin sensitizers. Acute data for gasoline gave comparable results. The inhalation acute toxicity read-across value for untested category members is LC50 > 5g/m^3

Repeated Dose toxicity: Gasoline blending streams have a low inhalation repeat dose hazard potential. The inhalation NOAELs and LOAELs in rats were similar between the different hydrocarbon classes of streams (PONA) and the formulated product, gasoline. Since there were no appreciable differences between paraffinic, olefinic, naphthenic, and aromatic streams, a range of values derived from all of the repeated dose inhalation studies will be used to read across to untested category members. The read-across values for untested category members are:

LOAEL: 6572 mg/m^3 – 27,800mg/m^3 (1864 – 7885ppm[a])
NOAEL: 1507mg/m^3 – 10,153mg/m^3 (427 – 2880ppm[a])

[[a] - upper range of NOAEL based on 211(b) Baseline Gasoline Vapor Condensate; Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]

In Vitro Genetic Toxicity – Gene mutation: Most naphthas are not mutagenic in mammalian cells except for materials with greater than 60% aromatic content where equivocal or in one case positive activity was seen with metabolic activation. Formulated gasoline tested in both bacterial and mammalian cell assays did not induce mutation in either test system. The read-across conclusions for all naphthas in this category are negative with and without metabolic activation with the exception of streams with a known aromatic content greater than 60%. Untested streams with an aromatic content greater than 60% can be classified as negative/equivocal without metabolic activation and equivocal/positive with metabolic activation.

In Vivo Genetic Toxicity – Cytogenetics: All gasoline blending naphthas are negative for induction of chromosome aberrations in rats. Gasoline did not induce cytogenetic damage in rats or adverse effects on the spermatogenic cycle in mice. Overall gasoline blending naphthas are not clastogenic. The read-across conclusion for untested substances in this category is negative for in vivo genetic toxicity.

Developmental Toxicity: Assessment of developmental toxicity was derived from inhalation developmental toxicity studies in which female rats were exposed to naphtha vapor during gestation and from the developmental portions of reproductive studies in which both male and female animals were exposed. Overall naphthas do not induce developmental toxicity up to the highest doses tested. The read-across range of values for developmental toxicity for untested substances in this category is NOAEL = 5970 to 27750mg/m^3

Reproductive Toxicity: Assessment of reproductive toxicity was derived from OECD 421 Inhalation Reproductive and Developmental toxicity screening tests and the reproductive portion of OECD 422 Combined Repeated Dose Toxicity Study with Reproductive/ Developmental Toxicity Screening Test. Two-generation studies with vapor recovery gasoline and baseline gasoline vapor condensate provided supporting data. All tested substances did not induce reproductive toxicity up to the highest doses tested. The read-across range of values for reproductive toxicity for untested substances in this category is NOAEL = 13650 to 27750mg/m^3

Carcinogenicity: No inhalation carcinogenicity studies have been performed with gasoline blending naphthas. Long term [2-year] inhalation exposure to unleaded gasoline produced tumors in male rat kidneys, the result of alpha 2-microglobulin mediated nephropathy, also identified as light hydrocarbon nephropathy, a species and sex specific syndrome not relevant to human health (US EPA, 1991). In female mice there was an increase in hepatocellular adenomas and carcinomas.

Two year dermal carcinogenesis studies included evaluation for systemic toxicity after 12 months of exposure that did not show significant adverse systemic effects beyond skin irritation effects at the site of application. Skin tumors resulting from 2 years dermal exposure were induced primarily by naphtha streams derived from catalytic cracking. Other naphthas and formulated gasoline produced skin irritation but did not induce any or any significant incidence of skin tumors

Conclusions: Results from studies on gasoline blending streams demonstrate that these naphthas have similar low toxicity profiles for human health endpoints. Ecotoxicity results generally fall within the moderate toxicity range. Results from tests of formulated gasoline are consistent with results from these streams, thus supporting the conclusion that there is no distinction by hydrocarbon PONA class in the majority of the hazard endpoints evaluated. Therefore, the range of values from those studies can be used to characterize the untested substances in this category. In addition, exposure to these gasoline blending naphthas is minimal since they are production site limited and thus are unlikely to pose significant hazard to the environment or human health.

1. DESCRIPTION OF GASOLINE CATEGORY

The 81 substances in the Gasoline Blending Streams Category referred to as low boiling point naphthas are volatile liquids at standard temperature and pressure and are primarily used to blend unleaded motor gasoline. These naphthas are Class II substances on the Toxic Substances Control Act (TSCA) Chemical Inventory. Class II substances are defined as "Chemical Substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials. Appendix A is a complete list of substances included in this Category Assessment Document.

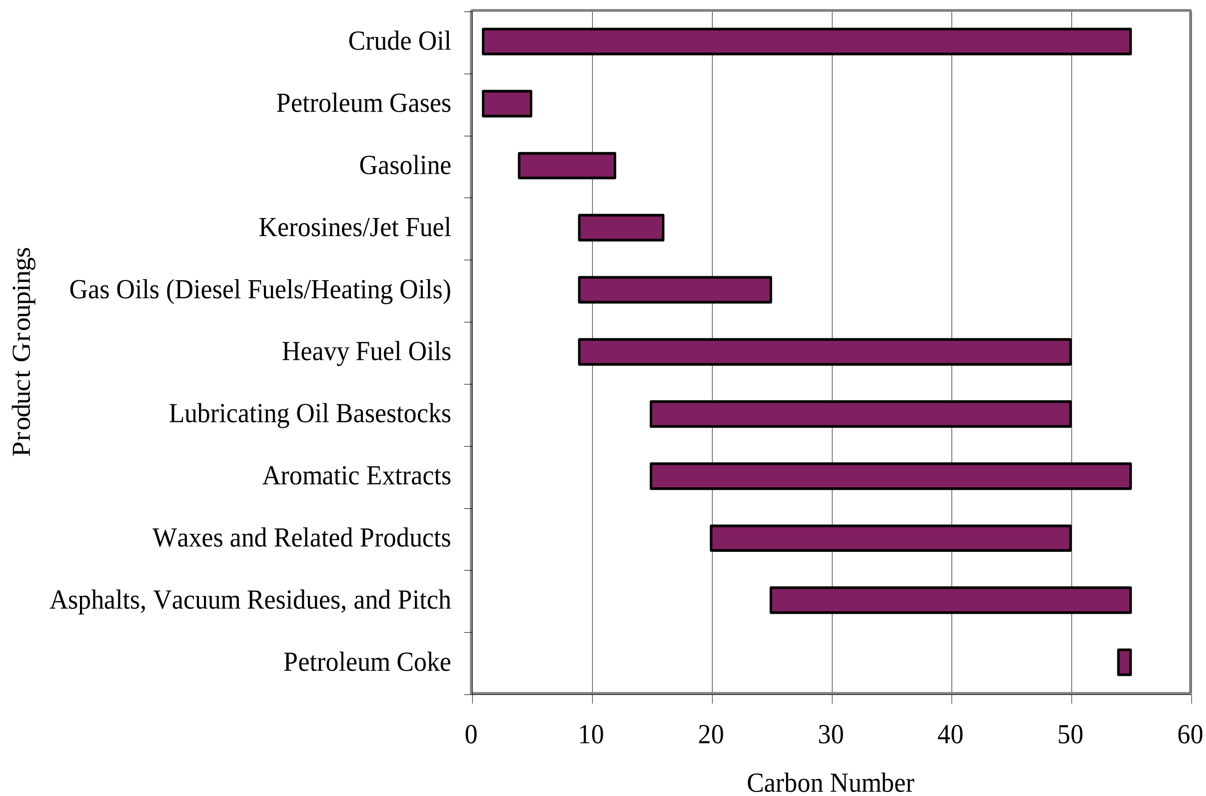
The substances in this category share many physical properties that make them suitable for gasoline blending but few, if any category member streams could be sold as finished gasoline. There is no significant human exposure to most of these substances, which are blended directly into gasoline and are not present outside the refinery pipelines. However, some narrower cut naphthas could be used as solvents and the International Hydrocarbon Solvents Consortium has sponsored several substances that are used for that purpose. Other naphthas from steam-cracking operations are being sponsored by the American Chemistry Council [ACC] Olefins Panel. The Petroleum HPV Testing Group has worked with ACC to ensure that there is no duplication of animal testing between the three groups.

To select test samples to characterize the range of naphtha streams blended into gasoline, the Petroleum HPV Testing Group used chemical-oriented groupings based on the four primary chemical classes found in naphthas. They are paraffins, olefins, naphthenes, and aromatics (PONA).

1.1. Petroleum Chemistry and Refining [Appendix B]

Gasoline blending streams are refined from petroleum, or crude oil, an extremely complex substance. The hydrocarbon molecules in crude oil may include from one to 50 or more carbon atoms. At room temperature, hydrocarbons containing one to four carbon atoms are gases; those with five to 19 carbon atoms are usually liquids; and those with 40 or more carbon atoms are typically semi-solids. Figure 1 below shows the typical carbon chain lengths found in substances from petroleum refining that make up the Petroleum HPV Testing Program and demonstrates the overlaps that occurs.

Figure 1.



Petroleum refining uses distillation as well as chemical treatment, catalysts, and pressure to separate and combine the basic types of hydrocarbon molecules into petroleum "streams" which have the characteristics needed for blending commercial petroleum products. Distillation is not a precise procedure and refining processes vary from refinery to refinery. As a consequence there is not a sharp cut-off between each of the streams that have been separated, which results in an overlap of substances that occurs in each of the streams. However, streams used in the blending of gasoline must generally fall in a boiling range of –4 to 446^{0}F (–20 to 230^{0}C) and a carbon number distribution approximately in the range of C4-C12.

In addition to primary distillation, numerous refining processes produce the naphthas for blending gasoline. These processes include alkylation, catalytic cracking, catalytic reforming, hydrocracking, hydrodesulfurization, hydrotreating, isomerization, polymerization, sweetening, and thermal cracking. Application of various refining steps is determined by the quality of the initial petroleum crude and product specifications to produce naphthas with similar carbon numbers and boiling range but with differing molecular compositions.

1.2 Chemical Composition of Naphtha Streams

Gasoline is manufactured to meet property limits, which comply with performance specifications and government regulations, and those property limits, in turn, influence its chemical composition. Specifications limit the boiling range over which naphthas used to blend gasoline can be distilled. Each hydrocarbon boils at a specific temperature and boiling point increases with molecular size. The temperature limits for the gasoline distillation profile excludes smaller hydrocarbons with lower boiling points and larger hydrocarbons with higher boiling points. Figure 2 shows the carbon number distribution of a typical formulated gasoline (C4-C12) and demonstrates that the low and high ends of the carbon number spectrum exist at low levels. Figure 2A illustrates how the cumulative carbon number distribution parallels the distillation profile. As the temperature increases over the gasoline boiling range, molecules with higher carbon numbers are increased in the distillate mix (e.g. a sample collected at 200^0F would contain primarily C4-C6 hydrocarbons, while one collected at 400^0F would also contain C7-C10 hydrocarbons).

Figure 2

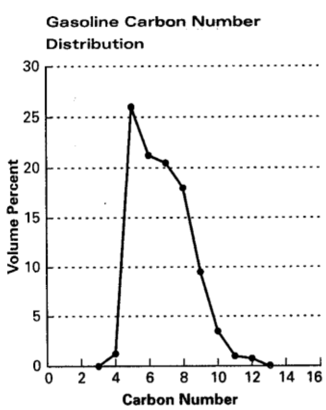
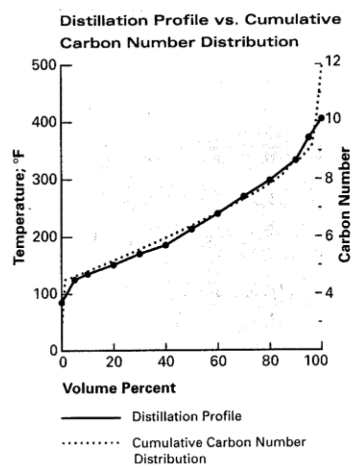
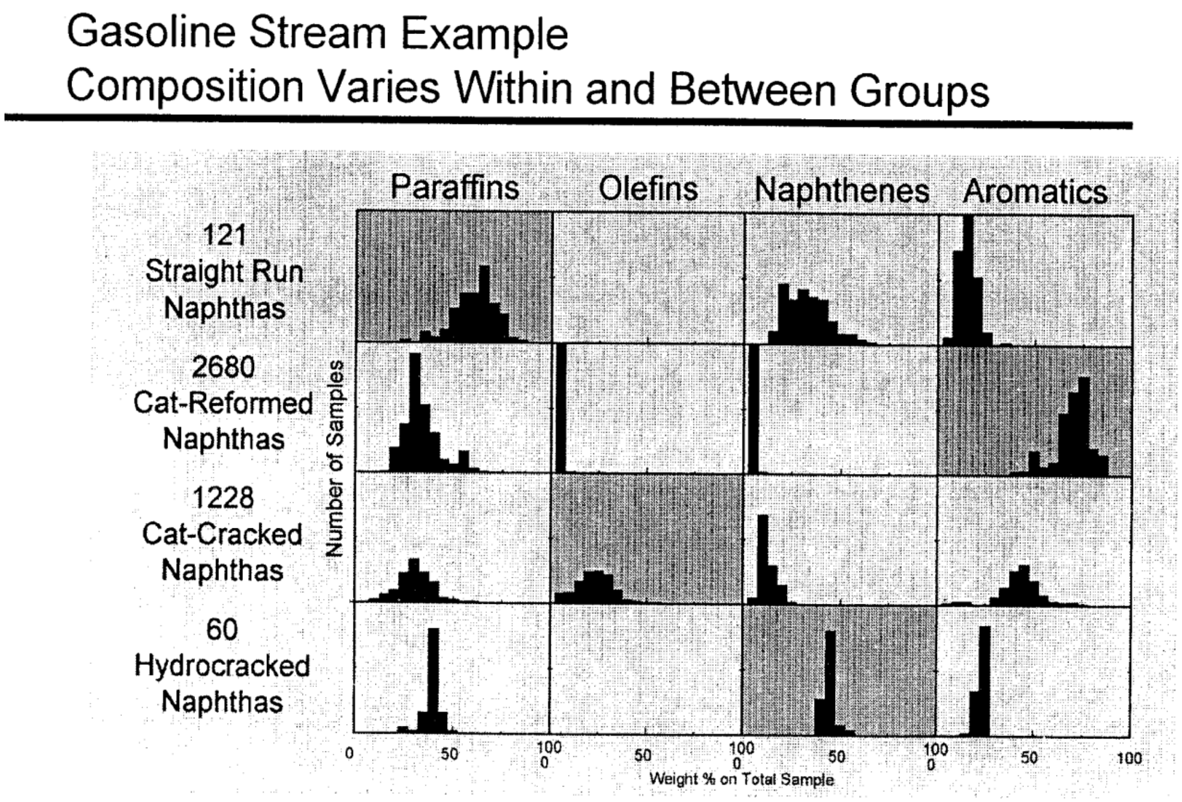


Figure 2A



The characteristic chemical composition of naphtha streams is described by PONA classification – the Paraffinic, Olefinic, Naphthenic and Aromatic classes in the stream; within each class, the hydrocarbons also vary in size. Figure 3 (below) illustrates distribution of PONA classes for gasoline blending streams from a major refiner: 121 straight run naphtha streams, 2680 catalytic reformed stream samples, 1228 catalytic cracked stream samples and 60 hydrocracked stream samples derived from a wide range of crude oils were analyzed, and the distribution of molecules by weight % for each class are presented. All streams contain paraffins, naphthenes and aromatics in varying concentrations while olefins are present almost exclusively in cracked stocks. These frequency diagrams show quantitatively the spectrum of PONA that can occur in different naphthas. A key point of this analysis is that even naphthas that have significant levels of one chemical class (i.e. aromatics in catalytic reformed naphtha) usually contain some molecules from the other chemical classes.

Figure 3



Some refining processes create naphtha that contain predominately one or two of these classes. For example, naphtha from catalytic reforming typically contains high concentrations of aromatics, while alkylation naphtha typically contains no aromatics. Other refinery processes do not significantly influence the chemical composition of the naphtha. Primary distillation and sweetening (desulfurization) would be examples of such processes. Many of the 81 HPV streams cannot be classified as high P, O, N, or A, rather, these hydrocarbon classes are more evenly represented in the respective stream.

2. CATEGORY DEFINITION AND JUSTIFICATION

The hydrocarbons that comprise gasoline and the 81 naphtha blending streams in this category – Paraffins, Olefins, Naphthenes (cycloparaffins) and Aromatics [PONA] - share some structural features but differ in the ratio of hydrogen to carbon atoms and the way in which these atoms are arranged. The basic strategy of this category for characterizing the human health and environmental hazards was to use data from naphthas that are relatively high in one of the four PONA classes to estimate the boundaries of toxicity and to predict the potential hazards of untested substances. Data from formulated gasoline (there is no CASRN for gasoline on the TSCA Inventory; the CAS No. for gasoline used in the EU is 86290-81-5, the EINECS No. is 289-220-8) was also used to characterize the potential hazards for the category members. The average composition of formulated gasoline was used to help identify blending streams that had significantly higher concentrations of paraffins, olefins, or aromatics than typical formulated gasoline (1990 Amendments to the Clean Air Act, 40 CFR 79.55(b)).

The naphthas selected to represent the four extremes of hydrocarbon composition were:

- Paraffinic: Light alkylate naphtha CAS # 64741-66-8 [approx. 100% paraffins]
- Olefinic: Light catalytic cracked naphtha CAS # 64741-55-5 [approx. >40% olefins]
- Naphthenic: Heavy straight run naphtha CAS # 64741-41-9 [approx. 30 % naphthenes]
- Aromatic: Full range catalytic reformed naphtha CAS #68955-35-1 [approx. 60% aromatic]
- Light catalytic reformed naphtha CAS #64741-63-5 [approx. 33% aromatic]

Detailed analysis of the data from new and existing studies on key high-PONA substances, combined with results of studies on formulated gasoline, demonstrated substantial similarities in the HPV hazard endpoint results. This has allowed a simplification of the category assessment to characterize the potential hazard for most human health and environmental endpoints without requiring the high-PONA class distinction. Consequently, prediction of values for untested category members consists of the range of values obtained from key studies on high-PONA substances and formulated gasoline.

3. TEST MATERIALS

The four streams selected to represent the four extremes of hydrocarbon composition are described in greater detail below. Data on at least one naphtha stream from each of the four chemical classes (PONA) are summarized here as key studies and in the Robust Summaries. In many instances data on several naphthas in the same chemical category are also presented in the Robust Summaries. In addition, data on gasoline as an integrator of the various naphtha blending streams are provided to assist in evaluating the toxicity of these substances.

Test Material**:** Naphtha, light alkylate
CAS # 64741-66-8
This test substance is virtually 100% paraffins, by analysis.

Test material: Naphtha, light catalytic cracked
CAS #64741-55-5
This test substance has greater than 40% olefins, by analysis.

Test Material: Naphtha, heavy straight-run
CAS # 64741-41-9
This test substance contains approximately 30% naphthenes by analysis.
Alternatively, where analytical data confirms similar high percentage of naphthenic content, existing test data for other naphtha streams (i.e., existing data for light straight run naphtha, CAS #64741-46-4, Concawe sample ID W94/809, 34% naphthenics) were also used.

Test Material: Naphtha, full range catalytic reformed
CAS # 68955-35-1
This test substance has greater than 60% aromatics, by analysis.
Alternatively, data from streams with an aromatic range of 30 to > 60% were evaluated where compositional analysis confirmed similar high percentage of aromatic content. Therefore existing test data for other naphtha streams (i.e., existing data for light catalytic reformed naphtha, CAS # 64741-63-5, Concawe sample W94/812, 63% aromatics or representative constituents) were also used.

Test Material: Naphtha, light catalytic reformed
CAS # CAS #64741-63-5
This test substance contains 33% aromatics and is more representative of currently blended gasoline streams (see Table 1).

The naphthas selected for hazard evaluation are used in the blending of gasoline and contain at least as much or substantially more of a given chemical class as is found in gasoline. These streams are usually refinery pipeline-contained and blended directly from the pipeline into gasoline so that human exposure is minimal. As illustrated in Table 1, naphthas enriched in one chemical class also contain components of other hydrocarbon classes to contribute to gasoline composition.

Table 1. Examples of Contribution of Chemical Classes From Refinery Streams to Gasoline

	1990 Industry Average Gasoline	Light Alkylate Naphtha API 83-19.	Light Catalytic Cracked Naphtha API 83-20	Sweetened Naphtha API 81-08	Full Range Catalytic Reformed Naphtha API 83-05
Carbon No.	C4-C12	C5-C10	C5-C10	C4-C10	C5-C12
Boiling range ^{0}C	31-192	37-175	37-168	39-114	58-200
PONA Classes (Volume %)					
Paraffins	52.8	99.4	30.6	72.1	32.1
Olefins	9.3	0.0	45.6	<0.1	0.5
Naphthenics	4.7	0.6	10.4	20.9	3.7
Aromatics	33.1	0.0	13.1	6.9	63.3

Thus, to determine effects attributable to paraffinic streams present in gasoline at >50%, a light alkylate naphtha, virtually 100% paraffins was tested. To predict the hazard of the contribution from olefins, which occur in average unleaded gasoline at approximately 9%, light catalytic cracked naphthas containing >40% olefin have been evaluated. Similarly for high aromatic streams present in gasoline at approximately 30%, data from streams with an aromatic range of 30 to > 60% were evaluated.

3.1 New Studies:

Data gaps for this category were identified for streams high in naphthenes (cycloparaffins). Although present in gasoline at 5-10%, high naphthenic streams are rarely isolated and are usually blended directly into gasoline. A sweetened naphtha (20.9 vol% naphthenic) is presented in Table 1 as a relatively high naphthenic stream. Available data for this stream addresses acute toxicity, genetic toxicity, and dermal repeat dose toxicity. Ecotoxicity data exist for other high naphthenic streams. To complete toxicity evaluation for the High Naphthenic class, a heavy straight run naphtha was tested in OPPTS 870.3650 [OECD 422] Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test and in a Ready Biodegradability Study [OECD 301F]. The PONA distribution for this material was 53% paraffins, 4.7% olefins, 28.4% naphthenics and 12.3% aromatics.

4. PHYSICAL-CHEMICAL PROPERTIES

The gasoline blending streams category is comprised of naphthas which are complex, multi-constituent substances composed of hydrocarbons having carbon number distributions generally of C4 to C12. Although their composition is highly variable, these streams consist of principal classes of hydrocarbon types that vary in relative proportions but fall within the cited range of carbon numbers. This similarity among the streams in this category allows the characterization of physical-chemical properties to be given as ranges of values for the different endpoints. Therefore, it was not considered necessary to create subgroups of category members based on PONA components as was originally proposed in the Test Plan. When the physical-chemical attributes are compared across the various streams that are characterized and described in the robust summaries, it is evident that their physical-chemical properties are similar.

For such complex substances it is not possible to measure or calculate a single numerical value for some of the physicochemical properties. For example, a complex substance containing a number of individual chemical constituents does not have a single boiling point. The boiling point range of a naphtha is reflective of distillation processes as described in Section 1.1. For the physical-chemical properties that cannot be defined for complex substances, ranges of endpoint values were reported for constituent hydrocarbons covering the principal hydrocarbon types and molecular weight ranges in these streams. Individual compounds were chosen from detailed hydrocarbon analyses of selected streams using gas chromatography. When available, measured data were reported. In the absence of empirical measurements, physical-chemical properties were estimated using the EPI-Suite™ computer subroutines (US EPA, 2000). When measured data for the whole substance were available, those data were presented.

4.1 Physical-Chemical Endpoints

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

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

4.1.1 Melting Point

Melting point values estimated by EPI Suite™ for various hydrocarbon constituents of gasoline blending streams having carbon numbers from C4 to C12 range from -138°C to 13.2°C. Gasoline blending streams typically exist as liquids at ambient temperatures.

4.1.2 Boiling Point

For petroleum substances produced under atmospheric distillation, the boiling range is typically measured by ASTM method D86. The blending streams described in the robust summaries had initial and final boiling point temperatures falling within the range of 37°C to 200°C (API, 1987; King et al, 1984). This range is expected to represent the approximate initial and final boiling points of the majority of individual constituents within these substances. The boiling range for one blended gasoline was cited as 34°C to 220°C (MacFarland et al, 1984).

4.1.3 Vapor Pressure

Vapor pressure measurements were reported for selected members of the gasoline blending streams category. The cited data reflect vapor pressure values measured following ASTM method D5191, which determines the total vapor pressure exerted in vacuum by air-containing, volatile, liquid petroleum products. Measurements indicate that a range of 1290 hPa to 9150 hPa may be considered typical vapor pressures for members of the gasoline blending streams category (CONCAWE, 1996a).

4.1.4 Octanol:Water Partition Coefficient

Partition coefficient (log K_{ow}) values were reported for individual hydrocarbon constituents found in gasoline blending streams. Constituents were selected from detailed hydrocarbon analyses of selected category members so that a range in molecular weights and hydrocarbon types was represented. Thus, the range of partition coefficient values reported reflects the typical range for log Kow of individual hydrocarbon structures in these substances. When measured values were found, these were included in the reported ranges. In the absence of empirical measurements, the computer program, KOWWIN, a subroutine in EPI-Suite™ (US EPA, 2000), was used to provide calculated values for individual structures. Based on the cited data, the partition coefficient values of the hydrocarbons in these streams are expected to fall within the range 1.23 to 4.85.

4.1.5 Water Solubility

Individual components of complex petroleum substances have specific and differing water solubility values. For example, constituent hydrocarbons of gasoline blending streams have measured and calculated solubility values ranging from <1 to 2000 mg/L (US EPA, 2000). However, for complex petroleum substances, the resulting aqueous concentration of each constituent hydrocarbon is a function of: 1) the loading rate (i.e., ratio of petroleum substance to water), 2) log K_{ow}, 3) the amount of component present, and 4) the maximum water solubility of each component. Initially, as the complex petroleum substance is added to water in amounts below the solubility limit of the least soluble component, the aqueous concentration increases proportionally until the least soluble component reaches a saturation concentration. As more product is added to water, only the more soluble components continue to dissolve, resulting in a two phase system. Further addition of the complex petroleum substance results in an aqueous concentration that is a non-linear function of the amount added.

Gasoline blending streams are complex substances that follow this pattern of component dissolution in an aqueous medium, which is shown by analyses of hydrocarbon components in the dissolved phase of water-accommodated fractions (WAFs). The data cited in the robust summaries varied in methodology with respect to loading rate (e.g., 50 to 1000 mg/L) and equilibration time (24 to 72 hours), but they provided a spectrum of solubility data for a number of gasoline blending streams containing different proportions of the major hydrocarbon classes (ABC Laboratories, 1998a,b; CONCAWE, 1995a,b; Springborn Laboratories, 1993; Stonybrook Laboratories, 1995a,b). Those studies show that in freshwater, the sum of the measured dissolved concentrations of selected hydrocarbon compounds ranged from 1.6 to 13.7 mg/L. Similar measurements made in saltwater WAF preparations ranged from 0.9 to 14 mg/L. For comparison, analysis of a freshwater WAF preparation of a blended gasoline showed a range of dissolved concentrations of selected hydrocarbons from <0.1 to 3.1 mg/L, with the sum of the individual components being approximately 7 mg/L. These concentrations are likely somewhat conservative in that not all dissolved hydrocarbon components in the WAFs were measured. Although the gasoline blending streams are comprised of constituents with low water solubility, streams higher in aromatic and naphthenic structures demonstrate greater solubility than other streams or formulated gasoline.

4.2 Assessment Summary for Physical Chemical Endpoints

The physical-chemical characteristics of the members of the gasoline blending streams category reveal that these substances are liquids composed of individual hydrocarbons that boil within the general range of 37°C to 200°C at atmospheric pressure. Measured vapor pressures on samples of whole product ranged from 1290 hPa to 9150 hPa, indicating a considerable tendency to volatilize. For constituent hydrocarbons making up these streams, partition coefficients ranged from 1.23 to 4.85, while water solubility values ranged from <1 mg/l to 2000 mg/L, respectively.

5. ENVIRONMENTAL FATE

When a complex substance such as one of the gasoline blending streams is released into the environment, the hydrocarbon constituents separate and partition to the different environmental compartments in accordance with their own individual physical-chemical properties. The ultimate partitioning of the individual components in gasoline blending streams are influenced by both abiotic and biotic processes, and the relative importance of these processes will depend upon the environmental compartment to which the individual components partition. Because these streams differ primarily in the proportions of various hydrocarbon classes that they contain, it was not necessary to characterize environmental fate properties based on PONA subgroups as was originally described in the test plan.

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. Thus, environmental fate endpoints include the following:

- photodegradation,
- stability in water (hydrolysis),
- environmental distribution (fugacity), and
- biodegradation.

In determining these fate characteristics for members of the gasoline blending streams category, a high reliance was placed on predicted properties of the individual hydrocarbon constituents of these complex substances. Because it would be impractical to conduct an environmental fate assessment on a component by component basis, specific hydrocarbons were selected from detailed hydrocarbon analyses of the whole product to serve as representative structures. These constituents were selected to span the typical ranges of molecular weights and hydrocarbon types that constitute these category members. Therefore, the package of computer programs contained in EPI Suite™ (US EPA, 2000) was used to estimate the properties of photodegradation, stability in water, and environmental distribution. Measured data, when available, were also included in the assessment.

For the assessments of biodegradation, the approach taken was to characterize the biodegradability potential of the whole product. Existing biodegradation data on these streams were collected and new testing was conducted to provide a characterization of the biodegradability of the category members.

5.1 Environmental Fate Endpoints

5.1.1 Photodegradation

5.1.1.1 Direct photodegradation

The direct aqueous 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 result in photochemical transformations in the environment, although absorption is not always sufficient for a chemical to undergo photochemical degradation (Harris, 1982a). In general, most representatives of the gasoline blending streams category do not contain component molecules that will undergo direct photolysis. Saturated hydrocarbons (paraffins and naphthenics), olefins with one double bond, and single ring aromatics, which constitute the majority of these components, do not absorb appreciable light energy above 290 nm. Therefore, this fate process will not contribute to a measurable degradative removal of chemical components in this category from the environment.

5.1.1.2 Indirect Photodegradation

Hydrocarbon constituents of gasoline naphtha streams that readily volatilize to air may undergo a gas-phase oxidation reaction with photochemically produced hydroxyl radicals (OH^{-}). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation (Schwarzenbach et al, 2003). The atmospheric oxidation potential (AOP) of individual hydrocarbon compounds was estimated using AOPWIN (atmospheric oxidation program for Microsoft Windows), a subroutine in the EPI Suite™ (US EPA, 2000) models and used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances). This program calculates a reaction rate constant (cm^3/molec-sec) and a chemical half-life (hour or days) of a compound based upon average atmospheric concentrations of hydroxyl radicals and a 12-h day at 25°C.

Olefinic hydrocarbons have the additional reaction possibility of the double bond with atmospheric ozone (O_3). AOPWIN calculates a separate O_3 reaction rate and half-life for olefinic structures.

AOPWIN calculations show that typical atmospheric hydroxyl radical reaction half-lives for the modeled hydrocarbons may range from 1.4 hours to 16 days. This range may be used as an overall assessment of the atmospheric fate of the principal hydrocarbon types making up gasoline naphtha blending streams. For olefinic compounds, rate constants for reaction with atmospheric ozone may range from 38 min to 23 hours.

Conclusion:
Direct photodegradation is not expected to play an important role in the environmental fate of gasoline naphtha streams. Indirect photodegradation via reaction with hydroxyl radicals and ozone (for olefinic constituents) may be important in the gas-phase degradation of hydrocarbons that volatilize to the troposphere. An overall range of half-lives expected for individual components of these streams is 38 min to 16 days.

5.1.2 Stability in Water

Hydrolysis is unlikely for gasoline and blending streams. Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkylhalides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982b). The chemical components that comprise the gasoline blending streams category are hydrocarbons, which are not included in these chemical groups, and they are not subject to hydrolysis reactions with water.

Conclusion:
The streams within the gasoline blending streams category do not undergo hydrolysis, and this reaction would not be expected to be an important fate pathway.

5.1.3 Transport Between Environmental Compartments (Fugacity Modeling)

Equilibrium models can provide information on where a chemical is likely to partition in the environment. These data are useful in identifying environmental compartments that could potentially receive a released chemical. A widely used fugacity model is the EQC (Equilibrium Criterion) model (Mackay et al., 1997). In its guidance document for HPV data development, the U.S. EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. The EQC model is a Level I model that describes the equilibrium distribution of a fixed quantity of conserved (i.e., non-reacting) chemical at steady state within a closed environment with assumed volumes of air, water, soil and sediment. The model assumes the chemical becomes instantaneously distributed to an equilibrium condition using physical-chemical properties to quantify the chemical’s behavior. The model does not include degrading reactions, advective processes or inter-media transport between compartments.

Results of Level I 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. One drawback of these and higher level models is their inability to predict the distribution of the entire set of constituents comprising complex petroleum streams. To gain an understanding of the potential environmental distribution for these complex substances, modeling was performed for individual hydrocarbon compounds that had been identified through detailed hydrocarbon analyses to exist in these streams. The hydrocarbons selected for modeling were not only those identified to exist in these substances, but they also spanned a wide range of molecular weights and hydrocarbon types. The resulting values represent the potential ranges of distribution to environmental media for those hydrocarbon constituents found in these streams:

Air	≥ 96.5%
Water	≤2.7%
Soil	≤1.2%
Sediment	≤0.03%
Suspended sediment	≤0.02%

Conclusion: Fugacity modeling for those constituents in gasoline blending streams indicates that, at steady state, these petroleum components partition ≥96.5% to air, while partitioning into soil or water does not exceed 1.2% or 2.7%, respectively. Partitioning to sediment or suspended sediment is minimal.

5.1.4 Biodegradation

Selected data for streams in this category show that they have the potential to biodegrade to a high extent. These data are based on test results for four streams; one composed primarily of isoparaffinic hydrocarbons (CAS #64741-66-8), a second consisted of isoparaffinic, olefinic, naphthenic and aromatic hydrocarbons (CAS #64741-55-5), and a third stream composed of linear paraffins, iso-paraffins and aromatic hydrocarbons (CAS #64741-63-5). These three streams were tested for inherent biodegradability by the modified ISO/DIS 14593 CO_2 evolution test (CO_2 headspace test) using acclimated inoculum (Springborn Laboratories, 1999a-c). The CO_2 headspace procedure employed a closed system, which is recommended when assessing the biodegradability of poorly water soluble and volatile substances like those in this category. The fourth stream contained approximately 30% naphthenes (CAS #64741-46-4) and was tested for ready biodegradability following the manometric respirometry OECD 301F guideline (ExxonMobil Biomedical Sciences, 2006).

Results of the inherent biodegradability tests indicated these substances have a high capacity to biodegrade when the inoculum has been allowed to optimize their enzymatic activity during an acclimation period. The inherent biodegradation tests were run for 56 days, and the biodegradation values were essentially asymptotic by day 28, illustrating the rapid and for some nearly complete biodegradation possible for these streams. Biodegradation on day 28 ranged from 42% to 96% and on day 56 from 48% to 85%.

Even under the stringent test conditions used in ready biodegradability procedures, these substances may occasionally pass ready biodegradability criteria. This was illustrated in the OECD 301F study with CAS #64741-46-4. Conducted as part of the Petroleum HPV program, the respirometry test achieved a mean percent biodegradation of 77% over the 28-day test period. Furthermore, 60% biodegradation was attained within the 10-day window, thus passing the criteria for ready biodegradability. While not all potential gasoline blending streams may pass the criteria for ready biodegradability, the data show that the substances in this category can demonstrate relatively high extents of biodegradability, and they are not expected to persist in the environment.

Various types of microbial organisms have demonstrated the capability of degrading gasoline-type hydrocarbons. Solano-Serena (1998, 1999) showed that under non-limiting conditions, both activated sludge microorganisms and an inoculum consisting of microbes from a native soil suspension achieved biodegradation levels in the range 89 – 94%. Prince et al. (2007) followed the biodegradation of 131 individual hydrocarbons. The authors identified a relatively consistent pattern of degradation. Larger n-alkanes, iso-alkanes, and simple and alkylated aromatic compounds were the most readily biodegraded compounds. Next were the smaller n-alkanes and iso-alkanes and the naphthenes. The last compounds to be degraded were butane, iso-butane, and 2,2-dimethylbutane.

Conclusion: The cited data demonstrated that members of the gasoline blending streams can biodegrade to a great extent. While some of these substances may occasionally pass ready biodegradability criteria, these substances would not be considered categorically readily biodegradable. However, the data illustrate the high capacity for these substances to degrade and not persist in the environment.

5.2 Assessment Summary for Environmental Fate

If gasoline blending streams are released to the environment, individual components will disperse and partition according to their individual physical-chemical properties. Their final disposition is shaped by both abiotic and biotic processes. Based on modeling individual structures encompassing the different types and molecular weights of hydrocarbons making up gasoline blending streams, volatilization to the atmosphere is an important process. Residence times in the atmosphere are relatively short due to indirect photodegradation reactions. In water, hydrolysis is not likely to occur, as the chemical linkages of hydrocarbons do not allow for these reactions. However, biodegradation data show that these products can exhibit a moderate to rapid rate of biodegradation and are considered at least inherently biodegradable.

6. ENVIRONMENTAL EFFECTS

6.1 Aquatic Toxicity

The Test Plan for the gasoline blending streams category originally proposed to create subgroups based on predominant PONA hydrocarbon classes within each category member. However, similarities in the aquatic toxicity of these streams allowed simplification of the category without using the PONA class subgroups. This is because the hydrocarbon constituents in these substances elicit acute aquatic toxicity through non-polar narcosis, for which the mechanism of action is disruption of biological membrane function (van Wezel and Opperhuizen, 1995). For this reason, gasoline blending streams share a common mode of action, and their acute toxicities would be expected to fall within a relatively narrow range that is independent of PONA distribution. Any differences between toxicities (i.e., LC/LL50, EC/EL50) can be explained by the differences between the target tissue-partitioning behavior of the individual chemicals (Verbruggen et al., 2000). For example, the existing fish toxicity database for hydrophobic neutral chemicals supports a critical body residue (CBR, the internal concentration that causes mortality) of approximately 2-8 mmol/kg fish (wet weight) (McCarty and Mackay, 1993; McCarty et al., 1991). When normalized to lipid content the CBR is approximately 50 µmol/g of lipid for most organisms (Di Toro et al., 2000).

Because petroleum hydrocarbons elicit toxicity through a common mode of action, quantitative structure activity relationships (QSARs) have been developed to calculate acute toxicity to aquatic organisms. Toxicity QSARs estimate the lethal loading rates (i.e., LL50, EL50) for hydrocarbons having similar hydrocarbon/water partition coefficients (Kp). Based on the hydrocarbon block method used in the risk assessment of petroleum substances (CONCAWE, 1996b, Hermens et al., 1985), the QSAR methodology utilizes knowledge of the composition of the hydrocarbon substance and the hydrocarbon/water partition coefficient (Kp) for each of the components. The details of this calculation approach have been published (Peterson, 1994) and refined (McGrath et al., 2005). In this procedure, the dissolved concentrations of individual hydrocarbons from a petroleum substance are estimated for a given loading rate and then normalized by their acute toxicity to yield Toxic Units (TU). The sum of the toxic units for the complex mixture will equal one at the LL50 of the substance. Considerable experimental support for this conceptual framework has been developed, which confirms that complex substances exerting toxicity via a common mechanism, are additive and further, that hydrocarbons act through a common mechanism of non-polar narcosis (Hermens et al, 1985; Deneer et al., 1988). In summary, given the compositional analysis (together with consideration of the variability of composition of the particular petroleum substance), acute toxicity can be calculated. This toxicity calculation is conservative in that it assumes that each component is maximally dissolved (completely equilibrated with un-dissolved phase and there is no competition for solubility between similar hydrocarbons) and that there are no losses from solution (due to adsorption to surfaces, absorption to test organisms or volatilization, etc.). Depending on the QSAR selected, the toxicity calculation may be performed for fish, daphnia or algae.

The acute aquatic toxicity of the gasoline blending streams is described below, and an overall range of acute toxicity values is provided for each trophic level that may serve as “read-across” for the untested members of this category.

6.1.1 Aquatic Endpoints – Acute Toxicity

The HPV Chemical Test Program includes acute toxicity to a freshwater fish, an invertebrate (*Daphnia magna*), and an alga. The substances in the Gasoline Blending Streams Category are expected to produce a similar range of toxicity for these aquatic species when studies using similar solution preparation and exposure techniques are compared. The endpoint values cited in the robust summaries and described below for the three trophic levels reflect the loading rates of the test substance added to exposure solutions. Termed water accommodated fractions (WAF), the WAF preparation is recommended as the appropriate procedure for testing complex substances having low water solubility (OECD, 2000).

All the studies reported here employed aqueous exposure solutions prepared as WAFs, and tests were conducted in closed vessels with minimal headspace. This was done to minimize loss of volatile components. For fish and *Daphnia magna* tests, most studies were semi-static with daily renewal of test solutions. Algal test solutions were not renewed, but the nutrient medium was fortified with sodium bicarbonate to avoid CO_2-limited growth that can occur when testing algae in closed systems.

The acute toxicity tests on aquatic organisms described in the robust summaries consists of the following:

- twelve key studies of four different gasoline blending streams (Stonybrook Laboratories, 1995c-h; ABC Laboratories, 1998c-h) described as:
    - CAS No. 64741-66-8, naphtha (petroleum), light alkylate
    - CAS No. 64741-55-5, naphtha (petroleum), light catalytic cracked

  - CAS No. 64741-46-4, naphtha (petroleum), light straight-run
  - CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed

- data from CONCAWE (1996c) on four blending streams,
  - CAS No. 64741-70-4, naphtha (petroleum), isomerization
  - CAS No. 64741-54-4, naphtha (petroleum), heavy catalytic cracked
  - CAS No. 64741-46-4, naphtha (petroleum), light straight-run
  - CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed

- two samples of a blended gasoline and identified as CAS No. 86290-81-5 (CONCAWE, 1995a,c-g).

The experimental methods described for the 12 key acute studies and the two studies of gasoline included chemical analysis of the WAF solutions for dissolved petroleum components. Gas chromatographic analysis was used to measure the concentrations of a suite of hydrocarbon compounds in the dissolved fraction. Measured values are not reported here because the suite of compounds is not identical among the tests, nor did they constitute 100% of the total possible hydrocarbon components in the dissolved phase. While this precluded an even comparison among studies, the analytical measurements verified that petroleum substance was present in the dissolved phase thus exposure of the organisms was confirmed.

Aquatic toxicity also can be predicted based on the known carbon number range, measured or calculated toxicity of hydrocarbon blocks, and knowledge of the hydrocarbon composition of those substances (see discussion in Section 6.1). As an example of this calculation, acute lethal loading rates for fish and *Daphnia magna* were calculated using the hydrocarbon block model (Peterson, 1994; McGrath et al., 2005). Calculations cited in Peterson, 1994 were made using the detailed hydrocarbon analysis of CAS No. 64741-63-5 and information on acute toxicity of specified hydrocarbon blocks making up this substance.

6.1.1.1 Acute Toxicity to Aquatic Vertebrates

The results of the studies described in detail in the robust summaries are provided in the following table.

Table 2. Acute Toxicity Values for Gasoline Blending Streams to Freshwater Fish

Test Substance	Toxicity Endpoint	Endpoint Value, mg/L (loading rate)	Reference
CAS No. 64741-66-8, naphtha (petroleum), light alkylate	96-h LL50	8.2	Stonybrook Laboratories (1995c) (key study)
CAS No. 64741-55-5, naphtha (petroleum), light catalytic cracked	96-h LL50	46	Stonybrook Laboratories (1995d) (key study)
CAS No. 64741-46-4,	96-h LL50	15	ABC Laboratories

naphtha (petroleum), light straight-run	96-h LL50	18	(1998c) (key study) CONCAWE (1996c)
CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed	96-h LL50 96-h LL50 96-h LL50	34 12 2.09 (calculated)	ABC Laboratories (1998d) (key study) CONCAWE (1996c) Peterson (1994)
CAS No. 64741-70-4, naphtha (petroleum), isomerization	96-h LL50	10	CONCAWE (1996c)
CAS No. 64741-54-4, naphtha (petroleum), heavy catalytic cracked	96-h LL50	15	CONCAWE (1996c)
CAS No. 86290-81-5 (Gasoline) Sample #1 Sample #2	96-h LL50 96-h LL50	11 16	CONCAWE (1995a,c)

Based on the key studies, the range of measured fish acute toxicity values (expressed as lethal loading rates) for exposure to gasoline blending streams was 8.2 – 46 mg/L (Stonybrook Laboratories, 1995c,d; ABC Laboratories, 1998c,d) when exposure solutions were prepared as WAFs. The data cited in Table 2 from CONCAWE (1996c) which employed similar WAF preparation and exposure techniques, ranged 10 – 18 mg/L. For an additional comparison, the range of acute toxicity values for WAF studies cited in CONCAWE (2001), which included some marine/estuarine species, was 8.3 – 27 mg/L.

Acute toxicity to fish when exposed to blended gasoline would not be expected to vary from the individual stream data, and this was demonstrated by measured acute toxicity values of 11 and 16 mg/L (as lethal loading rates) for two studies on a gasoline (CONCAWE, 1995a, c). Finally, a reasonable prediction of acute toxicity may be calculated based on known composition and acute toxicity of hydrocarbon blocks. This was shown by the calculated value of 2.09 mg/L for one of the streams.

In summary, fish acute toxicity values for members of the gasoline blending streams category are expected to be similar based on the common mode of action for acute toxicity of petroleum hydrocarbons. As demonstrated by the present data, these values may be expected to fall within the range of calculated and measured values cited in the robust summaries, and those values were in agreement with the studies cited by CONCAWE (2001).

CONCLUSION: The range of acute toxicity values that may be used as read-across for members of the category is 2.09 – 46 mg/L (lethal loading rates) based on both calculated and measured data.

6.1.1.2 Acute Toxicity to Aquatic Invertebrates

The results of the studies described in detail in the robust summaries are provided in the following table.

Table 3. Acute Toxicity Values for Gasoline Blending Streams to Freshwater Invertebrates (*Daphnia magna*).

Test Substance	Toxicity Endpoint	Endpoint Value, mg/L (loading rate)	Reference
CAS No. 64741-66-8, naphtha (petroleum), light alkylate	48-h EL50	32	Stonybrook Laboratories (1995e) (key study)
CAS No. 64741-55-5, naphtha (petroleum), light catalytic cracked	48-h EL50	18	Stonybrook Laboratories (1995f) (key study)
CAS No. 64741-46-4, naphtha (petroleum), light straight-run	48-h EL50 48-h EL50	18 4.5	ABC Laboratories (1998e) (key study) CONCAWE (1996c)
CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed	48-h EL50 48-h EL50 48-h EL50	10 8.4 0.9 (calculated)	ABC Laboratories (1998f) (key study) CONCAWE (1996c) Peterson (1994)
CAS No. 64741-70-4, naphtha (petroleum), isomerization	48-h EL50	10	CONCAWE (1996c)
CAS No. 64741-54-4, naphtha (petroleum), heavy catalytic cracked	48-h EL50	13	CONCAWE (1996c)

CAS No. 86290-81-5 (Gasoline)	Sample #1 Sample #2	48-h EL50 48-h EL50	7.6 12	CONCAWE (1995d,e)

Based on the key studies, the range of invertebrate acute toxicity values (expressed as lethal loading rates) for exposure to gasoline blending streams was 10 – 32 mg/L (Stonybrook Laboratories, 1995e,f); ABC Laboratories, 1998e,f) when exposure solutions are prepared as WAFs. The data cited in Table 3 from CONCAWE (1996c) which employed similar WAF preparation and exposure techniques, ranged 4.5 – 13 mg/L. For an additional comparison, the range of acute toxicity values for WAF studies cited in CONCAWE (2001), which included some marine/estuarine species, was 2 – 32 mg/L.

Acute toxicity to invertebrates when exposed to blended gasoline would not be expected to vary from the individual stream data, and this was demonstrated by measured acute toxicity values of 7.6 and 12 mg/L (as lethal loading rates) for two studies on a gasoline (CONCAWE, 1995d,e). Finally, a reasonable prediction of acute toxicity may be calculated based on known composition and acute toxicity of hydrocarbon blocks. This was shown by the calculated value of 0.9 mg/L for one of the streams.

In summary, invertebrate acute toxicity values for members of the gasoline blending streams category are expected to be similar based on the common mode of action for acute toxicity of petroleum hydrocarbons. As demonstrated by the present data, these values may be expected to fall within the range of calculated and measured data cited in the robust summaries, and those values were in agreement with the studies cited by CONCAWE (2001).

Conclusion: The range of acute toxicity values that may be used as read-across for members of the category is 0.9 to 32 mg/L (lethal loading rates) based on both calculated and measured data.

6.1.1.3 Toxicity to Aquatic Plants

The results of the studies described in detail in the robust summaries are provided in the following table.

Table 4. Acute Toxicity Values for Gasoline Blending Streams to Freshwater Algae.

Test Substance	Toxicity Endpoint	Endpoint Basis	Endpoint Value, mg/L (loading rate)	Reference
CAS No. 64741-66-8, naphtha (petroleum), light alkylate	96-h EL50	CD	45	Stonybrook Laboratories (1995g) (key study)
CAS No. 64741-55-5, naphtha (petroleum), light catalytic cracked	96-h EL50	CD	64	Stonybrook Laboratories (1995h) (key study)

CAS No. 64741-46-4, naphtha (petroleum), light straight-run	96-h EL50 72-H IL50	CD GR AUC	6.4 4.1 3.6	ABC Laboratories (1998g) (key study) CONCAWE (1996c)
CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed	96-h EL50 72-h IL50	CD GR AUC	8.5 6.4 1.1	ABC Laboratories (1998h) (key study) CONCAWE (1996c)
CAS No. 64741-70-4, naphtha (petroleum), isomerization	72-h IL50	GR AUC	>50 25	CONCAWE (1996c)
CAS No. 64741-54-4, naphtha (petroleum), heavy catalytic cracked	72-h IL50	GR AUC	6.3 5.3	CONCAWE (1996c)
CAS No. 86290-81-5 (Gasoline) Sample #1 Sample #2	 72-h IL50 96-h IL50 72-h IL50 96-h IL50	 GR AUC GR AUC GR AUC GR AUC	 3.1 1.4 3.7 1.1 3.3 4.2 2.5 0.25	 CONCAWE (1995f,g)
CD = Cell density (cells/mL); GR = Growth rate (1/day); AUC = Area under the growth curve				

Based on the key studies, the range of toxicity values (expressed as lethal loading rates) for exposure of freshwater algae to gasoline blending streams was 6.4 – 64 mg/L (Stonybrook Laboratories, 1995g,h); ABC Laboratories, 1998g,h) when exposure solutions were prepared as WAFs and conducted for 96 hours. The stream data in Table 4 referencing CONCAWE (1996c) were based on studies employing similar WAF preparation and exposure techniques. The 72-h IL50 based on algal growth rate ranged 4.1 – >50 mg/L, while the 72-h IL50 based on area under the growth curve (i.e., biomass) ranged 1.1 – 25 mg/L. The CONCAWE (1996c) data indicate that growth rate may be a slightly less sensitive indicator of toxicity than area under the growth curve, but the values suggest that there is not a substantial difference in toxicity among the different blending streams.

Toxicity of blended gasoline to algae would not be expected to vary from the individual stream data, and the data cited in CONCAWE (1995f,g) suggest only slightly lower endpoint values compared to the blending streams. As described for the individual streams, toxicity endpoints based on growth rate were slightly higher than those based on area under the growth curve, and values calculated for 72-hour and 96-hour time periods were not substantially different. The 72-hour IL50 endpoints ranged from 1.4 to 4.2 (as lethal loading rates) for biomass (i.e., area under the growth curve calculation (AUC)), and 3.1 to 3.3 for growth rate, while the 96-hour IL50 endpoints ranged from 0.25 to 1.1 for biomass and 2.5 to 3.7 for growth rate.

Not all toxicity data reported in the published literature have fallen within the relatively narrow range cited in Table 4. Algal toxicity values as high as 30,000 mg/L (e.g., IL50 based on growth rate) were reported by CONCAWE (1996c, 2001) for a battery of five algal tests (four tests on individual streams, one on a blended gasoline; sample numbers CWE1 – CWE5). In a review of those data, CONCAWE (1996c) noted several limitations in test methodology that likely affected the algal cell growth. Among them were the broad spread of the test concentrations (concentrations were spaced by a factor of 10) and the development of a void in the sealed test vessels that allowed partitioning of volatile components to the headspace. Measurements of hydrocarbon constituents in the WAFs showing declines of 81% to 94% between initial and final analyses provided evidence of test substance loss in the test vessels. The reporting of the IL50 values also was contrary to conventional practice. For example, the maximum WAF concentration in those studies was 1000 mg/L, while the IL50 values for two tests were reported as 4,700 mg/L and 30,000 mg/L. The current convention for reporting data of this nature is to cite the IL50 as >1000 mg/L, the maximum exposure level used in the test. For these reasons, algal toxicity values for the purpose of read-across to other members of the gasoline blending streams category did not include the battery of the five tests described for sample numbers CWE1-CWE5 in the CONCAWE report (CONCAWE, 1996c).

In summary, algal toxicity values for members of the gasoline blending streams category are expected to be similar based on the common mode of action for petroleum hydrocarbons. As demonstrated by the present data, measured toxicity values were similar for the key studies cited in the robust summary (6.4 – 64 mg/L; lethal loading rates) and were in general agreement for other WAF test data cited in Table 4.

Conclusion: The acute toxicity of gasoline blending streams to freshwater algae is expected to fall within the approximate range 1.1 - 64 mg/L (lethal loading rates). This range of acute toxicity values can be used as “read-across” to untested members of the gasoline blending streams category.

6.1.2 Aquatic Endpoints – Chronic Toxicity

Chronic aquatic toxicity was measured for fish (OECD 204) and *Daphnia magna* (OECD 211) for three gasoline blending streams. These included the following category members:

- CAS No. 64741-66-8, naphtha (petroleum), light alkylate
- CAS No. 64741-55-5, naphtha (petroleum), light catalytic cracked
- CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed

As for the acute studies, the chronic tests employed WAF preparation and testing methods, and all tests were conducted in closed test vessels. The analytical measurements of selected hydrocarbon components in the dissolved phase of the WAFs also were subject to the same limitations as described in section 6.1.1. Therefore, test endpoints are presented as WAF loading rates.

6.1.2.1 Chronic Toxicity to Aquatic Vertebrates

The results of the chronic toxicity tests on fish are shown in the following table.

Table 5. Chronic Toxicity Values for Gasoline Blending Streams to Freshwater Fish

Test Substance	Toxicity Endpoint	Endpoint Value, mg/L (loading rate)	Reference
CAS No. 64741-66-8, naphtha (petroleum), light alkylate	Survival LL50 NOELR Growth EL50 NOELR	 8 2.6 ND 2.6	Springborn Laboratories (1999d)
CAS No. 64741-55-5, naphtha (petroleum), light catalytic cracked	Survival LL50 NOELR Growth EL50 NOELR	 23 6.4 ND 6.4	Springborn Laboratories (1999e)
CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed	Survival LL50 NOELR Growth EL50 NOELR	 5.2 2.6 ND 2.6	Springborn Laboratories (1999f)
ND = Not Determined			

For the 14-day chronic toxicity to fish, LL50 values ranged from 5.2 to 23 mg/L (expressed as lethal loading rates), while no observed effect loading rates (NOELR) ranged from 2.6 to 6.4 mg/L. There was no apparent difference in the concentrations eliciting survival and growth effects, as range of NOELR values for survival and growth were both 2.6 – 6.4 mg/L.

Conclusion: The chronic toxicity NOELR values for gasoline blending streams to fish were found to range from 2.6 to 6.4 mg/L (lethal loading rates).

6.1.2.2 Chronic Toxicity to Aquatic Invertebrates

The results of the chronic toxicity tests on invertebrates are shown in the following table.

Table 6. Chronic Toxicity Values for Gasoline Blending Streams to Freshwater Invertebrates (*Daphnia magna*).

Test Substance	**Toxicity Endpoint**	**Endpoint Value, mg/L (loading rate)**	**Reference**
CAS No. 64741-66-8, naphtha (petroleum), light alkylate	Survival EL50 NOELR Reproduction EL50 NOELR	 >40 16 10 2.6	Springborn Laboratories (1999g)
CAS No. 64741-55-5, naphtha (petroleum), light catalytic cracked	Survival EL50 NOELR Reproduction EL50 NOELR	 27 16 13 2.6	Springborn Laboratories (1999h)
CAS No. 64741-63-5, naphtha (petroleum), light catalytic reformed	Survival EL50 NOELR Reproduction EL50 NOELR	 26 16 14 <0.39	Springborn Laboratories (1999i)
ND = Not Determined			

For the 21-day chronic toxicity to *Daphnia magna*, EL50 values based on survival ranged from 26 to >40 mg/L (expressed as lethal loading rates), while the survival NOELR values were all 16 mg/L for the three tests. When toxicity was evaluated based on reproduction, EL50 values 10 to 14 mg/L, while NOELR values ranged <0.39 to 2.6 mg/L. The most sensitive test endpoint for chronic toxicity of gasoline blending streams to *Daphnia magna* was reproduction.

Conclusion: The chronic NOELR values for reproduction ranged from <0.39 to 2.6 mg/L (lethal loading rates).

6.2 Terrestrial Toxicity

Effects on terrestrial ecosystems were not evaluated for the gasoline blending streams category. Although terrestrial organisms may be exposed if spills occur, the physical-chemical properties of the individual hydrocarbons indicate that many of the constituents in these substances would tend to partition to the air. The hydrocarbon constituents absorbed to the soil would gradually biodegrade based on the inherent biodegradability properties of these substances. The rate of biodegradation would be affected by the types of hydrocarbon compounds present, the amount of available nutrients as well as contact with, and adaptation by, the indigenous microbial community.

6.3 Assessment Summary for Environmental Effects

Based on the studies identified to represent the acute toxicity of gasoline blending streams to aquatic organisms, the range of acute toxicities was generally similar for the three trophic levels (fish, invertebrates, and algae). The proposed “read-across” ranges of toxicity endpoints (expressed as lethal loading rates) that are expected to represent the potential acute toxicity to fish, invertebrates, and algae were 2.09 to 46 mg/L, 0.9 to 32 mg/L, and 1.1 to 64 mg/L, respectively. Impacts to terrestrial ecosystems from spill events may occur, but constituents in gasoline blending streams are at least inherently biodegradable, show appreciable volatility, and eventually these constituents would be expected to partition to environmental compartments consistent with their physical-chemical characteristics, particularly into air, and eventually dissipate.

7.0 HUMAN HEALTH ENDPOINTS

Results of studies on naphthas high in paraffinic, olefinic naphthenic and aromatic constituents are summarized in this section. Although some studies were performed by the oral and dermal routes of exposure, inhalation has been identified as the route most relevant to human exposure for purposes of hazard and risk assessment. The mammalian toxicology and environmental profiles on these blending streams are supported by comparable test results on gasoline from studies in the US and Europe. In addition, a testing program currently in progress mandated by the Clean Air Act 211(b) statute on an EPA designated “industry average” gasoline vapor condensate provides even more current data on mammalian toxicity of gasoline. Data submitted to the HPV program have been developed with the goal of facilitating international harmonization of hazard and risk characterization worldwide. The EU categories for gasoline components [Appendix C], organized by the definitive processing step to produce those components are generally complementary to the approach employed in this program.

7.1 Human Health Effects

7.1.1 Acute Toxicity

Light alkylate naphtha (API 83-19; CAS #64741-66-8; approx 100% paraffinic) is not acutely toxic by the oral (rat > 7000mg/kg), dermal; (rabbit > 2000mg/kg) and inhalation (rat > 5mg/l, 4 hr exposure) routes and is non-irritating to the rabbit eye 24 hrs after exposure. It is a moderate skin irritant in rabbits but is not a skin sensitizer in guinea pigs. (API, 1986a, 1987a)

Light catalytic cracked naphtha (API 83-20; CAS #64741-55-5, approx. 46% olefinic) is not acutely toxic by the oral (rat > 5000mg/kg), dermal (rabbit > 3000mg/kg) and inhalation (rat > 5.3mg/l, 4 hr exposure) routes and is not irritating to the rabbit eye 24 hrs after exposure. It is a moderate skin irritant in rabbits but is not a skin sensitizer in guinea pigs. (API, 1986b, 1987b)

Sweetened naphtha (API 81-08, CAS #64741-87-3, approx. 21% naphthenics) is a light straight run naphtha in which a sweetening process has converted mercaptans and removed acidic impurities. It is not acutely toxic by the oral (rat > 5000mg/kg), dermal (rabbit >

2000mg/kg) and inhalation (rat > 5.2mg/l, 4 hr exposure) routes and is not irritating to the rabbit eye 24 hrs after exposure and only a mild skin irritant in rabbits. (API, 1986c, 1987c)

Full range catalytic reformed naphtha (API 83-05, CAS #68955-35-1, approx. 63% aromatics) is not acutely toxic by the oral (rat = 3500-9800mg/kg), dermal (rabbit > 2000mg/kg) and inhalation (rat > 5.22mg/l, 4 hr exposure) routes. Eye irritation observed within 1 hour of instillation gradually resolved over 7 days and was not apparent at 14 days. It is a moderate skin irritant in rabbits but is not a skin sensitizer in guinea pigs. (API 1984, 1985a, 1986d)

Gasoline (supplemental chemical)

Unleaded gasoline (API PS-6) is similar to its component blending streams. It is not acutely toxic by the oral (rat > 18.75ml/kg [14g/kg]), dermal (rabbit > 5ml/kg [3.9g/kg]) routes and is not irritating to the rabbit eye 24 hrs after exposure. It is a mild skin irritant in rabbits and is not a skin sensitizer in guinea pigs. (API 1980a)

Conclusion: Results of testing naphtha blending streams for acute toxicity indicate that these materials demonstrate consistently low toxicity by the oral [Rat LD50 >5g/kg], dermal [Rabbit LD50 >2g/kg] and inhalation [Rat LC50 >5g/m^3] exposure routes, are mild to moderate eye and skin irritants and are not skin sensitizers. Acute data for gasoline gave comparable results. The inhalation acute toxicity read-across value for untested category members is LC50 > 5g/m^3

7.1.2. Repeated Dose Toxicity

Key inhalation studies are described in detail below. The focus is on inhalation studies as inhalation is the most relevant route for human exposure. Studies by the dermal route are summarized in the Supplemental Studies section**.**

Light alkylate naphtha (LAN, CAS #64741-66-8; approx 100% paraffinic) has been tested as a vapor distillate fraction (approx. 100% paraffinic) by inhalation in the rat for systemic toxicity and neurotoxicity, and in the rabbit by dermal exposure.

Sprague Dawley rats [12males/12 females/group] were exposed to a LAN light end distillate at concentrations of 0, 668, 2220, and 6646ppm (2438, 8102 and 24300mg/m^3), 6 hours/day, 5 days/wk for 13 weeks, according to OECD guideline 413. The test material (LAN-D) was prepared to be representative of the fraction of light alkylate naphtha to which man would be exposed during normal handling and use. It was obtained by the distillation of light alkylate naphtha (LAN) and collection of that fraction that boiled over the temperature range 78 to 145°F. The maximum exposure level was 75% of the lower explosive limit for LAN distillate. Extra groups of 12 rats of each sex exposed to the high dose level and a recovery control group were maintained untreated for 28 days following cessation of the 13 weeks exposure. Neurobehavioral evaluations of motor activity and functional activity [FOB] were performed pretest and during weeks 5, 9, 14 and week 18 for recovery groups. Animals were not exposed to LAN-D during these tests. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 14 weeks and18 weeks (recovery groups). Body weights and food consumption were measured throughout the study. Blood samples were taken from 12 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemical measurements. At termination (after 13 weeks exposure for the main study and after 18 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymides), thymus and uterus. Brain lengths and widths were measured for each rat. Thirty nine tissues removed from the control and high dose animals, were fixed, stained with hemotoxylin-eosin and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were collected from the nervous system (central and peripheral) of all animals and nervous system tissues were selected randomly from 6 rats per sex/group in the high dose and controls at the end of 13 weeks for microscopic examination. Specific brain regions examined were forebrain, cerebral cortex, hippocampus, basal ganglia, midbrain cerebellum and pons and medulla.

Neurobehavioral studies included motor activity, monitored as the number of beam breaks in an activity box, at pretest, and during weeks 5, 9, 14, and at the end of the 4- week recovery period. The Functional Operational Battery [FOB] was comprised of home cage evaluations, handling and open field behaviors and reflex assessment. Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

There were no mortalities during the study and there were no treatment related signs of toxicity with the possible exception of an increased incidence of red facial staining in rats of both sexes in the high dose group. Mean body weights, body weight gains and food consumption were unaffected by treatment. Hematologic changes were a 5% decrease in hemoglobin and a 7% decrease in erythrocyte counts. Hemoglobin was still decreased 4% after the 4 week recovery period. However all these decreases were small and within historical control range for the laboratory. Decreases in AST and ALT in high dose females were not considered toxicologically significant because several control females had AST and ALT levels that were elevated relative to the other control females and relative to the historical control range. Comparison of values from high dose females with these elevated control values indicates that some were different by statistical criteria, but these differences were not toxicologically important. Organ weight changes were few. Statistically significant increases in kidney weights in high dose males correlated with microscopically observed hyaline droplet formation and degeneration of proximal renal tubules were observed, indicative of alpha 2-microglobulin mediated nephropathy, also identified as light hydrocarbon nephropathy, a species and sex specific syndrome not relevant to humans (US EPA, 1991). Increased liver weights in high dose rats of both sexes had no microscopic correlate and appeared reversible after 4 weeks of recovery. Absolute and relative liver weights were observed in the high dose males and females at 13 weeks but the differences had disappeared after the recovery period. There were no pathological findings associated with this increase. In the neurobehavioral studies no treatment-related effects were observed in the functional operational battery. In the study of motor activity there were some statistically significant differences, but overall they did not occur in a dose related manner and furthermore were smaller than some of the differences seen during the pre-dosing period. The systemic LOAEL = 6646ppm (24300mg/m^3) based on increased liver weight and red facial staining and the NOAEL = 2220ppm (8200mg/m^3). The Neurotoxicity NOAEL = 6646ppm (24300mg/m^3). (Schreiner et al., 1998)

Light catalytic cracked naphtha (LCCN, CAS #64741-55-5, approximately 46% olefinic) was tested by inhalation in three 13-week studies and one 21-day study. In the three 13 week studies, concentrations were 147 - 2136ppm (530-7690 mg/m^3) partially vaporized LCCN to rats and mice (Dalbey et al, 1996); 1500 – 4500ppm (5474-16423 mg/m^3) wholly vaporized LCCN to rats (API, 1987d); and 750 – 7500ppm (2336-23364 mg/m^3) light ends distillate to rats (Lapin et al., 2001). In the 21-day study (15 actual exposures), wholly vaporized LCCN was administered to male and female Sprague Dawley rats at concentrations of 55, 567, and 3628ppm (200, 2040, and 13060mg/m^3) (Halder et al., 1984). Only kidney effects in male rats were reported in detail in the Halder et al. studies [see Supplemental studies below]. Of the three 13 week studies, two discussed in detail here most accurately reflect effects of exposure to LCCN.

Sprague Dawley rats [16 males/16 females/group] were exposed to an LCCN light end distillate (approx. 60% olefinic) at concentrations of 0, 750, 2500, and 7500ppm (2340, 7700 and 23400mg/m^3), 6 hours/day, 5 days/wk over 15 weeks, according to OECD guideline 413, for a total duration of at least 65 exposures. The test material (LCCN-D) was prepared to be representative of the fraction of light catalytic cracked naphtha to which man might be exposed during normal handling and blending. The maximum exposure level was 75% of the lower explosive limit for LCCN distillate. Extra groups of 16 rats of each sex exposed to the high dose level and a recovery control group were maintained untreated for 28 days following cessation of the 15 weeks exposure. Neurobehavioral evaluations of motor activity and functional activity [FOB] were performed pretest and during weeks 5, 9, 15 and 19 for recovery groups. Animals were not exposed to LCCN-D during these tests. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 15 weeks and 20 weeks (recovery groups). Body weights and food consumption was measured throughout the study. Blood samples were taken from 10 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemistry measurements. At termination (after 15 weeks exposure for the main study and after 19 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. Ten rats/sex/group were selected for non-neuropathologic examination and 6 rats/sex/group for neuropathologic examination. The following organs were weighed from the non-neuropathologic animals: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymides), thymus and uterus. Brain lengths and widths were measured for each rat. Thirty-nine tissues removed from the control and high dose animals, fixed and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were collected from the nervous system (central and peripheral) of all animals and nervous system tissues were selected randomly from 6 rats per sex/group in the high dose and controls at the end of 15 weeks for microscopic examination. Specific brain regions examined were forebrain, cerebral cortex, hippocampus, basal ganglia, midbrain cerebellum and pons and medulla.

Neurobehavioral studies included motor activity, monitored as the number of beam breaks in an activity box, at pretest, and during weeks 5, 9, 14, and at the end of the 4 week recovery period. The Functional Operational Battery [FOB] was comprised of home cage evaluations, handling and open field behaviors and reflex assessment. Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

No exposure-related clinical observations were noted either during exposure or during non-exposure periods and no ocular abnormalities were observed. Slight differences in weight gain were seen in high dose animals but weights were comparable to controls at the end of the recovery period. During the 4-week recovery period, the high dose males and females had food consumption that was greater (statistically significant) than controls. At 15 weeks, hematologic changes in the high dose group were decreased hemoglobin (8%) and hematocrit (7%) in males and decreased MCHC (3%) in females and in the mid-dose group males decreased MCHC (4%). After 4 weeks recovery, all hematologic values were comparable to controls. No abnormal clinical chemistry values were observed after 15 weeks exposure. Although glucose and albumin levels were elevated in high dose females after the recovery period, the values fell within normal historical range and were not considered toxicologically significant. Statistically significant increases in absolute and relative kidney weights in high dose males and relative kidney weights in mid-dose males correlated with microscopically observed hyaline droplet formation and degeneration of proximal renal tubules in high dose males, indicative of alpha 2-microglobulin mediated nephropathy, also identified as light hydrocarbon nephropathy, a species and sex specific syndrome not relevant to humans (US EPA, 1991). Increased relative liver weights in high dose rats of both sexes had no microscopic correlate, although the absolute liver weight in recovery high dose females was elevated possibly correlated with increased food consumption. Decreased relative brain weight in recovery females had no microscopic correlate. With the exception of kidney findings in males, the only treatment related microscopic observations were nasal mucosa hyperplasia and hypertrophy/ hyperplasia of goblet cells indicative of exposure to a mild irritant, the incidence of which was comparable to controls after the recovery period.

In the neurobehavioral studies there was no evidence of any effect on motor activity either after 15 weeks exposure or after the 4-week recovery period. There was no evidence of a treatment-related effect in the functional operational battery. The systemic toxicity LOAEL = 7500ppm (23400mg/m^3) based on increased organ weight and nasal epithelium changes and the NOAEL = 2500ppm (7700mg/m^3). The Neurotoxicity NOAEL = 7500ppm (23400mg/m^3). (Lapin et al., 2001)

A 13 week inhalation study was conducted in Sprague Dawley rats [10/sex/group] and CD-1 mice [10/sex/group] exposed to a 40% vapor of LCCN at concentrations of 0, 147, 572, 2136ppm (0, 530, 2060 & 7690 mg/m^3), 6 hours/day, 5 days/week. Extra groups of 10 rats and mice of each sex served as sham and untreated controls. Food and water was available ad-lib, except during the exposure periods. Clinical observations were made regularly and body weights were recorded weekly. At the end of the 13 weeks exposure, blood was drawn from fasted animals for hematological and clinical chemistry measurements. All animals were then sacrificed and necropsied. Organs were weighed and a wide range of tissues fixed for subsequent histology and microscopic examination. The wet and dry weights of the right apical and right middle lung lobes were also recorded. The cauda epididymis of the control and high dose male rats was used to determine the morphology and number of sperm and the left testis was used to determine the number of testicular spermatids. The following tissues from the high dose and sham treated animals were examined histologically: adrenals, kidney, bone and marrow (sternum), pancreas, brain, submaxillary salivary gland, eye, optic nerve, spleen, heart, stomach, colon, testes or ovaries, duodenum, kidneys, thymus, thyroid, liver, tracheobronchial lymph nodes, lung (left lobe), nasal turbinates, muscle, urinary bladder, sciatic nerve, and any gross lesions. Additional sections included lung from untreated controls and kidney from 0, 572 and 2136ppm (2060 and 7690 mg/m^3) exposure groups.

No treatment-related changes were observed in either species in clinical signs, body weight, clinical chemistry or hematology except four male rats in the high dose group that had lesions on the skin in the scrotal area. This was attributed possibly to an interaction between abrasions of the skin against the floors of the cages and the whole-body exposure to high concentrations of LCCN. Organ weights were unaffected in either species, except for uterus weights. Uterine weights in the rats were less than untreated controls for all exposed groups, but not less than the sham controls. Uterine weight changes were not considered to be related to LCCN because they were not dose-related, and there was no difference between the sham and untreated controls. Additionally, no similar effect was observed in the mice. No treatment-related abnormalities were observed in any of the organs examined microscopically. The occurrence of hyaline droplets in dilated renal tubules was similar in the controls and the high dose male rats. The number of sperm per gram of cauda epididymis was significantly lower in the 2136ppm (7690 mg/m^3) rat group than in the sham controls but not the untreated controls. The actual number of epididymal sperm was not significantly affected by exposure. Also, the number of testicular spermatids and the percentage of abnormal sperm in the cauda epididymis were not affected by exposure to 2136ppm (7690 mg/m^3) in rats compared to either control group. The toxicological significance of the decrease in sperm/gram in the epididymis is unknown since it was not supported by other male parameters, but the occurrence has been used to set the LOAEL. The rat systemic toxicity LOAEL = 2136ppm (7690 mg/m^3) NOAEL = 572ppm (2060 mg/m^3). The mouse systemic NOAEL = 2136ppm (7690 mg/m^3). (Dalbey et al, 1996)

Another 13 week inhalation study on a high olefinic test material similar to those used in the Dalbey et al (1996) and Lapin et al (2001) studies conducted in rats at exposure concentrations of 1510, 2610, 4520 ppm (0, 5475, 9500, -16425 mg/m^3) had results similar to the Lapin et al study. Additionally, a trace centrilobular hepatocellular hypertrophy was observed in 50% of the males and 25% of the females in the 4520 ppm group. This study was performed at vapor concentrations above that of the 40% vapor study of Dalbey et al, and did not show any effects on sperm numbers in the epididymis. (API 1987d).

The 21 day inhalation study set of Halder et al, 1984 confirmed the findings in male kidneys that had been observed in other studies with rats [see Supplemental studies below]. However, since these kidney effects are not considered relevant to man and study duration is less than the 13 week studies, this is not considered a key study and is not included in establishing toxicity ranges. Only abbreviated robust summaries are supplied.

Heavy straight run naphtha (CAS # 64741-41-9, HNN, approximately 30% naphthenic) was tested by inhalation in an OECD 422 Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test. General study procedures and the result of the systemic repeated dose section are presented here. Details of the reproductive/developmental segment are found in Section 7.1.5. Concentrations of HNN were generated by flash evaporation of the test material. Groups of male and female Sprague Dawley rats [12/sex/group] were exposed to 0, 100, 500 or 3000ppm (0, 455, 2275, or 13650mg/m^3) for 30 [males] and 31 [female] days. Satellite groups of 12 young, nulliparous, nonpregnant female rats were exposed to 0, 100, 500, or 3000 ppm during a premating period of approximately 2 weeks, a cohabitation period of approximately 2 weeks, and a gestation period of approximately 3 weeks. The animals were not exposed after gestation day 19, or during the approximately 4-day lactation period. Females without evidence of mating continued to be exposed for 26 days after the end of the cohabitation period. Body weights, clinical signs, and food consumption were recorded throughout the study. After approximately 30 days, from all male and all subchronic female rats and on lactation day 4 from satellite females, blood samples were collected for haematology and clinical chemistry measurements. An abbreviated neurobehavioral evaluation was conducted on all males, subchronic females, and satellite females prior to test substance administration in order to obtain baseline measurements, and again following approximately 4 weeks of test substance administration for males and subchronic females and on lactation day 4 for satellite females. Neurobehavioral evaluation consisted of motor activity and a modified Functional Observational Battery [FOB] of open field (approach and touch response, auditory response and tail pinch), papillary response, and fore and hind limb grip strength. Males and subchronic females were sacrificed after approximately 30 days of exposure, organs (liver, kidneys, lungs, adrenal glands, thymus, brain, spleen, heart, testes with epididymides, prostate, ovaries with oviducts, and uterus with cervix) were weighed, and 36 selected tissues were evaluated microscopically. On postpartum day 4, lactating females and offspring were sacrificed, organs (liver, kidneys, lungs, ovaries with oviducts and uterus with cervix) were weighed, and reproductive organs were evaluated microscopically. Offspring were evaluated for external abnormalities.

Mortality did not occur at any exposure concentration. Test substance-related increases in the incidence of stained and wet fur in males, subchronic females, and satellite females were observed in the 3000ppm group; however, they did not adversely impact the health of the animals. Adverse, test substance-related, decreases in body weight, weight gain, and food efficiency occurred in 3000ppm subchronic females. Slightly decreased body weight and/or weight gain occurred in 3000ppm males and satellite females; however, the magnitude of the effects was not statistically significant. There were no adverse or test substance related effects on neurobehavioral parameters, haematology or clinical chemistry parameters. Liver weight parameters were increased in 3000ppm males and subchronic females, which correlated with hepatocellular hypertrophy. Kidney weight parameters were increased in 500ppm and above males and in 3000ppm subchronic females. In males, the increased absolute/relative kidney weights correlated with hyaline droplet accumulation observed in 100ppm and above males, indicative of alpha 2-microglobulin mediated nephropathy also identified as light hydrocarbon nephropathy, a species and sex specific syndrome not relevant to humans (US EPA, 1991). In 3000ppm subchronic females, the increased kidney weight parameters were not associated with any functional or microscopic change, and therefore were considered secondary to non-adverse enzyme induction. Minimal hypertrophy of thyroid follicular epithelium occurred in 3000ppm males and subchronic females, possibly secondary to liver enzyme induction The systemic toxicity LOAEL exclusive of kidney effects = 3000ppm (13650mg/m^3) based on decreased body weight, weight gain and decreased food efficiency in females and hypertrophy of thyroid follicular epithelium in 3000ppm animals of both sexes. The systemic NOAEL excluding male kidney effects = 500ppm (2275mg/m^3). The Neurobehavioral NOEL = 3000ppm (13650mg/m^3). (API, 2008a)

Full range catalytic reformed naphtha (CAS # 68955-35-1, FR-CRN, approximately 63% aromatic) was tested as a 30-40% vaporized sample in Sprague Dawley rats [15 rats/sex/group] at nominal concentrations of 0, 96, 464, 1894ppm (0, 410, 1970 and 8050 mg/m^3), 6 hours/day, 5 days/week. Two extra groups of 15 rats/sex served as sham and untreated controls. Water was available ad lib, but food was withheld during the exposure periods. Clinical observations were made regularly and body weights were recorded weekly. At the end of the 13 weeks exposure, blood samples were taken for hematological and clinical chemistry measurements. The rats were then sacrificed and necropsied. Organs were weighed and a wide range of tissues fixed for subsequent histology and microscopic examination. The wet weight of the right middle lung lobe was also weighed. The lobes were then dried and their dry weights determined. The cauda epididymis of the control and high dose male rats was used to determine the morphology and number of sperm and the left testis was used to determine the number of testicular spermatids. The following tissues from the high dose animals were examined histologically: adrenals, bone and marrow (sternum), pancreas (head), brain (three sections), submaxillary salivary gland, eye, optic nerve, spleen, heart, stomach (squamous and glandular), colon, testes or ovaries, duodenum, kidneys, thymus, thyroid, liver, tracheobronchial lymph nodes, lung (left lobe), nasal turbinates (four sections), thigh muscle, urinary bladder, sciatic nerve, and any gross lesions. In addition, tracheobronchial lymph nodes and any gross lesions from untreated control animals were also evaluated.

There were no treatment-related clinical signs during the study. No effects on serum chemistry values or parameters of the male reproductive system at terminal sacrifice were reported. Body weights of males exposed at the mid and high dose were higher than the controls throughout the study and the differences were statistically significant in the high dose group from week 10 onwards. WBC count was significantly lower in sham treated controls and all three treated groups in both sexes compared to untreated controls. Additionally the WBC count was decreased by approximately 24% in the high dose females when compared to the sham controls. No other parameters were affected. The only organ weights affected were the liver and kidney. In the male high dose group, mean kidney weight was approximately 13% greater than the sham treated animals (but not the untreated controls), and the liver weight was approximately 14% greater. No treatment-related gross lesions were observed at necropsy and no treatment-related abnormalities were noted during microscopic examination. The results of this study are consistent with the study performed with the light catalytic reformed naphtha distillate fraction.

The LOAEL = 1894ppm (8050mg/m^3) based on increased liver and kidney weights in males, decreased WBC in females. NOAEL = 464ppm (1970mg/m^3). (Dalbey and Feuston, 1996).

Light catalytic reformed naphtha (CAS #64741-63-5, LCRN, 33% aromatic) was tested as a light end distillate in Sprague Dawley rats [16 males/16 females/group] at concentrations of 0, 750, 2500, and 7500ppm (0, 2775, 9250 and 27750mg/m^3), 6 hours/day, 5 days/wk over 15 weeks, according to OECD guideline 413, for a total duration of at least 65 exposures. The test material (LCRN-D) was prepared to be representative of the fraction of light catalytic reformed naphtha to which man might be exposed during normal handling and blending. The maximum exposure level was 75% of the lower explosive limit for LCRN distillate. Extra groups of 16 rats of each sex exposed to the high dose level and a recovery control group were maintained untreated for 28 days following cessation of the 15 weeks exposure. Neurobehavioral evaluations of motor activity and functional activity [FOB] were performed pretest and during weeks 5, 9, 14 and 19 for recovery groups. Animals were not exposed to LCRN-D during these tests. Ophthalmoscopic evaluations were performed pretest and just prior to the scheduled sacrifices at 14 weeks and 19 weeks (recovery groups). Body weights and food consumption was measured throughout the study. Blood samples were taken from 10 fasted rats/sex/group at 14 and 18 weeks for hematological and clinical chemical measurements. At termination (after 13 weeks exposure for the main study and after 19 weeks for the recovery animals) all animals were killed and subjected to a complete macroscopic examination. Ten rats/sex/group were selected for non-neuropathologic examination and 6 rats/sex/group for neuropathologic examination. The following organs were weighed from the non-neuropathologic animals: adrenals, brain, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes (with epididymis), thymus and uterus. Brain lengths and widths were measured for each rat. Thirty-nine tissues removed from the control and high dose animals, fixed, stained with hematoxylin eosin and examined histopathologically. Additionally, kidneys from selected animals were stained with Mallory-Heidenhain and examined. Tissues were collected from the nervous system (central and peripheral) of all animals and nervous system tissues were selected randomly from 6 rats per sex/group in the high dose and controls at the end of 15 weeks for microscopic examination. Specific brain regions examined were forebrain, cerebral cortex, hippocampus, basal ganglia, midbrain cerebellum and pons and medulla.

Neurobehavioral studies included motor activity, monitored as the number of beam breaks in an activity box, at pretest, and during weeks 5, 9, 14, and at the end of the 4-week recovery period. The Functional Operational Battery [FOB] was comprised of home cage evaluations, handling and open field behaviors and reflex assessment. Animals were also evaluated for fore limb and hind limb grip strength, landing foot splay and air righting ability.

There were no mortalities during the study and there were no treatment-related signs of toxicity. The ophthalmic examinations did not reveal any treatment-related effects. Mean body weights, body weight gains and food consumption were unaffected by treatment. After 13 weeks exposure there was a significant decrease in total WBC count (36%) and lymphocyte counts in the high dose males and a slight decrease in neutrophil counts for the mid dose males. A trend towards decreased WBC (2.1%) and lymphocyte counts was also seen in the mid dose males and high dose females. After the 4 week recovery period, leukocyte values were comparable to control values. However, MCV was slightly decreased (2.8%) in the high dose males. It was concluded that these changes were suggestive of a reversible slight effect of the LCRN-D. Clinical chemistry parameters were unaffected by treatment. After 13 weeks exposure relative kidney weights in the high dose males were increased (15.9%) and this correlated with the occurrence of hyaline droplets in the proximal convoluted tubules. This finding has been described as alpha 2-microglobulin mediated nephropathy, also identified as light hydrocarbon-induced nephropathy and is sex and species specific and is not relevant for human health risk assessment. (US EPA, 1991) In the high dose males decreased absolute (25.7%) and relative (22%) spleen weights were also recorded. It was concluded that this was associated with the minor hematological changes that had been observed. These differences were not apparent after the recovery period and no abnormal microscopic findings were found in either the spleen or bone marrow.

No treatment-related effects were recorded in the Functional Operational Battery. In the examinations of motor activity, there were no treatment-related effects recorded during the 13-week exposure period but a slight increased activity was found in the high dose males after the 4-week recovery period. Brain length and width measurements were unaffected by treatment and there were no abnormal microscopic findings in the brain, spinal cord or peripheral nerves. The systemic toxicity LOAEL exclusive of kidney effects = 7500ppm 927750mg/m^3) based on decreased WBC and lymphocyte counts, and decreased male spleen weight. The systemic NOAEL = 2500ppm (9250mg/m^3). The Neurobehavioral NOEL = 2500ppm (9250g/m^3) due to increased motor activity in high dose recovery males. (Schreiner et al., 2000)

Supplemental studies

In the 21 day inhalation studies, male Sprague Dawley rats were exposed to a light reformate naphtha (31% aromatics) and a heavy reformate naphtha (93% aromatics) at concentrations of 0, 544, 1591, and 5522ppm (0, 2000, 5850 and 20300mg/m^3) LCRN or 0, 215, 587, and 2132ppm (1030, 2810, and 10200mg/m^3) HCRN for 15 actual exposures. These studies focused on nephropathy in male rats; other systemic effects are not described in detail in the publication. Alpha 2-microglobulin mediated nephropathy also identified as light hydrocarbon induced nephropathy in male rats is sex and species specific; it does not occur in female rats or other species, including humans. Alpha 2-microglobulin mediated nephropathy is not relevant to human hazards (US EPA, 1991). LCRN induced small concentration related increases in necrosis of renal tubules and an increase in incidence and severity of hyaline droplets, typical of alpha 2-microglobulin mediated nephropathy. The exposure levels at which they occur are not included in establishing ranges for inhalation repeat dose toxicity. Exposure to HCRN did not cause adverse effects in the kidney but lung irritation was apparent. Results indicate that naphthas high in aromatics do not induce hydrocarbon nephropathy in male rats. (Halder et al., 1984).

Dermal studies:

Dermal treatment of New Zealand White rabbits, 3 times/week for 4 weeks with **Light alkylate naphtha** (LAN, CAS #64741-66-8, approximately 1000% paraffinic) at concentrations of 200, 1000, and 2000mg/kg/day on the shaved backs of rabbits resulted in mild skin irritation at the lowest dose and moderate skin irritation at the mid and high doses in both sexes, in association with granulopoiesis of bone marrow in the highest dose group. Significantly lower body weights were observed in both sexes at 2000mg/kg; organ wt changes included increased adrenal weights in males and decreased ovary weight in females at the highest dose. Adrenal weight changes and granulopoiesis are related to skin irritation induced stress. (API, 1986e)

Light catalytic cracked naphtha (LCCN, CAS #64741-55-5, approximately 46% olefinic) was tested for 13 weeks in Sprague Dawley rats. LCCN was applied undiluted to the clipped backs of rats (15/sex/group) at concentrations of 0, 30, 125 or 3000mg/kg/day, 5 days/week for 90 days. Rats were fitted with Elizabethan collars to minimize ingestion of test material. Percutaneous absorption was assessed by applying LCCN containing radiolabeled n- octane in a non-occlusive Bronaugh cell to untreated animals and animals treated for 90 days with cold LCCN. The percent of applied dose was recovered in urine, feces and tissue over 96 hours. No systemic toxicity expressed as changes in body or organ weights, clinical observations, hematology or clinical chemistry parameters, gross pathology or histopathology with the exception of skin irritation at treated sites resulted from exposure to LCCN. Sperm morphology in treated rats was comparable to controls. Skin irritation, erythema and edema at treated sites and histopathologic correlates of hyperplasia, inflammation and ulceration in all groups in a dose related pattern were the only effects reported. Approximately 1% radiolabeled LCCN penetrated the skin over 96 hours. Bioavailability was similar for untreated rats and those pre-treated for 90 days with LCCN. (Mobil 1988a)

Full range catalytic reformed naphtha (FR-CRN CAS # 68955-35-1, approximately 63% aromatic) was tested in a 28-day dermal study. FR-CRN was applied to the shaved backs of New Zealand White rabbits, 3 times a week for 4 weeks at doses of 200, 1000 and 2000mg/kg/day. Three males (2 high dose, 1 mid dose) died. The kidneys of the two high dose animals contained slight to moderate tubular degeneration. FR-CRN was a moderate-severe skin irritant. Inhibition of body weight and weight loss occurred at 2000mg/kg. Some differences observed between the control and treated groups for a few hematological and clinical chemistry parameters fell within the normal range for the laboratory, and were not regarded as treatment related. Histopathologic examination revealed slight-moderate proliferative and inflammatory changes in skin at the highest dose concurrent with granulopoiesis of bone marrow, attributed to stress and other factors associated with skin irritation. No other significant findings were reported. LOAEL = 2000mg/kg/day based on decreased body weight and weight loss, irritation; NOAEL excluding I death = 1000mg/kg/day. (API, 1986f)

Six naphtha streams were tested in a series of 28 day dermal irritation pilot studies with Sprague Dawley rats. The test materials were: Hydrodesulfurized heavy naphtha (CAS #64742-82-1; UBTL, 1992a); Heavy reformate naphtha (CAS # 64741-68-0; UBTL 1992b), Sweetened naphtha (CAS #64741-87-3; UBTL 1994a), Full range coker naphtha (Merox Feed

F-250, CAS # 68513-02-0; UBTL, 1994b) and two naphtha streams without CAS numbers – Light naphtha Isohexane Rich (UBTL, 1992c) and Light naphtha N-hexane rich (UBTL, 1992d). The test materials were applied undiluted to the clipped backs of Sprague Dawley rats (10/sex/group) at concentrations of 0, 0.05, 0.25 and 1.0ml/kg followed by 6 hours occlusion, 5 days/week for 4 weeks. Slight to moderate skin irritation in a concentration-related progression from low to high dose was seen in all studies at clinical observation and confirmed with histopathological evaluation. No other significant adverse effects in clinical signs, body weight, organ weights, hematology or clinical chemistry parameters or histopathology were observed. Although animals treated with full range coker naphtha demonstrated changes in hematology (% neutrophils, lymphocytes), clinical chemistry (globulin levels and albumin/ globulin ratio) and myeloid hyperplasia in bone marrow and lymph node hyperplasia, these effects were considered secondary to the severity of dermal irritation and not a direct effect of the test material. The NOEL level for systemic toxicity for all streams was 1.0ml/kg, the highest concentration tested in each study.

Gasoline (supplemental chemical)

Thirteen week inhalation toxicity studies were performed with wholly vaporized leaded and unleaded gasoline at target concentrations of 0, 100 and 400ppm, or 0, 400, 1500ppm (0, 1570, 6350 mg/m^3) [actual concentrations: 0, 384, 1552ppm; (0, 1507, 6570 mg/m^3)] respectively, in Sprague Dawley rats and squirrel monkeys (Kuna and Ulrich, 1984). Only the results of the unleaded gasoline studies are relevant to the HPV program. Twenty rats and 4 monkeys of each sex were housed in 1m^3 glass and stainless steel exposure chambers 24 hours a day and were only removed for cleaning purposes. Blood was taken from 10 rats of each sex at the end of the study from the highest dose groups only for hematological evaluation. Blood was taken from all monkeys in the highest dose group at 1.5, and 3 months. Urine samples were analyzed for all animals at 1.5 and 3 months for levels of protein, glucose, ketones, bilirubin, and blood. CNS measurements and pulmonary function tests were performed on monkey and are summarized in the robust summary. All animals that died or were sacrificed at termination of the study were subjected to a gross necropsy. Organ weights were recorded and lungs, kidneys, spleen, heart, brain and bone marrow from the control and high dose groups were evaluated for histopathology. All male and female animals from the control and high exposure groups were also evaluated for the presence of IgG in the renal glomerulus and lungs.

Alpha 2-microglobulin mediated nephropathy also identified as light hydrocarbon induced nephropathy was observed in kidneys of all examined male rats exposed to leaded or unleaded gasoline but not in kidneys of squirrel monkeys. In rats, slight increases in thrombocyte and reticulocyte counts and liver weights of high dose males occurred with exposure to both gasolines, with increases in tissue and urinary lead levels for animals given leaded gasoline. Monkeys showed a small increase in respiratory rate with exposure to the highest concentration of unleaded gasoline, 6570mg/m^3.

Unleaded gasoline LOAEL, excluding alpha 2-microglobulin mediated nephropathy = 1552ppm (6570mg/m^3) based on increases in thrombocytes and reticulocytes, increased liver weights in males; NOAEL = 384ppm (1507mg/m^3)

Baseline Gasoline Vapor Condensate [BGVC], a 20% light fraction of a whole unleaded gasoline sample was evaluated in a 13- week inhalation study according to OPPTS 870.3465. This test material was a representative evaporative emission tested under the US EPA 211(b)

Fuels and Fuel Additives Health Effects Testing Program (1994b, 1998). BGVC was administered to Sprague Dawley rats (10/sex/group) at target concentrations of 0, 2000, 10000, and 20000mg/m^3 (actual concentrations 0, 2050, 10,153 and 20,324 mg/m^3) 6hr/day, 5 days/week for 13 weeks. Additional groups of control and high dose rats (10/sex/group) were also exposed and retained untreated for an additional 4- week recovery period (API, BGVC, 2005b). Clinical signs, body weights and body weight changes, and food consumption were recorded throughout the study. Opthalmoscopic evaluations were performed pretest and at exposure termination. Hematology, coagulation and clinical chemistry parameters were measured at week 4 and week 13. Neurobehavior evaluation of motor activity and functional activity [FOB] were performed on 10 rats/sex/group pretest and during weeks 3, 7, and 12 of exposure according to OPPTS 870.6200. After 13 weeks exposure rats were sacrificed except for recovery animals sacrificed 4 weeks later. Fourteen selected organs were weighed. Histopathologic examination was performed on 31 tissues from rats in the control and high dose groups and on kidneys from rats in all groups. Five rats/sex/group were perfused for neuropathology and sections of brain, eye, spinal cord, peripheral nerves and ganglia were examined microscopically. Satellite groups of animals were exposed to BGVC with the subchronic rats for immunotoxicology, genetic toxicity and glial fibrillary acidic protein (GFAP) analyses. The genetic toxicology studies are presented in Section 7.1.4 of this document. The immunotoxicology and GFAP report details are provided in robust summaries, and are not considered further here other than to state that BGVC was not toxic in these two satellite studies.

Test animals were generally unremarkable in exposure chambers and during non-exposure periods except for a slight increase in red nasal discharge seen in 20324mg/m^3 animals during 13 weeks of exposure but not during recovery. No adverse effects were induced by BGVC on ophthalmology, body weights, feed consumption or blood chemistry parameters. No toxicologically significant changes were observed in organ weights although male absolute and relative kidney weights were slightly elevated at the mid and high dose levels. Gross abnormalities were not seen at terminal sacrifice. Dose related microscopic findings included eosinophilic material in the nasolacrimal ducts in high dose rats consistent with reported red nasal discharge and renal histopathologic changes in kidneys of all treated male rats. These renal changes were consistent with alpha 2-microglobulin mediated nephropathy, a species and sex-specific change not considered relevant to human health (US EPA, 1991). Kidneys of recovery 20324mg/m^3 male rats had nearly complete resolution of these changes. BGVC did not cause adverse neurobehavioral or neuropathologic effects. The systemic LOAEL [excluding male kidney effects] = 20324mg/m^3 and NOAEL = 10153mg/m^3. NOAEL for neurotoxicology = 20324mg/m^3. The NOAEL/LOAEL values for this material are similar to those reported for refinery streams in the 4 chemical classes.

Conclusion: Results of repeated dose studies have demonstrated fairly similar profiles of toxicity across the 4 chemical classes. Inhalation studies which reflect the most relevant route of human exposure were performed with distillates prepared from the range of likely human exposure or as vapor generated directly from the liquid blending stream. Because of limitations imposed by the lower explosive limits, vapor studies were performed at concentrations much lower than those possible with distillates. Exposure whether to distillate or vapor fractions could result in alpha 2-microglobulin mediated nephropathy in kidneys of male rats, also identified as light hydrocarbon induced nephropathy, a species and sex specific syndrome not relevant to human health (US EPA, 1991). Other systemic toxicity was minimal and in general, included increased weight of the liver in most studies and of spleen in one aromatic sample, and some decreases in body weight or small changes in clinical pathology parameters. One 40% vapor olefinic sample induced a decrease in sperm number per gram of epididymis, an effect not supported by other male parameters in this study or other studies. In studies where neurotoxicity was evaluated none of the streams induced significant neurobehavioral or neuropathologic effects.

Therefore, gasoline blending streams have a low inhalation repeat dose hazard potential. The inhalation NOAELs and LOAELs were similar between the different hydrocarbon classes of streams (PONA) and the formulated product, gasoline in rats. Since there were no appreciable differences between paraffinic, olefinic, naphthenic, and aromatic streams, a range of values derived from all of the repeated dose inhalation studies will be used to read across to all untested category members. These read-across values are:

LOAEL: 6572 mg/m^3 – 27,800mg/m^3 (1864 – 7885ppm[a])
NOAEL: 1507mg/m^3 – 10,153mg/m^3 (427 – 2880ppm[a])

[[a] - upper range of NOAEL based on 211(b) BGVC; Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]

The majority of streams tested induced alpha 2-microglobulin mediated nephropathy in male rats. It has been demonstrated that this syndrome is specific for male rats, and does not occur in female rats or other species, including humans. Since this species and sex specific syndrome is not relevant to humans (US EPA, 1991), alpha 2-microglobulin mediated nephropathy has been excluded for developing LOAELs and NOAELs in all rat studies.

When applied dermally, gasoline blending streams induced skin irritation with the only systemic effects related to skin damage and accompanying stress.

7.1.3. Genetic Toxicity *In Vitro*

Light alkylate naphtha (LAN, approx. 100% paraffinic) diluted in acetone, has been tested in a mouse lymphoma (L5178Y TK+/-) forward mutation assay. For the mutation assay the lymphoma cells were exposed for 4 hours to test material at concentrations ranging from 0.005 to 0.08 µl/ml without activation and 0.00004 to 0.8 µl/ml with Aroclor-induced rat liver S-9 activation. After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; trifluorothymidine (TFT) was used as the restrictive agent. The non-activated cultures treated with 0.005 to 0.04µl/ml LAN were cloned, resulting in a range of growth of 6 to 97%. The activated cultures treated with 0.0002 to 0.75 µl/ml LAN were cloned, resulting in a range of growth from 24 to 109%. Plates were prepared from TFT-restricted and from the Viable cultures (VC) and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. Several trials were performed to verify the absence of genetic toxicity in this assay system. Light alkylate naphtha did not induce mutagenicity with or without metabolic activation from rat liver homogenate. (API, 1985b)

Three samples of light catalytic cracked naphtha (LCCN, approx. 46% olefinic) have been tested in a mouse lymphoma (L5178Y TK+/-) forward mutation assay. The results for API 83-20 are described here (API, 1987e). A cytotoxicity study carried out prior to the mutagenicity assay established that LCCN was highly toxic at 500nl/ml without activation and lethal at the same concentration in the presence of metabolic activation. For the initial mutation assay the mouse lymphoma cells were exposed for 4 hours to LCCN at treatments from 50 to 800 nl/ml LCCN without activation and with treatments from 25 to 500 nl/ml LCCN with Aroclor-induced rat liver S-9 activation. After exposure to LCCN, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection. Plates containing colonies of selected cells were incubated for 10 to 14 days after which they were scored for total number of colonies per plate. A mutation frequency was then determined. Due to a wide range of toxicity in the first assay with and without metabolic activation, a second assay was performed over a narrower dose range of 50 to 150nl LCCN without S9 activation and 200 to 300nl LCCN with activation. LCCN sample API 83-20 was not mutagenic with or without metabolic activation. Of the two other LCCN samples tested, one API 81-03 (API, 1985c) was not mutagenic and one API 81-04 (API, 1986g) was not mutagenic without metabolic activation but gave equivocal results with metabolic activation. Equivocal results are defined as a situation in which one or more doses exhibit a 2-fold mutant frequency greater than background level but there is no dose response. Overall LCCN is not considered mutagenic in this mammalian cell assay.

An *in vitro* sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells with and without metabolic activation [a non-SIDs endpoint] was performed with LCCN. CHO cells were seeded in duplicate for each treatment condition and were incubated at 37°C in a humidified atmosphere for 16 to 24 hours. Treatment was carried out by re-feeding two complete sets of flasks with complete medium for the non activation study or with Aroclor-induced rat liver S-9 reaction mixture for the activated study to which was added 50 µl of dosing solution of test control or article in solvent or solvent alone. CHO cells were exposed to solvent alone and to nine concentrations of LCCN ranging from 1 to 0.0001µl/ml in the absence and presence of an S-9 reaction mixture. Based on the growth inhibition and cell cycle delay, dose levels of 0.3, 0.2, 0.1 and 0.05 µl/ml LCCN were selected for use in the assay without metabolic activation and at concentrations of 0.2, 0.1, 0.05 and 0.03 µl/ml LCCN in the assay with metabolic activation. A harvest time of 30 hours after treatment initiation was selected to assure collection of enough analyzable second division metaphases at the high dose. In the non-activation study the cells were exposed for 28 hours. Two hours after exposure 0.01 mM BrdU was added to the treatment medium. At the end of the treatment period, the treatment medium was removed, the cells were rinsed and were then exposed to colcemid (0.1µg/ml) for a further 2 hours. In the activation study exposure was for 2 hours. After the exposure period, the treatment medium was removed; the cells were washed with PBS, re-fed with medium containing BrdU and then incubated for a further 28 hours. Colcemid was added at a final concentration of 0.1µg/ml for the last 2 hours of incubation. For activated and non-activated assays, metaphase cells were harvested 2 hours after addition of colcemid. Cells were collected and fixed and stored until slides were prepared. Slides were coded and scored without regard to treatment group. Only cells with 20 ≤ 2 centromeres were selected for evaluation of SCEs. A total of 4 doses were scored including the highest test article dose where sufficient second-division metaphase cells were available. SCEs were scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. The percentage of cells in first (M1), second (M2) or third division (M3) metaphase was also recorded for a total of 100 metaphase cells scored. Triethylenemelamine (TEM) was used as positive control at a concentration of 0.025 µg/ml. in the non-activated assay. In the activated assay cyclophosphamide (CP) was used at a concentration of 2.5µg/ml. API 81-03 did not induce an increase in sister chromatid exchanges in CHO cells when tested in the absence of metabolic activation. However the test material did induce a small but statistically significant increase in SCEs at two intermediate dose levels in the presence of metabolic activation, a result that was concluded to be equivocal. (API 1988a)

Sweetened naphtha (SN, CAS # 64741-87-3; approx 21% naphthenic) diluted in ethanol, has been tested in a mouse lymphoma (L5178Y TK+/-) forward mutation assay. For the mutation assay the mouse lymphoma cells were exposed for 4 hours to SN at concentrations ranging from 0.005 to 0.08 µl/ml without activation and 0.00004 to 0.8 µl/ml with Aroclor-induced rat liver S-9 activation. After exposure to SN, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection; trifluorothymidine (TFT) was used as the restrictive agent. The non-activated cultures treated with 0.005 to 0.04µl/ml SN and activated cultures treated with 0.0002 to 0.75 µl/ml SN were cloned and produced a range of growth from 24 to 109%. Plates were prepared from TFT-restricted and from the Viable cultures (VC) and after 10 to 12 days incubation these plates were scored for total number of colonies per plate. Five trials were performed due to wide ranges of toxicity and sporadic increases in mutant frequencies in order to verify the absence of genetic toxicity in this assay system. Overall Sweetened naphtha did not induce mutagenicity with or without metabolic activation from rat liver homogenate. (API, 1985d)

Three samples of catalytic reformed naphthas have been tested in a mouse lymphoma (L5178Y TK+/-) forward mutation assay. The results for API 83-05, a full range catalytic reformed naphtha (FRCRN, 63% aromatics) are described here. Other samples were a light catalytic reformed naphtha (LCRN, 42% aromatics) and a heavy catalytic reformed naphtha (HCRN, 90% aromatics). A cytotoxicity study carried out prior to the mutagenicity assay established that FRCRN was lethal to all cultures at 500µl/ml and highly toxic at 250 µl/ml. For the mutation assay the mouse lymphoma cells were exposed for 4 hours to FRCRN dissolved in acetone at treatments from 6.25 to 500 µl/ml without activation and at treatments from 3.13 to 400µl/ml with Aroclor-induced rat liver S-9 activation. After exposure to FRCRN material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection. Plates containing colonies of selected cells were incubated for 10 to 14 days after which they were scored for total number of colonies per plate. Cultures selected for cloning at doses of 6.25 to 100 µl/ml FRCRN without S-9 had growth rates of 30-97% and cultures selected for cloning with S-9 had growth rates of 4.6 to 67.9%. A mutation frequency was then determined. Full range catalytic reformed naptha was not mutagenic without metabolic activation but did induce dose related increases in mutant frequency with metabolic activation. (API, 1985e)

The light catalytic reformed naphtha containing 42% aromatics was not mutagenic with or without metabolic activation (API, 1985f). A heavy catalytic reformed naphtha containing 90% aromatics produced negative/equivocal results without metabolic activation and equivocal/positive results with metabolic activation in two separate laboratories (API, 1985g). Interestingly, this heavy catalytic reformed naphtha did not induce dermal tumors in a 2 year mouse skin painting study (see Section 7.2.1.2 Dermal Carcinogenesis). These results suggest that the aromatic content of these streams may influence the degree of mutagenic activity induced in this *in vitro* mammalian cell test system. However, the absence of dermal tumors from exposure to the heavy catalytic reformed naphtha in a two-year study indicates that the toxicological significance of the results of mouse lymphoma studies of CRN are unclear

Gasoline (Supporting chemical)

Unleaded gasoline was tested in the Ames Microbial mutation assay in *Salmonella typhimurium* and *Saccharomyces cerevisiae* with and without metabolic activation from an Aroclor-induced rat liver homogenate mixture. Salmonella strains TA100, TA1535, TA1537, TA1538, TA98 and yeast strain D4 were employed. Based on preliminary cytotoxicity assays, concentrations of gasoline in dimethylsulfoxide were administered to all 5 Salmonella tester strains at doses of 0.375, 0.75, 1.5 and 3.0% and to yeast at doses of 0.625, 1.25, 2.5, and 5.0%. For plate assays, test material was added to cells in broth. The contents of the test tubes of broth plus test material were poured over selective agar plates. Plates were incubated at 37^0C for 48 hours, then removed from the incubator and revertant cells were counted. In the suspension tests, bacteria and yeast cultures were grown in complete broth. The cells were removed, washed and exposed to the test material. For the yeast cells exposure to gasoline was for 4 hours and bacterial cell exposure was for 1 hour. Aliquots of the cells were plated onto the appropriate complete media. After suitable incubation periods, the number of revertant colonies was counted.

In the plate test, there was no increase in revertant colonies caused by exposure to gasoline at any concentration. The results in this assay were negative both with and without metabolic activation. In the suspension test without activation, Slight increases were observed at the high dose levels with TA100, TA1537 and TA1538. However the responses were not adequate to be considered positive. The increases with TA98 could not be reproduced in a repeat trial. In the suspension test with activation, scattered increases were found at one or more dose levels but were not reproducible in a repeat trial. Therefore, gasoline was not a mutagen in this test system. (API, 1977a)

Gasoline diluted in acetone, has been tested in a mouse lymphoma (L5178Y TK+/-) forward mutation assay. For the mutation assay the lymphoma cells were exposed for 5 hours to test material at concentrations ranging from 0.065 to 1.04 µl/ml with and without metabolic activation from Aroclor-induced rat liver S-9 homogenate mixture. After exposure to the test material, the cells were allowed to recover for 3 days and then cultures were selected for cloning and mutant selection. Surviving cell populations were determined by plating diluted aliquots in non-selective growth medium. A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and negative control values. Positive control compounds were ethyl methane sulfonate (EMS) for non-activated cultures and dimethylnitrosamine (DMN) for metabolically activated cultures.

Little toxicity was observed with the test material. All results for gasoline from the non-activation assay were negative. The results from the activation assay were also considered to be negative. There was an increase in the number of mutants at the 0.52 µl/ml concentration but this appeared to result from a slight increase in the number of viable clones. There was no trend indicating a dose-related response and therefore, the increases were not believed to be compound related. Gasoline was not mutagenic in this mammalian cell system. (API, 1977a)

Gasoline tested in an Unscheduled DNA synthesis assay in rat hepatocytes with and without metabolic activation, did not cause DNA damage requiring repair in this assay system. (API 1988b) This study is not part of the SIDS data set and is not described in Robust Summaries.

Conclusion: Results from representative samples from each of the PONA categories indicate that most gasoline blending streams are not mutagenic in mammalian cells except for those substances with fairly high aromatic content where equivocal or in one case positive activity was seen with metabolic activation. Gasoline tested in both bacterial and mammalian cell assays did not induce mutation in either test system. The read-across conclusion is that all streams in this category are negative with and without metabolic activation with the exception of streams with aromatic content greater than 60% that can be classified as negative/equivocal without metabolic activation and equivocal/positive with metabolic activation.

7.1.4. Genetic Toxicity *In Vivo*

Light alkylate naphtha (approx. 100% paraffinic) was tested in a Sprague Dawley rat chromosome aberration assay [15/sex/group] at doses of 0.3, 1.0, and 3.0g/kg in corn oil, administered intraperitoneally in a single dose. Two to four hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (1 mg/kg). Animals [5/sex/group/time] were sacrificed at 6, 24 and 48 hrs post dose. A group of 5 animals of each sex to be used as positive controls was dosed with triethylenemelamine (TEM) at a level of 0.5 mg/kg and these animals were killed at 24 hours postdose. Deaths occurred in both male [5/18] and females [4/18] in the highest dose group and a 9-10% body weight loss was observed in surviving rats of both sexes. Other signs of toxicity included piloerection, crusty eyes and noses and excess lacrimation. Bone marrow was harvested from the femurs of treated rats, processed and stained for cytogenetic examination [a minimum of 50 metaphase spreads per animal]. No chromosome aberrations, rearrangements, or cell cycle disruption were observed in any dose group (API, 1985h**).**

Samples of light catalytic cracked naphtha (approx. 46% olefinic) were tested in two rat chromosome assays [intraperitoneal and inhalation] and in an *in vivo* mouse sister chromatid exchange (SCE) assay. LCCN (API 81-04) was tested in a Sprague Dawley rat chromosome aberration assay [15/sex/group] at doses of 0.3, 1.0, and 3.0g/kg in corn oil, administered intraperitoneally in a single dose. Two to four hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (1 mg/kg). Animals [5/sex/group/time] were sacrificed at 6, 24 and 48 hrs post dose. A group of 5 animals of each sex to be used as positive controls was dosed with triethylenemelamine (TEM) at a level of 0.5 mg/kg and these animals were killed at 24 hours postdose. Bone marrow was harvested from the femurs of treated rats, processed and stained for cytogenetic examination [a minimum of 50 metaphase spreads per animal]. There was a 9% weight loss in males 48 hours after receiving 3 g/kg API 81-04 and a 2% weight loss in females at the same time and dose level. Clinical signs of toxicity in the 3g/kg group included lethargy in both sexes and increased tearing as indicated by a crusty appearance of fur around the eyes of the male animals. No chromosome aberrations, rearrangements, or cell cycle disruption were observed in any dose group (API, 1985i**).**

In a separate study in which exposure was by inhalation at 63, 297 and 2046 ppm, 6hr/day for 5 days, there was no evidence that light catalytic cracked naphtha (API 81-03) caused chromosomal aberrations in rats. (API, 1985c)

LCCN (API 81-03) was tested in a mouse [B6C3F1; 5/sex/group] sister chromatid exchange (SCE) assay [a non-SIDS endpoint] at doses of 0.2, 1.2, and 2.4 g/kg in corn oil, administered intraperitoneally in a single dose. Four hours prior to administration of test material, the mice were anesthetized with Metofane and an agar-coated 50mg BrdU pellet was implanted subcutaneously in the lower abdominal region. The positive control (cyclophosphamide) was injected ip at a dose level of 10 mg/kg. A second positive control (API 81-15, catalytic cracked clarified oil) was administered at a dose of 4 g/kg, which was administered by ip injection at a rate of 10 ml/kg. Colchicine, used to arrest dividing cells in metaphase, was administered ip at 1 mg/kg to all mice two to four hours prior to sacrifice. 24 to 26 hours after BrdU pellet implantation the mice were sacrificed. Marrow was collected from both femurs. After washing and fixing bone marrow cells slides were prepared for subsequent staining and examination. Two to five slides were prepared from each animal. A minimum of 50 second-division metaphase spreads from each animal were examined and scored for SCEs and chromosome number. The mitotic index was recorded as the percentage number of cells in mitosis based upon 500 cells counted. The percentage of first, second and third-division metaphase cells was also recorded as the number per 100 cells counted. There was a significant increase in SCEs/cell when analyzed by sex. Pairwise comparisons by sex of each treatment group with its vehicle control were significantly different. (API, 1988c)

Although the SCE assay demonstrated interaction of LCCN and DNA, it was not considered definitive for clastogenic activity since no genetic material was unbalanced or lost. SCE can be regarded as more a biomarker of exposure rather than as indicator of mutagenic effect. Negative results in two assays, which visualize actual cytogenetic damage demonstrate that LCCN is not a clastogenic material (API, 1985i,c)

Sweetened naphtha (CAS # 64741-87-3, approx. 21% naphthenic; API 81-08) was tested in an inhalation rat chromosome aberration assay. Groups of 10 male and 10 female Sprague Dawley rats were exposed (whole body) to nominal concentrations of 0, 65, 300 and 2050 ppm of test material, 6 hours/day for 5 consecutive days. A positive control group of 10 rats/sex was given a single dose (0.8 mg/kg) of triethylenemelamine (TEM) intraperitoneally 24 hours before sacrifice. For the treated and negative control groups, bone marrow was harvested 6 hours after the final exposure. For the positive control group the bone marrow was harvested 24 hours after administration of TEM. Three hours prior to sacrifice by carbon monoxide the rats were given a single intraperitoneal dose of colchicine (4mg/kg). Bone marrow was collected from the tibiae of each rat and slides prepared for cytogenetic examination. Routinely 50 metaphase chromosome spreads were examined for each animal. The locations of cells bearing aberrations were identified. A mitotic index based on at least 500 cells counted was calculated by scoring the number of cells in mitosis per 500 cells on each slide read. Slides were scored for chromosomal aberrations.

No systemic toxicity was observed in this study. There was no evidence of a clastogenic effect of the test material and no significant increase in chromosomal aberration in the sweetened naphtha exposed animals when compared to the negative controls. (API, 1986h)

Three samples of catalytic reformed naphthas have been tested in a Sprague Dawley rat chromosome assay. The results for API 83-05, a full range catalytic reformed naphtha (FR-CRN, 63% aromatics) are described here. Other samples were light catalytic reformed naphtha (LCRN, 42% aromatics) and heavy catalytic reformed naphtha (HCRN, 90% aromatics). (API 1985j, k, l, respectively)

Sprague Dawley rats [15/sex/group] were given doses of 0.26, 0.82, and 2.42g/kg FR-CRN in corn oil, administered intraperitoneally in a single dose. Two to four hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (1 mg/kg). Animals

[5/sex/group/time] were sacrificed at 6, 24 and 48 hrs post dose. A group of 5 animals of each sex to be used as positive controls was dosed with triethylenemelamine (TEM) at a level of 0.5 mg/kg and these animals were killed at 24 hours postdose. One male each died in the 2.42g/kg and the 0.82g/kg groups, immediately after dosing. Toxic signs included lethargy and a moribund appearance at the high dose and slow uncoordinated movement in the mid dose group. Bone marrow was harvested from the femurs of treated rats, processed and stained for cytogenetic examination [a minimum of 50 metaphase spreads per animal].

No chromosome aberrations, rearrangements, or cell cycle disruption were observed in any dose group**.** Similar cytogenetic assays have been reported for two other aromatic naphtha samples, light catalytic reformed naphtha (LCRN, 42% aromatics) and heavy catalytic reformed naphtha (HCRN, 90% aromatics) and both have given negative results. Samples in the high aromatics class do not induce chromosome damage in laboratory animals.

Gasoline (Supplemental chemical)

Unleaded Gasoline has been tested for induction of chromosome aberrations in rat bone marrow cells, and for transmittable genetic effects in the mouse dominant lethal assay. In the rat chromosome assay, animals were given a single intraperitoneal dose of 18.5, 62.0, and 185mg/rat (0.024, 0.08 and 0.24ml/rat) diluted in acetone, 15 rats/group, (API, 1977a) or one dose each day for 5 days at concentrations of 7.7, 23.1, and 77mg/rat (0.01, 0.03, and 0.10ml/rat/day), 18 rats total [5/treated group, 3 for acetone control] (API, 1977b). Doses were not calculated in relation to body weight. Two hours prior to sacrifice the rats were given a single intraperitoneal dose of colchicine (1mg/kg). Animals [5/sex/group/time in the acute group] were sacrificed at 6, 24 and 48 hrs post dose. For the repeat treatment study, all rats were killed 6 hours after the last dose. A group of 5 animals of each sex to be used as positive controls was dosed with triethylenemelamine (TEM) at a level of 0.3 mg/kg and these animals were killed at 24 hours postdose. Bone marrow was harvested from the femurs of treated rats, processed and stained for cytogenetic examination [a minimum of 50 metaphase spreads per animal]. The results of both studies were negative. Gasoline did not induce chromosome aberrations or disruption of cell cycle kinetics in either dosage regime.

In the dominant lethal assay, gasoline was administered by inhalation to male mice (10/group) at concentrations of 400 and 1600ppm (1493 and 5970mg/m^3), 6hr/day, 5 days/wk for 8 weeks over the entire mouse spermatogenic cycle (API 1980b). On the final day of exposure a positive control group of 10 male mice were given 0.3mg/kg triethylenemelamine (TEM) dissolved in 0.9% saline as a single intraperitoneal dose. Chamber concentrations were monitored at least hourly during the exposure periods. After 2 days rest following termination of exposures each male was caged with 2 unexposed virgin female mice. At the end of 5 days, the females were removed. This weekly mating sequence was continued for 2 weeks. Each pair of mated females was transferred to a fresh cage and after 14 days after the midweek of being caged with the male was sacrificed (approximately 2/3 through pregnancy). The uterine contents of the females were examined and scored for the numbers of dead and living implants and total implants. Gasoline exposure of male mice did not cause any significant reduction in the fertility index, did not affect the number of total implants or number of dead implants per pregnant female**.**

Baseline Gasoline vapor condensate [BGVC], a 20 % light fraction of a whole unleaded gasoline was tested in the rat micronucleus assay according to US EPA OPPTS 870.5395 as a satellite study to the 13 week inhalation study described in Section 7.1.2 Repeated Dose Toxicity. Sprague Dawley rats (5/sex/group) were exposed by whole body inhalation to target concentrations of 0, 2000, 10000, 20000mg/m^3 (actual concentrations 0, 2050, 10,153 and 20,324 mg/m^3) BGVC for 4 weeks, 6hr/day, 5 days/week. A separate positive control group was treated with 40mg/kg cyclophosphamide by intraperitoneal injection 24 hours prior to sacrifice. Rats were killed 24 hours after the 20th exposure and bone marrow from both femurs of each rat was prepared as smears on microscope slides. Slides were stained by the modified Feulgen method. One smear from each rat was examined for the presence of micronuclei in 2000 immature erythrocytes and cytotoxicity was determined by the ratio of immature erythrocytes in at least 1000 erythrocytes. The incidence of micronucleated mature erythrocytes was also recorded. BGVC did not cause statistically significant increases in micronucleated immature erythrocytes or micronucleated mature erythrocytes at any dose level. There was no cytotoxicity or a decrease in the proportion of immature erythrocytes observed. Baseline Gasoline Vapor Condensate did not induce cytogenetic damage in this test system. NOAEL = 20324mg/m^3. (API, BGVC, 2005b)

BGVC was also tested with a separate satellite group for the induction of sister chromatid exchange [SCE- a non-SIDs endpoint], using an in vivo/in vitro protocol. Sprague Dawley rats (5/sex/group) were exposed by whole body inhalation to target concentrations of 0, 2000, 10000, 20000mg/m^3 (actual concentrations 0, 2050, 10,153 and 20,324 mg/m^3) BGVC for 4 weeks, 6hr/day, 5 days/week. A separate positive control group was treated with 5mg/kg cyclophosphamde by intraperitoneal injection 24 hours prior to sacrifice. Rats were killed 24 hours after the 20th exposure. Blood (2-4ml) was collected from the abdominal aorta, cultured within 24 hours and incubated at 37^{0}C for 21 hours. Cells were then exposed to 5µg/ml bromodeoxyuridine. After 68 hours from culture initiation, 0.2µg/ml colcimid was added to each culture flask to arrest cell division and incubation continued for 4 hours. At 72 hours total elapsed culture time, cells were collected, washed and fixed. Slides were prepared for microscopic evaluation. A minimum of 25 second-division metaphases per animal was scored for SCE. At least 100 consecutive metaphases per animal were scored for the number of cells in 1st, 2nd, and 3rd division metaphases as an indicator of toxicity (cell cycle delay) and 1000 cells were scored for mitotic index per rat. Statistically significantly increased SCE frequency was observed at all 3 dose levels in females and at the 10153 and 20324mg/m^3 levels for males. Increases in average generation time were also observed but no appreciable differences in mitotic indices were seen for any test group compared to controls. Although the SCE assay demonstrated interaction of BGVC and DNA, it was not considered definitive for clastogenic activity since no genetic material was unbalanced or lost, but rather a biomarker of exposure. Negative results in a parallel micronucleus assay, which visualizes actual cytogenetic damage demonstrate that BGVC is not a clastogenic material (API, BGVC, 2005c)

Conclusion: All PONA streams are negative for induction of chromosome aberrations in rats. One high olefinic sample induced sister chromatid exchanges in mice. Although the SCE assay demonstrated interaction of the LCCN sample and DNA, it was not considered definitive for clastogenic activity since no genetic material was unbalanced or lost, but rather a biomarker of exposure. Negative results in two assays in rats, which monitor actual cytogenetic damage demonstrated that LCCN was not a clastogenic material. Gasoline did not induce cytogenetic damage in rats or adverse effects on spermatogenic cycle in mice. Although SCEs were induced in cultured peripheral blood from rats exposed to baseline gasoline vapor concentrate, the parallel micronucleus study was negative. Overall gasoline refinery blending streams are not clastogenic. The read-across conclusion for untested streams in this category is negative for in vivo genetic toxicity.

7.1.4 Reproductive and Developmental Toxicity

Light alkylate naphtha (CAS #64741-66-8, LAN, approx. 100% paraffinic) was tested in rats in an OECD 421 Inhalation Reproductive and Developmental Toxicity Screening Test as a light end distillate. The test material (LAN-D) was prepared to be representative of the fraction of light alkylate naphtha to which humans would normally be exposed during normal handling and use. It was obtained by the distillation of LAN and collecting that fraction that boiled over the temperature range 78 to 145°F. Male and female Sprague Dawley rats (12/sex/group) were exposed to concentrations of 0, 137, 3425 and 6850ppm, 6 hours/day, 7 days/week for 2 weeks prior to mating. The maximum exposure level was 75% of the lower explosive limit for LAN distillate. Parental males were also exposed during mating, throughout the female gestation and post partum period and throughout the female necropsy period (8 consecutive weeks). During the mating period, females were exposed until evidence of mating was observed. If there was no evidence that mating had occurred the pairs were allowed to remain together up to a period of 2 weeks after which time the female was assumed to be pregnant. Presumed pregnant females were treated daily during gestation (GD days 0-19) until sacrificed on post-natal day 4. Parental females were killed on gestation day 25 if they had not delivered. Viability, clinical observations, body weights, feed consumption, and survival were evaluated in parental rats. At necropsy each parental animal was examined macroscopically for structural abnormalities and pathological changes with emphasis on reproductive organs. Lungs, trachea and larynx were removed in their entirety. The right middle lobe of the lung was weighed; the remaining lobes were fixed for subsequent histopathological examination. The testes and epididymides of the males were weighed and then fixed for histological examination, as were the ovaries of the females. Reproductive parameters (mating indices, pregnancy rates, male fertility indices, gestation length, number of implantation sites and corpora lutea, pre- and post-implantation loss, pups per litter, live born and stillborn pups, and incidence of dams with no viable pups) and developmental endpoints (pup physical examination, viability, weight, sex ratio, litter survival indices and mean pup survival indices) were evaluated.

No adverse reproductive or systemic effects were induced in treated male and female rats. All pregnant females had comparable delivery data and pups in all groups showed comparable birth weights, weight gain, and viability at postnatal day 4. No histopathological changes were seen at necropsy for adults or offspring, and reproductive organs of adult animals were normal histologically. NOAEL for all endpoints = 6850ppm [25000mg/m^3], the highest dose tested (Bui et al., 1998).

Light catalytic cracked naphtha (CAS #64741-55-5, LCCN, approx. 42% olefinic) was tested in rats in an OECD 421 Inhalation Reproductive and Developmental Toxicity Screening Test as a light end distillate (approx. 60% olefinic). Male and female Sprague Dawley rats (10/sex/group) were exposed to concentrations of 0, 750, 2500 and 7500pppm (2700, 9000 and 27000mg/m^3), 6 hours/day, 7 days/week for 2 weeks prior to mating. The maximum exposure level was 75% of the lower explosive limit for LCCN distillate. Parental males and females who failed to mate were exposed during mating, and 23 additional days following completion of the mating period. These animals were sacrificed shortly after the last litters were delivered reached post partum day 4. During the mating period, females were exposed until evidence of mating was observed. If there was no evidence that mating had occurred the pairs were allowed to remain together up to a period of 2 weeks. Pregnant females were treated daily during gestation (GD days 0-19) until sacrificed on post-natal day 4. Viability, clinical observations, body weights, feed consumption, and survival were evaluated in parental rats. At necropsy each parental animal was examined macroscopically for structural abnormalities and pathological changes with emphasis on reproductive organs. The following organs were weighed and organ/body weight ratios were calculated: adrenals, brain, heart, kidneys, liver, lung, spleen, epididymides, testes and thymus. Twenty-seven tissues were preserved from all adult animals in all dose groups. Ovaries, testes, epididymides, nose with nasal turbinates, and any grossly observed abnormalities were processed and sections examined histologically for all males and female parental animals in the control and highest dose group. Reproductive parameters (mating indices, pregnancy rates, male fertility indices, gestation length, number of implantation sites and corpora lutea, pre- and post-implantation loss, pups per litter, live born and stillborn pups, and incidence of dams with no viable pups) and developmental endpoints (pup physical examination, viability, weight, sex ratio, litter survival indices and mean pup survival indices) were evaluated. Pups were sacrificed on day 4 of lactation and underwent a complete macroscopic examination and a determination of sex by internal examination. All pups were preserved with viscera intact. Pups found dead at birth and that died prior to day 4 of lactation also underwent a gross external and internal examination. Dead pups were not eviscerated, but were preserved intact.

All groups had a fertility index of >90% and a live birth index greater than or equal to 98%. Offspring showed comparable body weights, weight gain, and viability index at postnatal day 4. Parental male rats had increased kidney weights and relative liver weights at the highest dose, and high dose females had increased spleen weights. Reproductive organs and nasal turbinates from high dose and control animals were examined by a pathologist and no histological changes were observed in tissue from treated rats. LOAEL parental toxicity = 7500ppm [27000mg/m^3] NOAEL parental toxicity = 2500ppm [9000mg/m^3]; NOAEL reproductive performance/ developmental toxicity = 7500ppm [27000mg/m^3] (Schreiner et al., 1999).

An Inhalation Developmental toxicity screening study in Sprague Dawley rats [10/sex/group] and CD-1 mice [15 presumed pregnant females/group] exposed to a 40% vapor of LCCN at concentrations of 0 [untreated controls], 0 [sham-treated controls], 597, 2128ppm [2150 & 7660 mg/m^3], 6 hours/day, for gestation days 0-19. The vapor contained approximately 41% olefins. All animals were observed daily and body weights were recorded on days 0, 6, 13 and 20 of gestation. On day 20 each female was sacrificed and all organs were examined grossly. Serum samples were analyzed for a variety of parameters, including serum iron and lactic dehydrogenase. The number of corpora lutea per ovary and the gravid uterine weights were recorded. Uterine contents were examined and the numbers of implantation sites, early resorptions and live and dead fetuses recorded. Each fetus was identified for its sex, was weighed and the crown-rump distance was measured. Each fetus was examined for external anomalies. Half the fetuses were fixed in Bouin's solution and examined for visceral anomalies and the remaining fetuses were prepared for examination for skeletal anomalies.

There were no treatment related clinical abnormalities or differences in body weights among dams or adverse effects on reproductive parameters with the exception of a statistically significant increase in resorptions and percent resorptions at the 2128ppm dose [10.4% vs 4.6% in untreated controls and 3.9% in sham controls] and an increased incidence of high dose dams with resorptions compared to sham but not to untreated controls. The authors considered the biological significance of the increase in resorptions to be uncertain because the number of viable fetuses (i.e. litter size) in this group were comparable to litter size in other groups and the mean incidence of resorptions in the control groups from previous studies at this facility ranged from 3.0 to 11.2% compared to the 10.4% in the 2128ppm group. No visceral abnormalities were observed. There were an increased number of skeletal variations in animals housed in the exposure chambers (exposed and sham treated controls) when compared to the untreated controls. The authors concluded that these alterations were not related to LCCN since they occurred at a similar incidence in the sham treated controls as well. Despite the reservations of the authors, the developmental LOAEL is identified as 2128ppm (7660 mg/m^3) based on increased resorptions and NOAEL = 597ppm (2150mg/m^3) (Dalbey et al., 1996)

Two additional studies on LCCN, a single dose oral developmental study and a dermal developmental study are described in the Supplemental studies section below.

Heavy straight run naphtha (HNN, CAS # 64741-41-9, approximately 30% naphthenic) was tested by inhalation in an OECD 422 Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test. This was the same study described in greater detail in the systemic repeated dose section (Section 7.1.2.3). Concentrations of HNN were generated by flash evaporation of the test material. Groups of male and female Sprague Dawley rats [12/sex/group] were exposed to 0, 100, 500 or 3000ppm [0, 459, 2296, or 13773mg/m^3] for approximately 28 days. Satellite groups of 12 young, nulliparous, non-pregnant female rats were exposed to 0, 100, 500, or 3000 ppm during a premating period of approximately 2 weeks, a cohabitation period of approximately 2 weeks, and a gestation period of approximately 3 weeks. The animals were not exposed after gestation day 19, or during the approximately 4-day lactation period. Females without evidence of mating continued to be exposed for 19 days after the end of the cohabitation period. Measurements of body weight, food consumption, and clinical signs of toxicity in females were conducted throughout premating, cohabitation, gestation, and lactation. On postpartum day 4, blood samples were collected from lactating females for haematology and clinical chemistry parameters. In addition, the neurobehavioral evaluation was conducted on lactating females on postpartum day 4, and subsequently, lactating females and offspring were sacrificed, and organs [liver, kidney, lungs, ovaries with oviducts, and uterus with cervix) were weighed. Microscopic evaluation was performed on reproductive organs from females that failed to produce a litter. Offspring were evaluated for external abnormalities. Reproductive parameters (mating indices, pregnancy rates, gestation length, fertility index, pre-coital interval, number of implantation sites and corpora lutea, post-implantation loss, pups per litter, live born and stillborn pups, and incidence of dams with no viable pups) and developmental endpoints (pup physical examination, viability, weight, sex ratio, litter survival indices and mean pup survival indices) were evaluated.

Test substance related effects on body weight and weight gain were observed in 3000ppm females during the three-week gestation period. Body weight on GD21 was 7% lower than controls and the weight gain from GD0-21 was 14% lower than controls and was considered an adverse effect. The statistically significantly lower maternal body weight in 3000ppm dams at LD0 correlated with the lower weight trend in high dose females during gestation. The overall weight gain from LD0-4 was comparable to controls although the 3000ppm female body weight did not fully return to control values but was not statistically significantly lower. Only one mating pair in the 3000ppm group failed to produce a litter, resulting in a mating index of 92% in that group and 100% in all other groups. There were no test substance-related or statistically significant differences in mean number of pregnant animals, number of animals delivering, mating index, fertility index, precoital interval, gestation length, number of corpora lutea, number of implantation sites, or percent of post-implantation loss for any exposure concentration. There were no test substance-related or statistically significant differences in number of fetuses born or born alive, live born index, viability index, sex ratio, incidence of clinical observations, or mean fetal body weight on postnatal days 0 or 4. There were no HNN related effects on neurobehavioral, clinical chemistry or haematology parameters for lactating females.

The NOAEL for developmental and reproductive endpoints and neurobehavioral endpoints for LD4 dams = 3000ppm (13650mg/m^3), the highest dose tested. Systemic toxicity values for all adult animals were LOAEL = 3000ppm (13650mg/m^3) and NOAEL = 500ppm (2275mg/m^3) (API, 2008a)

Light catalytic reformed naphtha (CAS #64741-63-5, LCRN, 33% aromatic) was tested in rats in an OECD 421 Inhalation Reproductive and Developmental Toxicity Screening Test as a light end distillate. The test material (LCRN-D) was prepared to be representative of the fraction of light catalytic reformed naphtha to which man might be exposed during handling and blending. Male and female Sprague Dawley rats (10/sex/group) were exposed to concentrations of 0, 250, and 7500pppm (2775, 9250 and 27750mg/m^3), 6 hours/day, 7 days/week for 2 weeks prior to mating. The maximum exposure level was 75% of the lower explosive limit for LAN distillate. Parental males and females that subsequently failed to mate were exposed during mating, and an additional 18 days following completion of the mating period. Males were killed shortly after the last litters reached day 4 of lactation. During the mating period, females were exposed until evidence of mating was observed. If there was no evidence that mating had occurred the pairs were allowed to remain together up to a period of 2 weeks. Presumed pregnant females were treated daily during gestation (GD days 0-19) until sacrificed on post-natal day 4. Viability, clinical observations, body weights, feed consumption, and survival were evaluated in parental rats. Unmated females or those who failed to produce a litter were killed 23 days after completion of the mating period. At necropsy each parental animal was examined macroscopically for structural abnormalities and pathological changes with emphasis on reproductive organs. Lungs, trachea and larynx were removed in their entirety. The right middle lobe of the lung was weighed; the remaining lobes were fixed for subsequent histopathological examination. The testes and epididymides of the males and the ovaries of the females were weighed and then fixed for histological examination. Reproductive parameters (mating indices, pregnancy rates, male fertility indices, gestation length, number of implantation sites and corpora lutea, pre- and post-implantation loss, pups per litter, live born and stillborn pups, and incidence of dams with no viable pups) and developmental endpoints (pup physical examination, viability, weight, sex ratio, litter survival indices and mean pup survival indices) were evaluated.

All parental animals survived to scheduled sacrifice and no treatment related clinical signs were observed. Except for a slight reduction in body weights in the high dose males there were no other effects on either body weight or food consumption. The only treatment related organ weight changes were an increase in relative kidney (15%) and relative liver (5%) weights in the high dose males. No other organ weight changes were recorded. There were no treatment-related microscopic changes in the testes, epididymides, ovaries or nasal turbinates in the animals in the high dose group.

All groups had a mating index and a fertility index of 100% and all animals in all groups had mated within 4 days of cohabitation. Delivery and litter data did not demonstrate any effects of treatment. External and internal examination of pups sacrificed on day 4 of lactation resulted in only one pup in a single litter of the control group with abnormalities.

Parental toxicity LOAEL = 7500ppm (27750 mg/m^3) based on slightly decreased body weight and increased relative liver weight; NOAEL parental toxicity = 2500ppm (9250 mg/m^3). NOAEL for reproductive performance/ developmental toxicity = 7500ppm (27750mg/m^3) (Schreiner et al., 2000).

Full range catalytic reformed naphtha (CAS # 68955-35-1, FR-CRN, >60% aromatics) was tested as a 40% vapor in a developmental toxicity study in rats. Groups of 11 or 12 presumed pregnant female rats were exposed 6 hours each day from days 6-19 of gestation to 0, 508, and 1835ppm (0, 2160 and 7800mg/m^3) partially vaporized FR-CRN. Two extra groups served as untreated and sham treated controls. All animals were observed daily and body weights were recorded on days 0, 6, 13 and 20 of gestation. On day 20 each female was sacrificed and blood samples removed for serum chemistry evaluations that included iron and lactic dehydrogenase. All maternal organs were examined grossly and liver and thymus weights were recorded. In addition, the number of corpora lutea per ovary and the gravid uterine weights were recorded. Uterine contents were examined and the numbers of implantation sites, early and late resorptions and live and dead fetuses were recorded. Each fetus was gendered, weighed and grossly examined for external abnormalities. Half the fetuses were fixed in Bouin's fluid and examined subsequently for soft tissue abnormalities. Remaining fetuses were stained with Alizarin red and examined for skeletal anomalies.

There were no adverse effects on maternal body weight gain, liver weight or thymus weight. In the high dose group, maternal serum glucose levels were slightly decreased (1.5%) and potassium levels slightly increased (1%) relative to the untreated controls. Reproductive performance during gestation and in-utero survival and development of concepti were unaffected by treatment. There were no treatment-related increases in gross abnormalities or anomalies of soft or skeletal tissues.

The NOAEL for maternal and developmental endpoints = 1835ppm (7800mg/m^3) (Dalbey and Feuston, 1996)

Results for both distillate and vapor studies were similar. No treatment related reproductive or developmental effects were reported for these aromatic samples. A dermal developmental toxicity study with a full range coker naptha is described in the Supplemental studies section.

Supplemental studies

Light catalytic cracked naphtha (LCCN, CAS # 64741-55-5) was tested in a dermal developmental toxicity study. LCCN was applied to the clipped backs of pregnant Sprague Dawley rats (10/group) at concentrations of 0, 30, 125, or 500mg/kg/day from GD0-19. An additional group contained pregnant animals treated with 500mg/kg/day LCCN from GD0-18, following which a one-day dermal dose of LCCN+^{14}C-octane and ^{3}H-benzo(a)pyrene was administered on GD19 to monitor bioavailability. Placental and fetal samples and maternal blood were combusted and radiolabel content in the residue was measured by liquid scintillation counting.

Slight to moderate skin irritation was observed at the application site at all dose levels. Maternal parameters [body weight and weight gain and food consumption] and serum chemistry parameters were comparable to controls. No treatment related effects were seen on reproductive endpoints [number of corpora lutea, implantation sites, resorptions, live and dead fetuses], fetal viability, fetal body weights or crown-rump length. No teratogenic findings were seen in soft tissue or skeletal evaluations. Up to 0.12% ^{14}C radioactivity and 1.3% ^{3}H radioactivity were identified in maternal blood, placenta and fetal tissue at study termination, demonstrating that LCCN passed the maternal barrier into the fetal system but did not induce adverse effects. The developmental NOEL was greater than 500mg/kg, the highest dose tested. (Mobil, 1988b)

In another study, a 2000mg/kg single oral dose of LCCN was administered to pregnant Sprague Dawley rats on Day 13 of gestation. This material was tested with a series of other refinery streams to identify and compare any potential direct teratogenic effects that might be obscured by maternal or fetal toxicity resulting from repetitive exposure. Clinical signs of moderate to severe toxicity were seen in the first rats treated with LCCN to the extent that, although no females died, fetal viability may have been compromised; the test group was thus limited to 5 animals. Caesarean sections were performed on GD20. Body weight and weight gain were significantly reduced following exposure but effects were transient and normal weight gain resumed for the remainder of the study. No adverse effects on reproductive parameters [number of corpora lutea, implantation sites, resorptions, live and dead fetuses] were observed and no teratogenic events in soft tissue or skeletal specimens were seen. LCCN was not a developmental toxicant in this system. (Stonybrook Laboratories, 1995i)

Full range coker naptha (CAS # 68513-02-0; ARCO F-250 Merox Feed) was tested in a dermal developmental toxicity study with pregnant Sprague Dawley rats. The undiluted test material was applied daily to the shaved backs to 12 rats/ treated group at concentrations 0 (15 control rats), 100, 500 and 1000mg/kg from GD0-20. Test sites were not occluded. Animals were allowed to deliver litters and nurse pups through lactation day 4. Irritation at the test application site was observed in maternal animals at all dose levels. There were no statistically significant differences in reproductive parameters [number of females delivering live litters, gestation length, number of implantation sites, number of litters with live pups] or offspring survival at lactation days 0 or 4, pup sex ratio or pup body weight. The NOEL for developmental toxicity was 1000mg/kg, the highest dose tested. (ARCO, 1994)

Gasoline (Supporting Chemical)

Unleaded gasoline and gasoline vapor have been tested for developmental and reproductive effects. Pregnant Sprague Dawley rats were exposed by inhalation to unleaded gasoline vapor at concentrations of 0, 400 and 1600ppm (0, 1493, and 5970mg/m^3) from day 6-15 of gestation; caesarean sections were performed on day 20 Mated females were weighed on days 0, 6, 15 and 20 of gestation. Food consumption was recorded daily during the periods 0-6, 6-15 and 15-20 days of gestation. Observations were made daily for clinical signs. On day 20 of gestation the female rats were anesthetized and their visceral and thoracic organs were examined. The uterus was removed and opened and the number of implantation sites, their placement in the uterine horns, live and dead fetuses and resorption sites recorded. The fetuses were removed, examined externally for abnormalities and weighed. One third of the fetuses from each litter were fixed in Bouin's solution and examined later for changes in the soft tissues of the head, thoracic and visceral organs. The remaining fetuses in each litter were stained with Alizarin Red S and examined for skeletal abnormalities.

There were no treatment-related effects on body weight or food consumption. There were no treatment related effects on any reproductive parameter (pregnancy ratio, live litters, implantation sites, litters with resorptions, dead fetuses, litter size, fetal weights), or fetal soft tissue or skeletal examination. NOEL for maternal and developmental toxicity = 1600ppm (5970mg/m^3). (API, 1978)

An unleaded gasoline vapor condensate (10.4% by volume of starting gasoline) was also evaluated for developmental toxicity in pregnant Sprague Dawley rats by inhalation at target concentrations of 0, 1000, 3000, and 9000ppm (0, 2653, 7960, and 23900mg/m^3) [actual concentrations 0, 1015, 2984, 8993ppm; 0, 2693, 7918, 23881mg/m^3] from day 6-19 of gestation according to US EPA TSCA test guideline 798-4350. No maternal toxicity was observed. At caesarean section on day 20 of gestation, no treatment related effects were identified for any reproductive parameter (pregnancy ratio, live litters, implantation sites, litters with resorptions, dead fetuses, litter size, fetal weights) or fetal malformations or variations. The NOAEL for maternal and developmental toxicity = 8993ppm; [23881mg/m^3]. (Roberts et al, 2001)

A developmental toxicity study in rats of Baseline Gasoline Vapor Condensate (BGVC), a 20% light fraction of whole unleaded gasoline was performed according to OPPTS 870.3600, 870.3700 and OECD 414 guidelines. This test material was a representative evaporative emission tested under the US EPA 211(b) Fuels and Fuel Additives Health Effects Testing Program (1994b). BVCG was administered to confirmed pregnant Sprague Dawley rats (25/group) at target concentrations of 0, 2000, 10000, and 20000mg/m^3 (analytical concentrations 0, 1979, 10,676 and 20,638mg/m^3) 6hr/day, from Gestation Day 5 through Gestation Day 20 the period of major organogenesis and fetal growth. There was no evidence of maternal toxicity. At caesarean section on day 21 of gestation, there were no statistically or biologically significant differences for uterine implantation data, and external, visceral, and skeletal observations in the fetuses. Statistically significant reduced mean fetal body weights were noted for all treatment groups. There was no dose response pattern in these decreased weights. However, the fetal body weights of the treated groups were within the historical control range of the laboratory while the mean fetal body weights of both sexes of the control group were greater than the historical range. Additionally, the litter weights and the weights of the male and female components of the litter weights did not correlate with this decrease. Therefore, the decreased fetal body weights while statistically significant were not considered biologically significant. The NOAEL for maternal and developmental toxicity = 20,638 mg/m^3. (API, BGVC, 2008b)

Reproductive toxicity was evaluated in a 2-generation inhalation study with Baseline Gasoline Vapor Condensate (BGVC), a 20% light fraction of whole unleaded gasoline according to OPPTS 870.3800. This test material was a representative evaporative emission tested under the US EPA 211(b) Fuels and Fuel Additives Health Effects Testing Program (1994b). BVCG was administered to Sprague Dawley rats (26/sex/group) at target concentrations of 0, 2000, 10000, and 20000mg/m^3 (actual concentrations 0, 2014, 10,319 and 20,004 mg/m^3) 6hr/day, 7 days/week for 10 weeks before mating and 2 weeks of mating. Exposure of parental females [P0] with confirmed matings was continued until Gestation Day [GD] 19 and suspended until postpartum day 5 to avoid inducing undue stress to the dams during birth and early lactation. P0 dams continued to be exposed to BGVC until sacrifice at weaning. At weaning of the F1 generation on postpartum day 28, one pup/sex/litter was chosen randomly to continue exposure as the F1 parental generation; littermates were never paired together. Exposure of the F1 parental generation to BGVC began at weaning with 10 weeks of premating exposure and continued on the same schedule as the P0 parental generation through mating gestation and lactation. Physical observations, body weights and food consumption were monitored at least weekly during the study. After approximately 16 weeks of exposure, all parental males [P0 and F1] were sacrificed and all parental females [P0 and F1] were sacrificed on their respective postpartum days 28. Females that failed to mate were sacrificed 25 days after the end of the mating period. Fourteen organs were weighed from all rats and tissues from these organs were examined microscopically from 10 rats from the control and 20000mg/m3 groups. Reproductive organs from all males and bred females in the control and high dose groups were examined. Sperm evaluations included motility, counts of testicular homogenization-resistant sperm and cauda epididymal sperm, and sperm morphology in the cauda epididymis. Ovary histopathology included evaluation of primordial follicle population, number of growing follicles and corpora lutea. Pups (F1 and F2 generations) were observed as soon as possible after delivery for sex, number of live and dead pups and pup abnormalities. Pups dead at delivery were identified as stillborn or liveborn/found dead based on lung floatation evaluation. Thereafter litters were observed twice daily. On LD 4, F1 litters with more than 10 pups were randomly culled to 10 pups with sex distribution equalized if possible. Pups were examined and weighed on LD1 (delivery day), 4 (preculled), 7, 14, 21 and 28. At weaning one pup/sex/group was selected for mating to produce the F2 generation. F1 pups [5/sex/group/assessment] not selected for F1 mating were evaluated for standard Tier 2 neuropathology [40 CFR79.66] or for glial fibrillary acidic protein (GFAP) assessments [40 CFR79.67] on postpartum day 28 [Results of the GFAP study are reported in a separate Neurotoxicity robust summary but the GFAP assay is considered beyond the scope of this document]. The remaining pups were sacrificed. Three pups/sex/litter in each group were selected for macroscopic examination and selected organs [brain, spleen, thymus] were weighed from one pup/sex/litter.

Exposure of rats to 2014, 10,319 and 20,004 mg/m^3 of vapor of test substance resulted in decreased body weight gains in the P0 females and F1 males prior to mating in the 20004 mg/m^3 exposed group. Increases in kidney weights in parental male animals exposed to the two higher exposure levels of vapor were consistent with alpha 2-microglobulin mediated nephropathy seen in these animals, a finding has been generally accepted not to be relevant to human risk assessment (US EPA, 1991). There was no effect at any of the exposure levels on reproductive performance in the study, including mating, fertility, parturition, lactation, offspring survival and development or maturation, in either the P0 or F1 generations. Pregnancy rates for control, 2014, 10,319 and 20,004 mg/m^3 groups were 96.0%, 96.2%, 92.3% and 100% respectively for P0 animals and 100%, 100%, 91.7% and 100%, respectively for F1 animals. There was no evidence of any neuropathology in F1 pups as a result of the exposures. The NOAEL for systemic toxicity [excluding kidney effects in male rats] is 10319mg/m^3. The NOAEL for neurotoxicity in F1 animals is >20,004mg/m^3. The Reproductive NOAEL is >20,004mg/m^3. These results are comparable to those seen in other gasoline studies and with the refinery streams representative of the 4 chemical classes. (API, BGVC, 2008c)

Reproductive toxicity was also evaluated in a 2-generation study with Vapor recovery gasoline Sprague Dawley rats (30/sex/group) at concentrations of 0, 1850, 3700 0r 7400ppm (0, 5000, 10000, and 20000mg/m^3) in accordance with OECD protocol 416 and US EPA OPPTS 870.3800 draft guideline for reproduction and fertility effects (1994). The test material was a condensate of gasoline vapor that had been collected from a vapor recovery unit during normal operations. This test material was selected since it was representative of the exposures that normally occur for the general public during self-service refueling. Analytical studies were conducted on the condensate and the results compared with exposure studies that had been carried out during refueling operations. The results confirmed that the vapor recovery condensate was similar in composition to the vapors to which the public is exposed during refueling.

Singly housed animals were exposed for 10 weeks prior to mating followed by a 3 week mating period. Mating was confirmed by either presence of sperm in a vaginal rinse or by the presence of a vaginal plug. Exposure of females was continued until gestation day 20 and was then suspended until post partum day 5 to avoid unduly stressing the dams during birth. Exposure was re-commenced and continued until sacrifice of parental females after weaning. The pups were culled on a random basis to approximately 5/sex/litter. At weaning on postnatal day 28, the F1 pups were selected for the second generation. Among the pups not selected, 3/sex/litter were sacrificed and examined for internal abnormalities. The remainder were examined for external abnormalities, sacrificed and discarded. Pups selected for F1 were exposed for a 13 week premating period and for a 3 week mating period as described above. The males were sacrificed at this time and the females continued to be exposed until gestation day 20. Exposures were resumed on post partum day 5 and were continued until weaning, when all remaining animals were sacrificed. Other than during the period from gestation day 20 until post partum day 5, all F1 offspring were exposed from conception to sacrifice. All animals were examined regularly for viability and clinical observations. Body weights and food intakes were also recorded regularly throughout the study. All pups were counted and examined externally on a daily basis and weighed at regular intervals until post-natal day 21. F1 pups were examined regularly between postnatal days 21 to 28 and were weighed on days 28 and 35. All surviving F1 and F2 pups were examined for developmental landmarks, including pinna detachment, hair growth, incisor eruption, eye opening and the development of the surface-righting reflex. Surviving F1 female offspring were monitored for vaginal opening and males were examined for preputial separation. Reproductive parameters evaluated included: male and female fertility indices, male mating index, female fecundity and gestational indices, mean litter size, mean days of gestation, female estrous cycle length and number of females cycling normally. Live birth index, survival index, survival indices (post partum days 1, 4, 7, 14 and 21), viability index at weaning, mean live and dead offspring on day 0, sex ratio at day 0, offspring in-life observations, offspring body weight and offspring gross postmortem findings were also assessed. Randomly selected culled pups were necropsied and the following organs weighed: ovaries, liver, adrenals, testes, kidneys, spleen and brain. Additionally a wide range of tissues was taken for histology. Similar evaluations were also carried out on all adults surviving to scheduled sacrifice. Tissues taken from the high dose group and controls were evaluated histologically. Samples of sperm from the left distal cauda epididymis were collected from all males at terminal sacrifice for evaluation of sperm parameters. These included assessments of total caudal epididymal sperm numbers, % progressively motile sperm and homogenization resistant spermatid count, % morphologically normal sperm and % sperm with an identified abnormality. An ovarian examination was carried out in the females that included confirmation of growing follicles and corpora lutea and quantification of primordial oocytes in the high dose and control groups. Since there were no abnormal findings in the high dose group, other groups were not evaluated.

There were no treatment related systemic effects in parental females and only the species and sex specific increased hyaline droplet formation consistent with alpha 2-microglobulin mediated nephropathy was observed in kidneys of male rats of both generations. These kidney lesions have been determined not relevant to humans (EPA, 1991) and were excluded in parental NOAEL determination. No reproductive parameters were affected and there were no deleterious effects on offspring survival and growth in either generation. Sperm count and quality in both P1 and P2 (F1) males were comparable in all dose groups. NOAEL for parental and reproductive toxicity = 7400ppm (20000mg/m^3) (McKee et al, 2000).

Conclusions: Developmental or reproductive toxicity was not observed in dermal studies, or by inhalation in rats for distillate or vapor samples in any PONA class with the exception of one 40% olefinic sample [chamber vapor content 41% olefins] developmental study in which increased resorptions were reported at the highest dose [2128ppm; (7660mg/m^3)]. Of note is that the authors were not sure of the biological significance of this occurrence. The distillate sample of the same CAS number with higher olefin content [chamber vapor content 61% olefins] run at higher exposure concentrations did not show any reproductive toxicity. In addition, no developmental effects were seen with wholly vaporized gasoline [NOAEL = 1600ppm (5970mg/m^3], a 10% distillate sample of unleaded gasoline [NOAEL = 8993ppm (23881mg/m^3)], or a baseline gasoline vapor condensate [NOAEL = 20,638 mg/m^3] nor in two 2 generation study reproduction studies with vapor recovery gasoline or baseline gasoline vapor condensate [NOAEL ≥ 20,000mg/m^{3}in both studies]. No increases in resorptions were reported in any of these studies. Based on the absence of increased resorptions with naphthas and gasoline in other study results and the opinion of the authors themselves, it was concluded that the increase in resorptions seen in the 40% vapor sample may have been unique to that test sample and is not considered representative of refinery streams in general. This study has not been used to establish the lower limit of the read-across range. As there were no other appreciable differences between paraffinic, olefinic, naphthenic and aromatic streams, a range of values derived from all developmental and reproductive toxicity studies have been used to read-across to untested category members. NOAEL values for developmental and reproductive effects reflect the maximum doses tested. Parental systemic LOAEL and NOAEL values over all studies reflect primarily decreases in body weights at maximum doses. The read-across ranges are:

Developmental NOAEL = 5970mg/m^3 to 27750mg/m^3,

Reproductive NOAEL = 13650 mg/m^3 to 27750 mg/m^3

Parental systemic toxicity LOAEL = 13650 mg/m^3 to 27750 mg/m^3:
NOAEL = 2275 mg/m^3 to 25000 mg/m^3

[Parental toxicity values were determined exclusive of male kidney effects indicative of alpha 2-microglobulin mediated nephropathy, also identified as light hydrocarbon induced nephropathy, a species and sex specific syndrome that does not occur in female rats or other species, including humans and is not relevant to humans (US EPA, 1991)]

7.2 Human Health Effects Other

7.2.1 Carcinogenicity

7.2.1.1 Inhalation Carcinogenesis

No inhalation carcinogenicity studies have been performed with Gasoline Blending Streams

Gasoline (Supporting chemical)

A two year inhalation carcinogenesis bioassay was performed with wholly vaporized unleaded gasoline at actual concentrations of 0, 67, 292 and 2056ppm (250, 1089, 7672mg/m^3) administered to rats and mice [100/species/sex/group]. Exposures were for 6 hours a day, 5 days each week for up to 113 weeks. All animals were individually housed and were allowed free access to food and water except during the exposure periods. All animals were observed twice daily, once before and once after the exposure period. Animals found moribund were removed from the study and sacrificed. All animals were examined once per month for clinical signs and palpable tissue masses. Body weights were recorded monthly for the first 17 months and bi-weekly thereafter. After approximately 18 and 24 months exposure blood was collected from 7 male and 7 female rats from each dose group for hematological and clinical chemistry evaluations. After 3, 6, 12 and 18 months exposure 10 rats and 10 mice of each sex from each dose group were sacrificed and underwent complete post mortem examinations. At study termination all surviving animals were sacrificed. Body weights were recorded and after gross examination a wide range of organs/tissues were removed, weighed and fixed for subsequent histopathological examination.

Mortality rates were unaffected in either species. Rats and mice in the highest dose group had lower body weights throughout the study. Hematology and clinical chemistry parameters were comparable to control for each species. Kidney weights of male rats were elevated accompanied by alpha 2 microglobulin mediated nephropathy also identified as light hydrocarbon nephropathy at interim sacrifices and dose related incidences of kidney tumor at terminal sacrifice. These kidney lesions have been determined to be species and sex specific and not relevant to humans (EPA, 1991). Rat LOAEL, excluding kidney lesions = 2056ppm (7672mg/m^3) based on decreased body weight; NOAEL = 292ppm (1089mg/m^3)

In mice, hepatocellular tumors were present in high dose females, although organ weights were unaffected. No nephropathy was present in male kidneys. Mouse LOAEL = 2056ppm (7672mg/m^3) based on decreased body weight and liver tumors in females; NOAEL = 292ppm (1089mg/m^3). (McFarland et al, 1984).

Conclusions: Excluding kidney effects in the male rat, wholly vaporized unleaded gasoline was not carcinogenic in rats or male mice. Wholly vaporized unleaded gasoline induced hepatocellular adenoma and carcinoma in female mice.

7.2.1.2 Dermal Carcinogenesis:

Mouse skin painting studies have been performed for over 50 years in the petroleum industry to identify potential hazard of skin cancer to workers and to establish safety procedures and guidelines for protective clothing. The standard skin painting study protocol involves applying the test material to the shaved backs of male mice (50 /group) at a concentration of 50µl twice weekly for approximately 2 years. The test material is usually applied undiluted or, if extremely viscous or irritating diluted in solvent (e.g. toluene). A positive control compound such as benzo(a)pyrene diluted in toluene may be included. Physical examinations for dermal irritation and tumors are performed weekly. In some studies body weights may be recorded weekly. All mice are examined at death or scheduled sacrifice and selected organs may be weighed. Application sites are fixed, stained and examined histopathologically. Tumors confirmed by histological examination are used in calculating tumor incidence. The average latency period, the average time to appearance of the first tumor in each animal affected, is calculated. The tumor data is compared statistically (e.g. Chi square test) with the data from the untreated and solvent controls. Table 7 summarizes the skin painting results for samples of Gasoline Blending Streams

Table 7. Mouse Skin Painting Results for Naphtha Streams and Gasoline

Stream	API ID	No. of mice with tumors	Latency (wks)	Comments	Results Reference
Gasoline, unleaded	81-24	2	123	Local skin toxicity, no systemic toxicity at 12 month. Toluene control 4 mice with tumors.	Not a carcinogen API, 1989a
Lt Alkylate naphtha	83-19	1 [0B; 1M]	103	Local skin toxicity, no systemic toxicity at 12 month.	Not a carcinogen API, 1989b
Sweetened naphtha	81-08	3 [2B; 1M]	113	Local skin toxicity No systemic toxicity at 12 months. Toluene control 4 mice with tumor. Not initiator or promoter	Not a carcinogen API, 1989a,c
Heavy Cat reformed naphtha	83-06	0	-0-	Local skin toxicity, no systemic toxicity at 12 month.	Not a carcinogen 1989b
Lt. Cat. cracked naphtha	81-03	7 [2B; 5M]	118	Local skin toxicity, no systemic toxicity at 12 month. Toluene control 4 mice with tumor.	Positive, weak dermal carcinogen API, 1989a
Heavy Cat. cracked naphtha	83-18	6 [1B; 5M]	72	Local skin toxicity, no systemic toxicity at 12 month.	Positive, weak dermal carcinogen API, 1989b
Heavy Thermally cracked naphtha	84-02	6 [3B; 3M]	88	Local skin toxicity, no systemic toxicity at 12 month.	Positive, weak dermal carcinogen API, 1989b

B = benign tumor; M = malignant tumor

Conclusions: Dermal carcinogenesis occurred primarily in gasoline blending streams derived from cracked stocks. All streams produced skin irritation at the site of application. The paraffinic light alkylate naphtha and the aromatic heavy catalytic reformed naphtha samples were not dermal carcinogens. Sweetened naphtha did not induce a significant incidence of skin tumors in the lifetime skin painting study (API, 1989a) and was demonstrated not to have initiating or promoting properties (API, 1989c). No systemic toxicity other than skin irritation was reported after 12 months of exposure for any of these materials.

7.3 Assessment Summary for Health Effects

A substantial body of data has been compiled on representative blending streams and on formulated gasoline. These naphthas demonstrate consistently low acute toxicity by oral, dermal and inhalation exposure, are only mildly irritating to the eye, are mild to moderate skin irritants and are not skin sensitizers. Results of repeat dose mammalian studies for naphtha streams have demonstrated fairly similar profiles of toxicity across the 4 chemical classes.

When applied dermally, representatives of gasoline blending streams induced skin irritation with the only systemic effects related to skin damage and accompanying stress. In inhalation studies, exposure was to distillate fractions typical of the most likely human exposure, or to vapor generated directly from the liquid blending stream. Due to the restrictions imposed by explosive limits, distillate studies could be performed at much higher concentrations than vapor studies. In most studies, minimal toxic effects were observed with the exception of alpha 2-microglobulin mediated nephropathy, also identified as light hydrocarbon nephropathy in male rats, a species and sex specific syndrome not relevant to human health (US EPA, 1991).
Other general systemic effects included increased liver weight, some decreases in body weight or small changes in clinical pathology parameters and a decrease in sperm/grams of epididymis in one olefinic sample, an effect not supported by other male parameters in that study or in other studies. Gasoline blending streams have a low inhalation repeat dose hazard potential. The inhalation NOAELs and LOAELs were similar between the different hydrocarbon classes of streams (PONA) and the formulated product, gasoline in rats. Since there were no appreciable differences between paraffinic, olefinic, naphthenic, and aromatic streams, a range of values derived from all of the repeated dose inhalation studies will be used to read across to all untested category members. These read-across values are:

LOAEL: 6572 mg/m^3 – 27,800mg/m^3 (1864 – 7885ppm[a])
NOAEL: 1507mg/m^3 – 10,153mg/m^3 (427 – 2880ppm[a])

[[a] - upper range of NOAEL based on 211(b) BGVC; Total hydrocarbon determined as parts-per-million (ppm) hexane equivalents.]

Gasoline blending streams are overall not genotoxic. Testing of PONA streams in mammalian cells gave generally negative results across chemical classes except for a few equivocal and one positive finding for samples with higher aromatic content. The read-across conclusions for all streams in this category are negative with and without metabolic activation with the exception of streams with a known aromatic content greater than 60% that can be classified as negative/equivocal without metabolic activation and equivocal/positive with metabolic activation.

In vivo cytogenetic studies by the intraperitoneal or inhalation routes did not result in chromosomal damage or cell cycle toxicity. Equivocal results for sister chromatid exchanges *in vitro* and a positive result *in vivo* were seen with two separate olefinic samples, indicative of interaction with DNA but not definitive for genetic toxicity since no genetic material was unbalanced or lost. SCE can be considered a biomarker of exposure rather than a direct indicator of mutagenic effect. The read-across conclusion for untested streams in this category is negative for in vivo genetic toxicity

Reproductive or developmental toxicity was not observed by dermal exposure or by inhalation of distillate or vapor samples in any PONA class with the exception of a 40% olefinic vapor sample developmental study in which increased resorptions were reported at the highest dose. Increased resorptions were not seen in a similar olefinic naphtha sample containing 60% olefins in the vapor nor in other naphthas tested. Thus this study was excluded from determining a read-across range because these results appeared specific to this sample and not representative of refinery streams in general. The NOAEL values for developmental and reproductive effects reflect the maximum doses tested. Parental systemic LOAEL and NOAEL values over all developmental and reproductive studies reflect primarily decreases in body weights at maximum doses. The read-across ranges for untested streams in this category are:

Developmental NOAEL = 5970mg/m^3 to 27750mg/m^3,

Reproductive NOAEL = 13650 mg/m^3 to 27750 mg/m^3

Parental systemic toxicity LOAEL = 13650 mg/m^3 to 27750 mg/m^3:
NOAEL = 2275 mg/m^3 to 25000 mg/m^3

Results from toxicity studies in gasoline for repeat dose, genetic toxicity and reproductive/ developmental endpoints are consistent with results from tests on these refinery blending streams. The formulated gasoline study results confirm the category hypothesis that there are not significant differences in toxicity between the PONA streams, and separation of streams for hazard characterization is not necessary. Additionally, these results justify the strategy of using all studies to establish endpoint ranges to read across to untested category members.

Long term inhalation exposure to gasoline produced tumors in male rat kidneys, the result of alpha 2-microglobulin mediated nephropathy, a species and sex specific syndrome not relevant to human health (US EPA, 1991). In female mice there was an increase in hepatocellular adenomas and carcinomas.

Skin tumors were induced after 2 years dermal exposure primarily by naphtha streams derived from cracked stocks. No systemic toxicity was reported after 12 months exposure. Other gasoline blending streams and gasoline produced skin irritation but did not induce any or any significant incidence of skin tumors.

8.0 HUMAN EXPOSURE SUMMARY

There are no studies available that monitor the exposure of workers or the public to the refinery streams in this category because these materials are primarily site-limited in refinery pipeline and are blended directly into gasoline. However, formulated gasoline emissions have been monitored in the refineries and at filling stations. Table 8 summarizes monitored exposure levels and likely health effects from these exposures. Reference 10 provides a compendium of gasoline exposure data from European facilities.

Table 8. Data On Exposure To Gasoline Vapor

Type of Exposure or Study	PPM of Total Hydrocarbon Vapor[a]	Health Effects
1) Typical urban roadway	0.01 – 0.03	None Expected
2) Gasoline odor detection	0.50	None Expected
Gasoline odor recognition	0.76	None Expected
3) Typical gas station perimeter (4 hour average)	0.26	None Expected
4) Self service fill-up (2 minutes)	10 - 100	None Expected

5a) Refueling attendants			
	(15 minutes)	0.5 - 48	None Expected
	(6 hours)	0.1 - 31	None Expected
5b) Mechanics	(15 minutes)	0.4 - 138	None Expected
	(6 hours)	0.1 - 17	None Expected
6) Occupational exposure standard for gasoline			
	(8 hours)	300	None Expected
	(15 minutes)	500	None Expected
7) Human volunteers exposed to gasoline			
	(30 minutes)	200	Slight eye irritation
		500	Eye irritation
		1000	Eye irritation
8) Medical Management Guidelines for gasoline		>200	Eye irritation
9) Medical Literature for gasoline exposure			
	(8 hours)	160 - 270	Eye irritation
	(1 hour)	500 - 900	Eye and throat irritation, dizziness.
	(1 hour)	2000	Mild anesthesia

[a]Total hydrocarbon as parts-per-million (ppm) hexane.

The publications that provide data for Table 8 are:

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9.0 CATEGORY ANALYSIS CONCLUSIONS

The eighty-one HPV substances in the Gasoline Blending Streams Category are appropriately grouped in a single category because of their similar carbon number and boiling point ranges. They are used primarily in the blending of gasoline. Gasoline is manufactured to meet property limits that comply with performance specifications and government regulations. Those specifications limit the boiling range over which naphthas used to blend gasoline can be distilled, excluding smaller hydrocarbons with lower boiling points and larger hydrocarbons with higher boiling points. The complex substances in the gasoline blending range contain four basic chemical classes – paraffins, olefins, naphthenes and aromatics in varying ratios. Studies employing samples representing the highest concentrations of each of the chemical classes have demonstrated substantial similarities in environmental and human health effects across the chemical classes making it possible to simplify evaluation and provide read-across values for untested substances in this category. The study endpoint measured data, and read-across values for untested category members are summarized in Appendix D . Matrix of Gasoline Blending Stream Category Data.

The hydrocarbons in the gasoline blending stream category boil in the general range of 37 to 200^0C at atmospheric pressure and have a vapor pressure range of 1290hPa to 9150hPa indicating a considerable tendency to volatilize. Partition coefficients of representative individual hydrocarbon constituents range from 1.23 to 4.85, while water solubility values ranged from <1 to 2000 mg/L. When released into the environment, gasoline blending streams and gasoline will disperse and partition according to individual physical-chemical properties. Volatilization into the atmosphere is an important process but residence time in the atmosphere is relatively short due to indirect photodegradation. In water, hydrolysis is not likely to occur. Gasoline blending naphthas have demonstrated a high capacity to biodegrade and may be considered inherently biodegradable.

Gasoline blending naphthas demonstrated similar ranges of acute toxicity for the aquatic organisms tested, fish, invertebrates and algae. Data described in the robust summaries showed that acute toxicity of the test category members to the test species was bounded by the range 0.9 mg/L to 64 mg/L when test solutions were prepared as WAFs (see section 6.1). For chronic toxicity to aquatic organisms, the cited studies reported a range of chronic NOELRs from <0.39 mg/L to 6.4 mg/L for fish and invertebrates. Impacts on terrestrial ecosystems from spills may occur but the inherent biodegradability and appreciable volatility with rapid indirect photodegradation of constituent hydrocarbons indicates that these substances will eventually dissipate, degrade, and not persist in the environment.

Human exposure to gasoline and gasoline blending streams occurs primarily through the inhalation route, although dermal and less frequently oral exposure also occur. Naphtha blending streams demonstrate consistently low acute toxicity by all routes, illustrated by an inhalation LC50>5g/m^3. These streams are mildly irritating to the eyes and mild to moderately irritating to skin but are not skin sensitizers.

Gasoline blending streams have a low repeat dose hazard potential. The NOAELs and LOAELs are similar in rats between the hydrocarbon classes of these streams and formulated gasoline. Testing of vapor and distillate fractions resulted in overlapping LOAEL and NOAEL ranges of 6350 to 27,800mg/m^3 and 1570 to 10,153mg/m^3 respectively.

Genetic toxicity is not an area of significant adverse effects with minimal to no occurrence of gene mutation in mammalian cells *in vitro* and no induced chromosome damage in treated animals.

Results from tests for developmental and reproductive effects with samples of representative naphtha streams showed consistent similarity in the absence of adverse effects resulting in NOAEL values at the maximum doses tested over ranges equal or higher than doses employed in repeat dose toxicity testing. The NOAEL range for developmental effects was 5970mg/m^3 to 27750mg/m^3, and for reproductive effects, 13650 mg/m^3 to 27750 mg/m^3

Only an unleaded gasoline has been tested for systemic carcinogenicity in a 2-year study by the inhalation route. The principal effect was a species and sex-specific alpha 2-microglobulin mediated nephropathy, also identified as light hydrocarbon nephropathy not considered relevant to human health, and increased hepatocellular adenomas and carcinomas in female mice. Testing of gasoline blending streams for skin cancer over two years of treatment demonstrated varying degrees of skin toxicity at the treatment site but skin tumors were induced primarily by streams derived by catalytic cracking. Appropriate personal hygiene and use of protective clothing eliminate this dermal hazard.

This testing program demonstrates that gasoline blending streams have similar low toxicity profiles for human health endpoints. Ecotoxicity results generally fall within the moderate toxicity range. Results from tests of the formulated gasoline are consistent with results from these streams, thus supporting the conclusion that there is no distinction by hydrocarbon PONA class in the majority of these hazard endpoints evaluated. Therefore, the ranges identified above can be used to classify the 81 substances in this category for toxicity. In addition exposure to these blending streams is minimal since they are site limited and thus are unlikely to pose significant hazard to the environment or human health.

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11.0 LIST OF APPREVIATIONS AND ACRONYMS

API – American Petroleum Institute
BOD – biological oxygen demand
Btu/lb – British thermal unit per pound
Btu/scf – British thermal unit per standard cubic feet
AUGC – area under the growth curve
CAS RN/CAS #/CAS No. - Chemical Abstract Service Registry Number
^oC – degrees Celsius
CONCAWE – Conservation of Clean Air and Water in Europe
d - day
DMSO – Dimethyl sulfoxide
EINECS – European Inventory of Existing Commercial Chemical Substances
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
g/cm^3 – grams per cubic centimeter
h - hour
HLS – Huntingdon Life Sciences
HPV – High Production Volume
IRDC – International Research and Development Corporation
^oK – degrees Kelvin
kPa - kilopascal
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
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
mg/kg – milligrams per kilogram
mg/L – milligrams per liter
mg/m^3 – milligrams per cubic meter
mL - milliliter
mm - millimeter
nm - nanometer
NOAEL – no observable adverse effect level
NOEC – no observable effect concentration
NOELR – no observable effect loading rate
OECD – Organization for Economic Cooperation and Development
OPPTS – US EPA Office of Prevention, Pesticides and Toxic Substances
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
UV - ultraviolet
WAF – water accommodated fraction
wt% - weight percent
μg - microgram
μg/L – microgram/liter
> greater than
< less than
= equal to

12. GLOSSARY

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

Alpha 2-microglobulin mediated nephropathy: also identified as light hydrocarbon-induced nephropathy (LHN) is a species and sex-specific syndrome induced in male rats resulting from repeated exposure to volatile petroleum naphthas in the gasoline blending stream range. The syndrome is characterized by excessive formation of hyaline droplets comprised of the unique sex-hormone dependent alpha 2-microglobulin, in the epithelium of the proximal convoluted tubules leading to degenerative changes in these tubules in the renal cortex and tubular dilatation and necrosis at the corticomedullary junction. Evaluation of nephrotoxicity of volatile hydrocarbons in male rats and comparison of effects in female rats and both sexes of other species (Alden et al., 1984) has confirmed the specificity of this syndrome for male rats and has resulted in the US EPA determination that alpha 2-microglobulin mediated nephrotoxicity is not relevant to health effects in humans. (US EPA, 1991).

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 that 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 2007)**

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 a 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. (**US EPA 1996).**

Formulated Gasoline: Unleaded automotive fuel formulated by blending paraffinic, olefinic, naphthenic and aromatic petroleum naphtha that does not contain oxygenates (e.g. methyl tertiary butyl ether, ethanol, etc.)**..**

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 1996)**

Light hydrocarbon induced nephrotoxicity (LHN): also identified as alpha 2-microglobulin mediated nephropathy. See definition above.

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)**.

Petroleum (crude oil): A naturally occurring mixture of gaseous, liquid, and solid hydrocarbon compounds usually found trapped deep underground beneath impermeable cap rock and above a lower dome of sedimentary rock such as shale; most petroleum reservoirs occur in sedimentary rocks of marine, deltaic, or estuarine origin **(Speight 2007)**.

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 1994a)**.

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 A – CATEGORY MEMBERS

The CAS numbers and descriptions for refinery streams were developed in response to Section 8(b) of the Toxic Substances Control Act which required identification and registration with the Environmental Protection Agency, before July 1979, of each "chemical substance" being manufactured, processed, imported or distributed in commerce. Due to analytical limitations and known variability in stream composition, identification of every specific individual molecular compound in every refinery stream process under all processing conditions was impossible. American Petroleum Institute (API) recommended to EPA a list of generic names for refinery streams covering all known processes used by refiners. A definition of each stream was included and published with CAS numbers by EPA as "Addendum I, Generic Terms Covering Petroleum Refinery Process Streams". In these definitions process history, specifically the final process step, and not chemical composition, was one of the primary criteria to differentiate streams and assign CAS numbers. As a result, streams with the same or substantially similar compositions may have different CAS numbers if they originate in different process units. Thus the 81 naphtha CAS numbers in the gasoline blending stream category do not mean there are large compositional differences between streams. It simply reflects the fact that these streams, comprised of the same basic hydrocarbons in varying concentrations, are produced by a large number of process units within a refinery. Organization of these naphtha streams by composition, based on Paraffin, Olefin, Naphthene and Aromatic content, regardless of CAS number, is the most practical way of evaluating for biological effects.

CAS Number	CAS Name
64741-41-9	Naphtha (petroleum), heavy straight-run
64741-42-0	Naphtha (petroleum), full-range straight-run
64741-46-4	Naphtha (petroleum), light straight-run
64741-47-5	Natural gas condensates (petroleum)
64741-48-6	Natural gas (petroleum), raw liq. Mix
64741-54-4	Naphtha (petroleum), heavy catalytic cracked
64741-55-5	Naphtha (petroleum), light catalytic cracked
64741-63-5	Naphtha (petroleum), light catalytic reformed
64741-64-6	Naphtha (petroleum), full-range alkylate
64741-65-7	Naphtha (petroleum), heavy alkylate
64741-66-8	Naphtha (petroleum), light alkylate
64741-68-0	Naphtha (petroleum), heavy catalytic reformed
64741-69-1	Naphtha (petroleum), light hydrocracked
64741-70-4	Naphtha (petroleum), isomerization
64741-72-6	Naphtha, petroleum, polymn.
64741-74-8	Naphtha (petroleum), light thermal cracked
64741-78-2	Naphtha (petroleum), heavy hydrocracked
64741-83-9	Naphtha (petroleum), heavy thermal cracked
64741-84-0	Naphtha (petroleum), solvent-refined light
64741-87-3	Naphtha (petroleum), sweetened
64741-92-0	Naphtha (petroleum), solvent-refined heavy
64741-99-7	Extracts, petroleum, light naphtha solvent
64742-22-9	Naphtha (petroleum), chemically neutralized heavy
64742-23-0	Naphtha (petroleum), chemically neutralized light
64742-48-9	Naphtha (petroleum), hydrotreated heavy
64742-49-0	Naphtha (petroleum), hydrotreated light
64742-73-0	Naphtha (petroleum), hydrodesulfurized light
64742-82-1	Naphtha (petroleum), hydrodesulfurized heavy

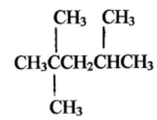
64742-89-8	Solvent naphtha (petroleum), light aliph.
64742-95-6	Solvent naphtha (petroleum), light arom.
67891-79-6	Distillates (petroleum), heavy aromatic
67891-80-9	Distillates (petroleum), light aromatic
68333-29-9	Residues, petroleum, light naphtha solvent extracts
68410-05-9	Distillates (petroleum), straight-run Light
68410-71-9	Raffinates (petroleum), catalytic reformer ethylene glycol-water countercurrent exts.
68410-96-8	Distillates (petroleum), hydrotreated middle, intermediate boiling
68410-97-9	Distillates (petroleum), light distillate hydrotreating process, low-boiling
68410-98-0	Distillates (petroleum), hydrotreated heavy naphtha, deisohexanizer overheads
68425-31-0	Gasoline, natural gas, natural
68475-79-6	Distillates (petroleum), catalytic reformed depentanizer
68476-43-7	Hydrocarbons, C4-6, C5-rich
68476-46-0	Hydrocarbons, C3-C11 catalytic cracker distillates
68476-50-6	Hydrocarbons, C5 and higher, C5-6-rich
68476-55-1	Hydrocarbons, C5-rich
68476-56-2	Hydrocarbons, cyclic C5 and C6
68477-34-9	Distillates (petroleum), C3-C5, 2-methyl-2-butene-rich
68477-63-4	Extracts, petroleum, reformer recycle
68477-89-4	Distillates (petroleum), depentanizer overheads
68478-12-6	Residues (petroleum), butane splitter bottoms
68478-15-9	Residues (petroleum), C6-8, catalytic reformer
68478-16-0	Residual oils (petroleum), deisobutanizer tower
68513-02-0	Naphtha (petroleum), full-range coker
68513-03-1	Naphtha, petroleum, light catalytic reformed, aromatic-free
68513-63-3	Distillates (petroleum), catalytic reformed straight-run naphtha overheads
68514-15-8	Gasoline, vapour-recovery
68514-38-5	Hydrocarbons, C4-10 unsatd.
68514-79-4	Petroleum products, hydrofiner-powerformer reformates
68526-52-3	Alkenes, C6
68526-55-6	Alkenes, C8-10, C9-rich
68527-21-9	Naphtha (petroleum), clay-treated full-range straight-run
68527-26-4	Naphtha (petroleum), light steam-cracked, debenzenized
68527-27-5	Naphtha (petroleum), full-range alkylate, butane-containing
68602-79-9	Distillates, (petroleum), benzene unit hydrotreater depentanizer overheads
68603-01-0	Distillates (petroleum), thermal cracked naphtha and gas oil, C5-dimer-containing
68603-08-7	Naphtha (petroleum), arom.-contg
68606-11-1	Gasoline, straight-run, topping-plant
68783-11-9	Naphtha, petroleum, light polymn.
68783-12-0	Naphtha (petroleum), unsweetened
68783-66-4	Naphtha (petroleum), light, sweetened
68919-15-3	Hydrocarbons, C6-12, benzene-recovery
68919-37-9	Naphtha (petroleum), full-range reformed
68919-39-1	Natural gas condensate
68920-06-9	Hydrocarbons, C7-9
68921-08-4	Distillates (petroleum), light straight-run gasoline fractionation stabilizer overheads
68921-09-5	Distillates (petroleum), naphtha unifiner stripper

68955-29-3	Distillates (petroleum), light thermal cracked, debutanized aromatic.
68955-35-1	Naphtha (petroleum), catalytic reformed
70955-08-7	Alkanes, C4-6
8006-61-9	Gasoline, natural
8030-30-6	Naphtha
92045-58-4	Naphtha, petroleum, isomerization, C6-fraction

APPENDIX B. PETROLEUM CHEMISTRY AND REFINING

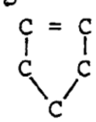
The hydrocarbons that comprise gasoline and its blending streams - paraffins, olefins, naphthenes (cycloparaffins) and aromatics – share some structural features but differ in the ratio of hydrogen to carbon atoms and how those atoms are arranged.

Paraffins: C_nH_{2n+2} where n= number of carbon atoms.
Carbons are joined by single bonds (e.g. butane, $CH_3CH_2CH_2CH_3$). Paraffins with 4 or more C atoms may have 2 or more structural arrangements or structural isomers for example: normal octane, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$ or isooctane

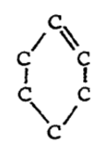


Olefins: C_nC_{2n} are similar to paraffins but have 2 fewer hydrogen atoms and contain at least one double bond (e.g. 2-butene, $CH_3CH{=}CHCH_3$). Olefins with 4 or more carbons can exist as structural isomers. Cyclic olefins are present in cracked products and are found mostly in motor gasoline, for example:

C_5H_8 Cyclopentene

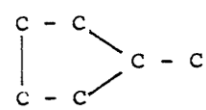


C_6H_{10} Cyclohexene

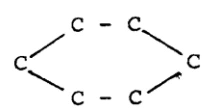


Naphthenes: Cycloparaffins in gasoline have 5 or 6 carbon atoms arranged in a ring and belong to either a cyclopentane or cyclohexane series, for example:

C_6H_{12} Methylcyclopentane

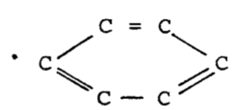


C_6H_{12} Cyclohexane

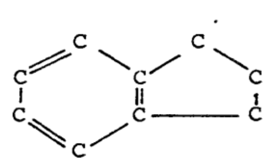


Aromatics: Some carbon atoms are arranged in a ring joined, not by single bonds, but by aromatic bonds that are evenly distributed around the ring,

for example: benzene, C_6H_6:



In polycyclic aromatics, some carbons are shared by 2 or more rings, for example, indane, C_9H_{10}



A Short Course in Gasoline Refining

Petroleum crude oils range in appearance from thin and light-colored to as thick and black as melted tar. Thin, light crudes contain more natural gasoline and lower sulfur and nitrogen content, making them easier to refine to high value products like gasoline; heavier thick crudes require more rigorous refining processes, more energy, and greater cost to produce high value products. All crudes are composed of hydrocarbons of the paraffinic, naphthenic and aromatic classes; olefins are produced during refining. Each class contains a broad range of molecular weights with a broad range of boiling points.

Distillation is the basic step in producing gasoline and other products from crude oil. Crude oil is heated and product is obtained by condensing the vapor that boils off over a specified temperature range at atmospheric pressure. In a distillation column, the vapor with the lowest boiling hydrocarbons (propane and butane) rises to the top. Straight run gasoline, kerosene and diesel fuel are drawn off at successively lower positions in the columns at higher boiling temperature. Hydrocarbons with boiling points higher than diesel fuel can't be vaporized; they remain as liquids in the bottom of the column (atmospheric bottoms). Application of a vacuum to the distillation column improves the high value product yield.

Cracking is a process used to produce higher quality products, including gasoline, from the atmospheric bottoms. Hydrocarbons with higher boiling points can be broken down (Cracked) by breaking carbon to carbon bonds into lower boiling hydrocarbons by subjecting them to very high temperature (Thermal cracking). Olefins are produced through the cracking process. When a catalyst is employed to supplement heating, this Catalytic cracking produces a gasoline of higher quality than thermal cracking. The catalyst speeds up or facilitates the chemical reaction without undergoing permanent chemical damage itself. Fluid catalytic cracking (FCC) is a standard method in modern refineries in which the solid catalyst is fluidized to allow circulation from the reaction section of the cracker to the regeneration section and back again.

Hydrocracking employs a catalyst in a hydrogen atmosphere to break down hydrocarbons resistant to catalytic cracking alone, and is used primarily to produce diesel fuel.

Reforming literally reorganizes the petroleum feed, converting straight chain paraffins into more complex aromatic hydrocarbons that contribute to octane level.

Octane quality defines the ability of gasoline to burn smoothly and uniformly without explosion (knock) in the engine. Octane rating is determined by measuring fuel performance in an engine against that of iso-octane (100 octane rating). The higher the octane rating the more efficiently the fuel burns, resulting in more power per gallon. Aromatics and olefins are high octane hydrocarbons but their content in gasoline has been reduced due to environmental concerns, so other methods of improving octane are employed.

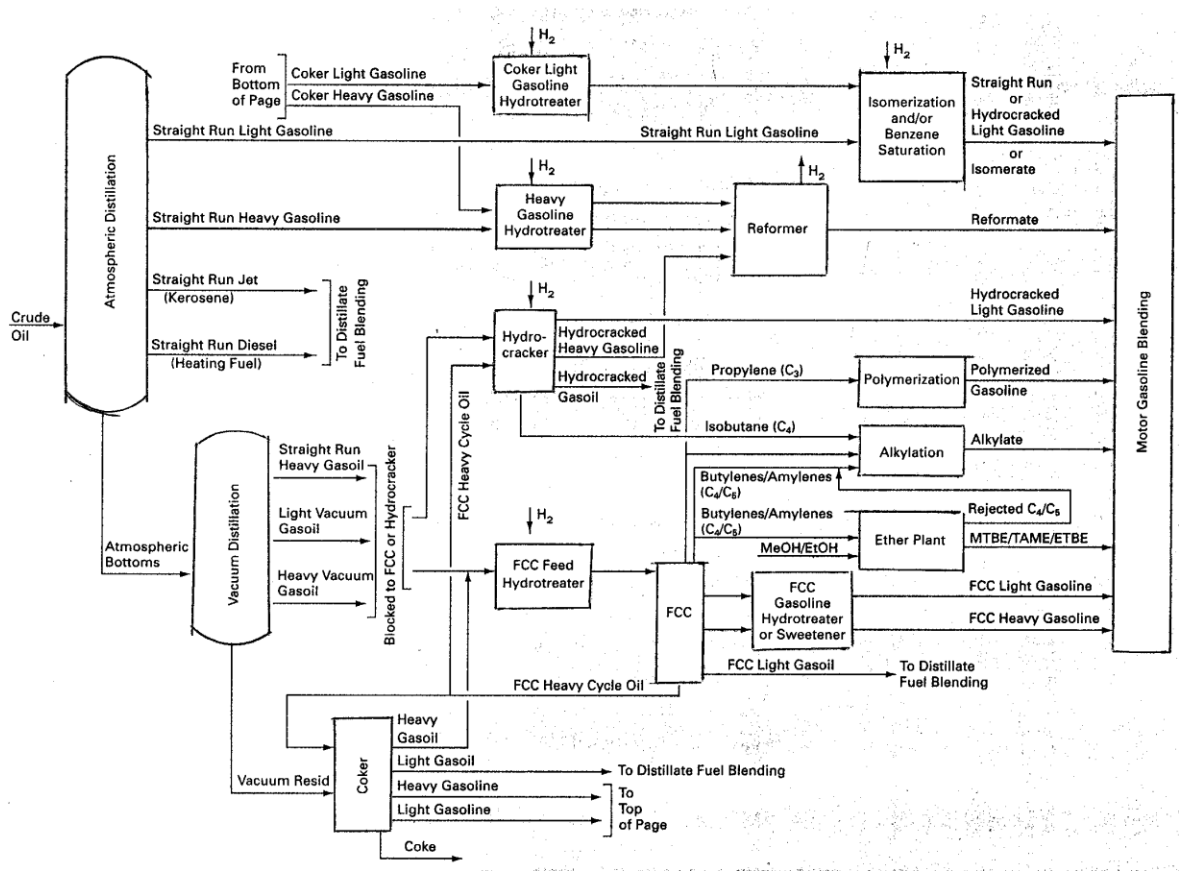
Alkylation combines small, gaseous hydrocarbons with boiling points too low for use in gasoline to form liquid hydrocarbons with higher boiling points. Alkylation is a key process in producing reformulated gasolines because the content of other classes of high octane hydrocarbons – olefins and aromatics- are limited by regulation.

Other conversion processes include polymerization that combines small olefins (C3, propylene) into larger olefins (C6, C9, C12) and isomerization that converts straight chain paraffins (C5, C6) into their branched isomers to improve octane value.

Hydrotreating identifies a range of processes that use hydrogen with catalyst to remove impurities from a refinery stream to improve the product. Mild, selective hydrotreating is used to remove highly reactive olefins, while heavy hydrotreating converts aromatic to naphthenes. Desulfurization, a form of hydrotreating known as sweetening, removes sulfur to comply with lower sulfur limits in reformulated gasolines, and to protect the catalyst that can be deactivated by excess sulfur in the stream.

The schematic layout of a modern refinery is shown in the figure below.

Gasoline Processing in a Modern Petroleum Refinery



Crude oil is fed to the distillation column where straight run light and heavy gasoline, jet and diesel are separated at atmospheric pressure. Straight run jet and diesel fuels are acceptable as is; straight run gasolines must be further processed before blending into gasoline product. Straight run light gasoline may be isomerized to increase octane, or hydrotreated to convert benzene to cyclohexane so that the final gasoline blend meets a benzene specification limit. Straight run heavy gasoline is hydrotreated to remove sulfur and then reformed to improve octane and generate hydrogen for the hydrotreaters.
The bottoms from the atmospheric column are vacuum distilled to produce gasoils for the FCC or hydrocracker feed. Gasoils are hydrotreated to reduce sulfur and nitrogen to levels that do not interfere with FCC cracking. The FCC product must also be sweetened to convert sulfur compounds (mercaptans) to more innocuous compounds to eliminate odor and instability in the gasoline blend.

The vacuum residuum is sent to a resid conversion unit (e.g. resid cracker, solvent extraction unit or coker) to produce more transportation fuel. These resid-derived streams require further processing before they can be blended into light fuels like gasoline or diesel.

APPENDIX C. EU Categorization of Gasoline Blending Streams

This categorization of petroleum substances was adopted by the European Union in their legislation (Official Journal of the European Communities, L84 Volume 36, 5 April 1993. Council Regulation (EEC) No 793/93 of 23 March 1993 on the evaluation and control of risks of existing substances). The organization of naphthas by PONA characteristics correlates well with the EU categorization, which is based on the definitive process step to produce the stream, not on the final process step. The representative PONA-selected samples are listed in boldface in the appropriate EU category. Although no samples were selected from Thermal Cracking (3E) and Hydrotreating (3F), these groups are adequately represented by the selected naphthas. Compositionally streams resulting from cracking under high temperature (3E) are similar to those derived from cracking using a catalyst (3D), and hydrotreating (3F) is employed with many streams to remove sulfur compounds and improve the quality of feedstock.

Gasoline Components from Crude Oil Distillation (3A)
Streams obtained from the atmospheric distillation of crude oil and containing saturated and aromatic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.
High Naphthenic: Heavy Straight Run Naphtha (CAS # 64741-41-9)

Gasoline Components from Alkylation, Isomerisation and Solvent Extraction (3B)
Streams obtained by alkylation (catalytic reaction), isomerization (catalytic conversion) and solvent extraction, and containing saturated hydrocarbons, mainly in the range C5 to C12 and boiling in the range ca. 35 to 230°C.
High Paraffinic: Light Alkylate Naphtha, CAS #64741-66-8

Gasoline Components from Catalytic Cracking (3C)
Streams obtained from the catalytic cracking of heavy distillates into lighter fractions, and containing saturated, olefins and aromatic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.
High Olefinic: Light Catalytic Cracked Naphtha, CAS # 64741-55-5

Gasoline Components from Catalytic Reforming (3D)
Streams obtained from the catalytic reforming of mainly n-alkane and cycloparaffinic feedstocks into aromatic and branched chain hydrocarbons, mainly in the range C5 to C12 and boiling in the range ca. 35 to 230°C.
High Aromatic: Catalytic Reformed Naphtha, CAS # 68955-35-1

Gasoline Components from Thermal Cracking (3E)
Streams obtained by the high temperature splitting of heavy distillates into lighter fractions, and containing saturated, olefinic and aromatic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.

Gasoline Components from Hydrotreating (3F)
Streams obtained by the catalytic reaction of feedstocks with hydrogen to remove unsaturated and organo-sulphur compounds, and containing mainly saturated hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca. -20 to 230°C.

Other Gasoline Components (3G)
Streams obtained by processes such as steam and hydrocracking and sweetening, and containing saturated, aromatic and olefinic hydrocarbons, mainly in the range C4 to C12 and boiling in the range ca -20 to 230°C.

Appendix D. MATRIX OF GASOLINE BLENDING STREAM CATEGORY DATA

Separate Document