AR201-13409A

GASOLINE BLENDING STREAMS TEST PLAN

Submitted to the US EPA

by

The American Petroleum Institute
Petroleum HPV Testing Group

Consortium Registration #

GASOLINE BLENDING STREAMS TEST PLAN

Table of Contents

GENERAL DESCRIPTION OF HPV SUBSTANCES

The 87 substances in the Gasoline Blending Streams Test Plan, which are volatile liquids at standard temperature and pressure and referred to as low boiling point naphthas, 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 I is a complete list of substances included in this Test Plan

The substances in this test plan share many physical properties that make them suitable for gasoline blending but few, if any, 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 naphthas could be used as solvents and the ACC Hydrocarbon Solvents Panel has sponsored several substances that are used for that purpose. Other naphthas from steam-cracking operations, are being sponsored by the ACC Olefins Panel. The Petroleum HPV Testing Group has worked with ACC to ensure that there is no duplication of testing between the three groups.

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

Refining of Gasoline Blending Streams

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 solids. Figure 1 below shows the typical carbon chain lengths found in the proposed HPV

test plans and demonstrates the overlap that occurs.

Figure 1.
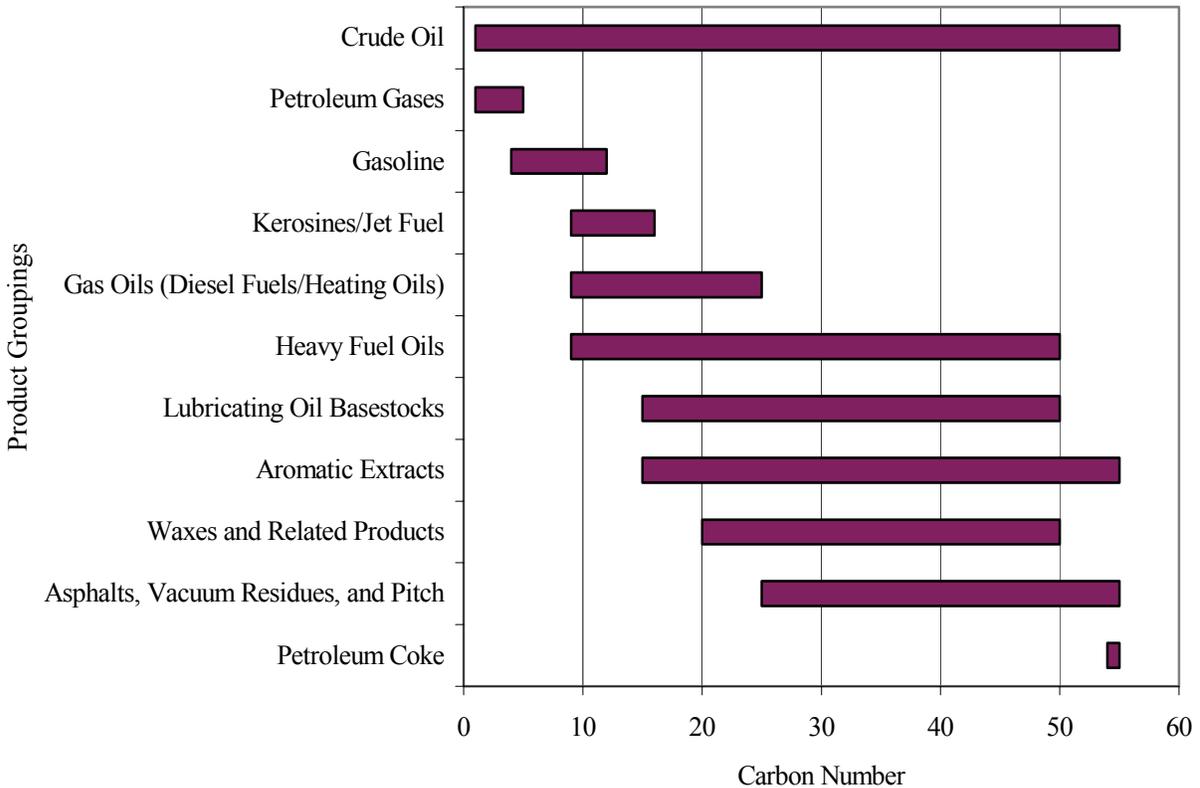
Petroleum refining uses distillation as well as chemical treatment, catalysts, and pressure to separate and combine the basic types of hydrocarbon molecules into petroleum "streams" which have the characteristics needed for blending commercial petroleum products. Distillation is not a precise procedure 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, and this 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 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, and produce naphthas with similar carbon numbers and boiling range but with differing molecular compositions. The characteristic chemical composition of naphtha streams is described by PONA classification – the **P**araffinic, **O**lefinic, **N**aphthenic and **A**romatic classes in the stream; within each class, the hydrocarbons also vary in size. All petroleum crude oils contain paraffins, naphthenes and aromatics; olefins are produced during cracking processes. 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 would be examples of such processes.

Category Rationale

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 gasoline (C4-C12). 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
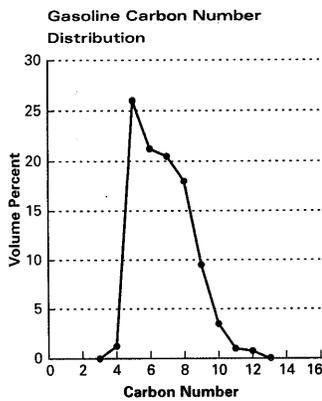
Figure 2A
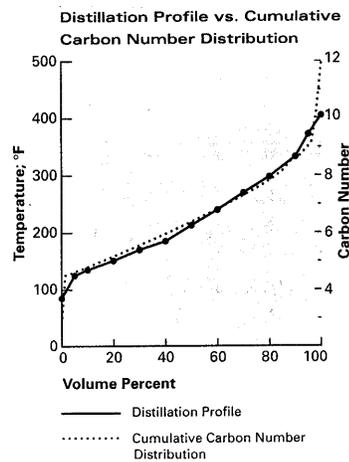
The hydrocarbons that comprise gasoline and its blending streams – paraffins, olefins, naphthenes (cycloparaffins) and aromatics - share some structural features but differ in the ratio of hydrogen to carbon atoms and how these atoms are arranged.

Figure 3 (below) illustrates distribution of PONA classes for gasoline blending streams from a major refiner: 121 straight run naphtha streams, 2680 catalytic reformed streams, 1228 catalytic cracked streams and 60 hydrocracked streams 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.

Gasoline Stream Example
Composition Varies Within and Between Groups
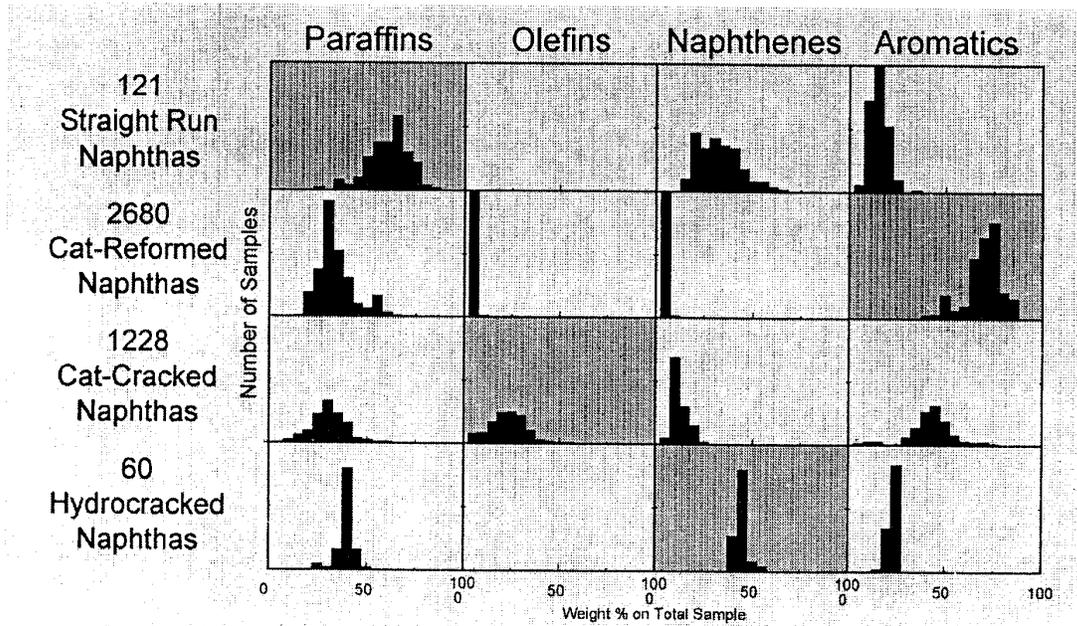
These frequency diagrams show quantitatively the spectrum of PONA that can occur in different naphthas. These differences can be exploited to efficiently test the range of potential chemical composition of all 87 substances in this test plan. A key point of this analysis is that even naphthas that have significant levels of one chemical class (i.e. aromatics in catalytic reformed naphtha) usually contain some amount of hydrocarbon from the other chemical classes. It should also be noted that not all of the 87 HPV streams can be classified as either high P, O, N, or A – these hydrocarbon classes may be more evenly represented in the mixture. However, knowledge of the biological activity of representative naphtha streams enriched in an individual PONA class combined with data on the gasoline product make it possible to predict toxicity potential for untested streams with defined PONA characteristics. These data can also be employed internationally to contribute to hazard and risk characterization, preventing unnecessary duplication of testing and reducing animal usage.

PONA CLASSES AND TEST MATERIALS

The Petroleum HPV Testing Group will select four streams to represent the four extremes of hydrocarbon composition. Evaluation of existing data and future testing of these streams will be used to provide relevant information on the 87 HPV substances. Data on at least one naphtha stream from each of the four chemical classes (PONA) will be summarized or obtained through additional testing. In many instances, data on several naphthas in the same chemical category are already available and will be included in the robust summaries. In addition, existing data on

several different samples of gasoline will also be included in the robust summaries. The four representative naphthas are arrayed below within the appropriate PONA category.

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

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

Category: HIGH NAPHTHENIC
Test Material: Naphtha, heavy straight-run
CAS # 64741-41-9)
This test substance must be obtained and should contain in the range of 30% naphthenes. Alternatively, where analytical confirmation 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) are acceptable.

Category: HIGH AROMATIC
Test Material: Naphtha, catalytic reformed
CAS # 68955-35-1
This test substance has greater than 60% aromatics, by analysis.
Alternatively, where analytical confirmation confirms similar high percentage of aromatic content, 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) are acceptable.

The naphthas selected for 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. Thus, to predict the hazard of the contribution from olefins, which occur in average unleaded gasoline at approximately 9%, a light catalytically cracked naphtha containing >40% olefins has been selected. To determine possible effects attributable to aromatic compounds, present in gasoline at 33%, a catalytically reformed naphtha containing >60% aromatics is evaluated. There is a substantial body of data currently available from testing of refinery streams and gasoline samples to address potential toxic effects for many endpoints related to paraffins, olefins and aromatics. As illustrated in Table 1, naphthas enriched in one chemical class also contain components of other classes to contribute to gasoline composition. Since naphthenes (cycloparaffins) are present in gasoline and most finished blending streams at similar concentrations (5-10%), data for a cycloparaffin-rich stream will have to be acquired using an intermediate refinery stream such as a hydrocracked naphtha, (20-26% naphthenes).

Table 1 Contribution of Chemical Classes From Refinery Streams to Gasoline

	Gasoline	Light Alkylate Naphtha.	Light Catalytic Cracked Naphtha	Light Hydrocracked Naphtha	Sweetened Naphtha	Full Range Catalytic Reformed Naphtha
Carbon No.	C4-C12	C5-C10	C5-C10	C4-C9	C4-C10	C5-C12
Boiling range ^{0}C	30-260	90-160	-20 to 190	65-230	39-200	30-220
PONA Classes (Volume %)[a]						
Paraffins	52.8	99.4	30.6	71.3	72.1	32.1
Olefins	9.3	0.0	45.6	0.0	<0.1	0.5
Naphthenics	4.7	0.6	10.4	26.1	20.9	3.7
Aromatics	33.1	0.0	13.1	2.67	6.9	63.3

a- Definitive chemical analysis by Mass Spectroscopy

EVALUATION OF EXISTING HEALTH EFFECTS DATA AND PROPOSED TESTING

Results of studies on naphthas high in paraffinic, olefinic and aromatic constituents are summarized in this section. 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 (see Appendix 3). 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 will provide even more current data on mammalian toxicity of gasoline. Detailed study information is available in the Robust Summaries organized in the IUCLID data set format employed by the European Union (Appendix 5). The currently available data submitted to the HPV program and any additional testing will be developed with the goal of facilitating international harmonization of hazard and risk characterization worldwide. The EU categories for gasoline components, organized by the definitive processing step to produce those components and complementary to the PONA approached employed in this plan are presented in Appendix 4.

Acute Toxicity

 (Paraffinic)

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.

(Olefinic)

Light catalytically 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.

(Naphthenic)

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.

(Aromatic)

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 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.

Summary: Results of testing naphtha blending streams for acute toxicity indicate that these materials demonstrate consistently low toxicity by the oral, dermal and inhalation exposure routes, are only mildly irritating to the eyes, are mild to moderate skin irritants and are not skin sensitizers. Acute data for gasoline gave comparable results. Since a heavier stream with a higher naphthenic content will be used for testing in place of sweetened naphtha, acute toxicity information for this stream will be derived as read-across from the existing data. **There is sufficient data to characterize the acute toxicity endpoints of all four categories and no additional testing is necessary**

Repeat Dose Toxicity

(Paraffinic)

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

Dermal treatment of New Zealand White rabbits, 3 times/wk for 4 wks. at concentrations of 200, 1000, and 2000mg/kg/day 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.

Sprague Dawley rats were exposed to a LAN light end distillate at concentrations of 0, 668, 2220, and 6646ppm (2438, 8102 and 24300mg/m^3) 5 days/wk for 13 weeks, according to OECD guideline 413. No test material related mortality or effects on physical signs, body weight. or food consumption, in neurobehavioral tests or neuropathology were observed. 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 light hydrocarbon nephropathy, a species and sex specific syndrome not relevant to humans (EPA, 1991). Increased liver weights in high dose rats of both sexes had no microscopic correlate and appeared reversible after 4 weeks of recovery.

(Olefinic)

Light catalytically cracked naphtha (LCCN, CAS #64741-55-5, approximately 46% olefinic) was tested by inhalation in one 21-day and three 13-week studies. In the 21-day study (15 actual exposures), wholly vaporized LCCN was administered to male Sprague Dawley rats at concentrations of 55, 567, and 3628ppm [200, 2040, and 13060mg/m^3] (Halder et al., 1984). In the three 13 week studies, dosage concentrations were 147 - 2136ppm (530-7690 mg/m^3) partially vaporized LCCN to rats and mice (Dalbey and Feuston, 1996); 1500 – 4500ppm (5474-16423 mg/m^3) wholly vaporized LCCN to rats (API, 1987); and 750 – 7500ppm (2336-23364

mg/m^3) light ends distillate to rats (Lapin et al., 2001). In all studies, over a wide range of doses and exposure durations of 3-13 weeks, the most significant treatment related effect was an increase in male kidney weights with increased incidence of hyaline droplets and degeneration of proximal renal tubules indicative of light hydrocarbon nephropathy, at the highest dose. In the 1987 API study, increases in liver weights in both sexes was accompanied by centrilobular heptocellular hypertrophy at the highest dose (4500ppm) in males only. Results of the Lapin et al, 2001 study which employed OECD guideline 413 (Combined subchronic toxicity and neurotoxicity screening), demonstrated that exposure to LCCN vapor did not induce neurobehavioral effects or neuropathologic damage to brain, spinal cord or peripheral nerves. Hyperplasia and hypertrophy of nasal epithelium observed at the high dose was not evident after 4 weeks of recovery.

(Naphthenic)   -

The only repeat dose study performed in this category was a 2 year mouse skin painting study to evaluate the dermal carcinogenesis of sweetened naphtha (CAS #64741-87-3). Sweetened naphtha caused skin irritation, but did not induce cancer or other indications of target organ toxicity.

(Aromatic)

Repeat dose studies have been performed on three materials in this stream category: Full range catalytic reformed naphtha (FR-CRN, CAS #68955-35-1) – 28 day dermal study in rabbits (API, 1986), and a 13 week inhalation study in rats with a partially vaporized sample (Dalbey and Feuston, 1996); Light catalytic reformed naphtha (LCRN, CAS #64741-63-5)- 21 day inhalation study in rats with a fully vaporized sample (Halder et al., 1984), and a 13 week inhalation study in rats with light end distillate sample (Schreiner et al., 2000); Heavy catalytic reformed naphtha (HCRN, CAS #64741-68-0) – 21 day inhalation study in rats with a fully vaporized sample (Halder, 1984).

FR-CRN: In the 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. Test material was a moderate-severe skin irritant. Inhibition of body weight and weight loss occurred at 2000mg/kg. 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.

For the 13-week inhalation study, Sprague Dawley rats were exposed to FR-CRN, partially vaporized (30-40%) to produce a vapor with composition similar to human exposure, at concentrations of 0, 96, 464, and 1894ppm (0, 410, 1970, 8050mg/m^3), 5 days/wk. No significant biological effects were observed with the exception of higher liver and kidney weight in high dose males. No treatment related abnormalities were seen in any tissue examined histologically.

LCRN and HCRN: 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/m3) LCRN or 0, 215, 587, and 2132ppm (1030, 2810, and 10200mg/m3) HCRN for 15 actual exposures. LCRN induced small concentration related increases in necrosis of renal tubules and an increase in incidence and severity of hyaline droplets, typical of light hydrocarbon nephropathy. Exposure to HCRN did not cause adverse effects in the kidney but lung irritation was apparent. In the 13 week study, male and female Sprague Dawley rats were exposed to a light vapor fraction of LCRN at concentrations of 750, 2500, and 7500ppm (2775, 9250 and 27,750mg/m^3). No test material related mortality or effects on physical signs, body weight, food consumption or clinical

chemistry were observed. In males exposed to 7500ppm, a statistically significant decrease in white blood cell and lymphocyte counts, and a decrease in spleen weight were observed at terminal sacrifice, but were not present in animals after a 4-week recovery period. Statistically significant increase in kidney weight relative to body weight in high dose males correlated with microscopically observed light hydrocarbon nephropathy. The only effect on neurobehavioral parameters was significantly higher motor activity in high dose males after the 4-week recovery period without exposure to LCRN distillate, but there was no evidence of hyperactivity or abnormal behavior from the functional observational battery and no microscopic changes in neural tissue (Schreiner et al., 2000).

Summary: Results of repeat-dose dermal studies of blending streams and gasoline indicate that these materials are generally skin irritants with the only systemic effects related to skin damage and related stress at high doses. Inhalation studies with naphtha streams demonstrated minimal toxic effects with the exception of light hydrocarbon nephropathy in the kidneys of male rats at the highest dose. Subsequent research with naphthas and gasoline demonstrated light hydrocarbon nephropathy to be a species and sex specific syndrome, not relevant to human health (EPA, 1991). This nephrotoxic activity appears attributable to the alkane constituents. Streams that contain a higher proportionate content of aromatic components including benzene and toluene, such as heavy catalytic cracked naphtha, do not produce this syndrome. Streams tested for neurotoxicity did not induce any significant neurobehavioral or neuropathologic effects. There is sufficient data from repeat-dose studies to characterize the paraffinic, olefinic and aromatic blending steams, supported by comparable data for gasoline product (Appendix 3). Only the naphthenic stream category has insufficient repeat dose data. **A repeat dose inhalation study in rats (OECD protocol 422) using a naphtha stream high in naphthenic content (e.g. heavy straight run naphtha or heavy hydrocracked naphtha) is proposed to complete this toxicity endpoint.**

In Vitro Genetic Toxicity

(Paraffinic)
   Light alkylate naphtha diluted in acetone, has been tested in a mouse lymphoma (L5178Y TK+/-) forward mutation assay and did not induce mutagenicity with or without metabolic activation from rat liver homogenate.

(Olefinic)
   Three samples of light catalytically cracked naphtha have been tested in the mouse lymphoma (L5178Y TK+/-) forward mutation assay and did not induce mutagenicity with or without metabolic activation from rat liver homogenate, with the exception of equivocal results with metabolic activation for one sample with a higher ratio of aromatic constituents (20.3%) compared to 10-13% aromatics in other samples.
   A sister chromatid exchange assay in Chinese hamster ovary cells with and without metabolic activation with LCCN produced negative results without, but equivocal results with activation.

(Naphthenic)
   Sweetened naphtha, diluted in ethanol, tested in the mouse lymphoma (L5178Y TK+/-) forward mutation assay, did not induce mutagenicity with or without metabolic activation from rat liver homogenate.

(Aromatic)
Light catalytic reformed naphtha (42% aromatics) did not induce mutagenic events in the mouse lymphoma (L5178Y TK+/-) forward mutation assay with or without metabolic activation from rat liver homogenate. Full range, catalytic reformed naphtha (63% aromatics) did not induce mutagenicity without metabolic activation but did induce a dose responsive positive mutagenic effect with metabolic activation. Heavy catalytic reformed naphtha (90% aromatics) was also positive with metabolic activation and induced equivocal results without activation.

Summary: Gasoline blending streams and gasoline show little if any mutagenic activity in *in vitro* test systems. Where activity is present, it occurs with metabolic activation and can be correlated with a higher ratio of aromatics in the test sample (60-90%) than is characteristic of the distribution of aromatics in gasoline, the product (approx. 30% aromatics). *In vitro* genetic toxicity potential of a high naphthenic stream selected for testing will be estimated from chemical composition and by read-across from sweetened naphtha and gasoline test results. **There is sufficient data to characterize the *in vitro* genetic toxicology endpoint for all four PONA categories and no additional testing is necessary.**

In Vivo Genetic Toxicity

(Paraffinic)
Light alkylate naphtha was tested in a rat chromosome aberration assay at doses of 0.3, 1.0, and 3.0g/kg in corn oil, administered intraperitoneally in a single dose. Animals were sacrificed at 6, 24 and 48 hrs post dose. Deaths occurred in both male and females in the highest dose group and a 10% body weight loss was observed in surviving rats of both sexes. No chromosome aberrations, rearrangements, or cell cycle disruption were observed in any dose group.

(Olefinic)
Samples of light catalytically cracked naphtha were tested in both *in vivo* mouse sister chromatid exchange (SCE) and rat chromosome aberration assays. In the SCE assay, mice were given a single intraperitoneal dose at concentrations of 0.2, 1.2 and 2.4g/kg in corn oil and bone marrow lymphocytes were evaluated for evidence of exchange of genetic segments between sister strands, indicative of DNA perturbation; LCCN induced SCE in this assay. In one rat chromosome aberration assay, when animals were treated with a single intraperitoneal dose at concentrations of 0.3, 1.0, and 3.0g/kg and sacrificed at 6, 24, and 48 hrs post-dose, LCCN did not induce chromosome aberrations or cell cycle disruption. In a separate chromosome aberration assay, rats were exposed by inhalation to 63, 297, and 2046ppm (230, 1084, and 7467mg/m^3) for 5 days. LCCN did not induce chromosome aberrations or inhibit normal cell cycle kinetics. Although the SCE assay demonstrated interaction of LCCN and DNA, it is not definitive for clastogenic activity since no genetic material is unbalanced or lost. Negative results in two assays, which visualize actual cytogenetic damage demonstrate that LCCN is not a clastogenic material.

(Naphthenic)
Sweetened naphtha was tested in a rat chromosome aberration assay by inhalation at concentrations of 65, 300, and 2050ppm (215, 993 and 6788mg/m^3) for 5 days. Animals were sacrificed 6 hrs after the final dose. Sweetened naphtha did not induce chromosome aberrations or disruption to cell cycle kinetics.

(Aromatic)
Full range, catalytic reformed naphtha (FR-CRN), light catalytic reformed naphtha (LCRN) and heavy catalytic reformed naphtha (HCRN) were tested in rat chromosome aberrations assays

with a single intraperitoneal injection of test material in corn oil at concentrations of approximately 0.3, 1.0 and 2.5-3.0g/kg. Rats were killed at 6, 24 and 48hrs post-dose to evaluate all stages of cell cycle in bone marrow lymphocytes. None of these materials induced chromosome aberrations or disruption of cell cycle kinetics in these assays.

Summary: Gasoline blending streams and gasoline are not clastogenic. Gasoline also did not induce heritable effects in male mice reflected in post-implantation deaths or reduced fertility (Appendix 3). Potential for cytogenetic damage for the high naphthenic stream selected for repeat dose testing will be estimated from existing data on sweetened naphtha (21% naphthenic) and on gasoline (approximately 5% naphthenic) as well the absence of activity shown by other naphtha streams. **There is sufficient data to characterize the *in vivo* genetic toxicology endpoint and no additional testing is necessary.**

Reproductive And Developmental Toxicity

(Paraffinic)
   Light alkylate naphtha: A light vapor fraction of LAN administered to rats by inhalation at target concentrations of 0, 500, 12500 and 25000mg/m^3 (0, 137, 3425, and 6850ppm) according to OECD protocol 421: Reproductive/developmental toxicity screening test, did not induce reproductive or systemic effects 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= 25000mg/m^3 (Bui et al., 1998).

(Olefinic)
   Light catalytically cracked naphtha was tested for reproductive and developmental effects in two assays. In a developmental toxicity screen, presumed pregnant Sprague Dawley rats were exposed to 0, 2150, and 7660mg/m^3 (0, 597, and 2128ppm) partially vaporized LCCN from day 0-19 of gestation. Females were sacrificed on day 20. Number of resorptions was increased at the highest dose level but no other treatment related changes were observed (Dalbey et al., 1996). A distillate of LCCN administered to rats by inhalation at target concentrations of 0, 2700, 9000, and 27000mg/m^3 (0, 750, 2500, and 7500ppm) according to OECD protocol 421, did not affect reproductive performance, delivery data, or live pups/litter. 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.   NOAEL parental toxicity = 9000mg/m^3; NOAEL reproductive performance/ developmental toxicity = 27000mg/m^3 (Schreiner et al., 1999).

(Napththenic)
   No reproductive/developmental studies are available for this category.

(Aromatic)
   Full range, catalytic reformed naphtha: A developmental toxicity screen was performed with partially vaporized (30-40%) FR CRN administered by inhalation to presumed pregnant Sprague Dawley rats at concentrations of 0, 2160, and 7800mg/m^3 (0, 508, and 1835ppm) on gestation days 6-19. Animals were sacrificed on day 20 of gestation. Maternal body weights, serum chemistry and organ weights were unaffected. No adverse effects were observed on fetal

parameters at sacrifice (viability, fetal body weight, external development) or subsequent skeletal and visceral examinations (Dalbey and Feuston, 1996).

A distillate of light catalytic reformed naphtha administered to Sprague Dawley male and female rats by inhalation at target concentrations of 0, 2775, 9250, and 27750mg/m^3 (0, 750, 2500, and 7500ppm) according to OECD protocol 421, did not affect reproductive performance, delivery data or live pups/litter. Offspring showed comparable body weights, weight gain and viability index at postnatal day 4. Parental systemic effects observed at the highest dose were slightly reduced body weights for males, increased kidney to body weight and liver to body weight ratios. Reproductive organs and nasal turbinates from high dose animals and controls were examined by a pathologist and no histological changes were observed in tissue from treated rats. NOAEL parental toxicity = 9250mg/m^3; NOAEL reproductive/developmental toxicity = 27750mg/m^3 (Schreiner et al, 2000).

Summary: There is sufficient data to characterize developmental and reproductive toxicity of paraffinic, olefinic and aromatic blending streams. The absence of naphtha-induced significant toxicity for these endpoints is supported by comparable data on gasoline (Appendix 3). However, there is no test data for any stream representative of the high naphthenic category; therefore **screening for developmental and reproductive effects in rats as part of OECD protocol 422, using a stream high in naphthenic content (e.g. heavy straight run naphtha or heavy hydrocracked naphtha) is proposed to complete this toxicity endpoint.**

EVALUATION OF EXISTING PHYSICOCHEMICAL AND ENVIRONMENTAL FATE DATA

The physicochemical endpoints for the EPA HPV chemical program include melting point, boiling point, vapor pressure, water solubility, and octanol/water partition coefficient (Kow). Environmental fate endpoints include biodegradation, photodegradation, hydrolysis, and fugacity. Because the HPV substances covered under the testing plan are mixtures of differing compositions, it is not possible to measure or calculate a single numerical value for some of the physicochemical properties. For example, a product that is a mixture of chemicals does not have a melting point, but rather a melting point range. Melting point, boiling point and vapor pressure range will be reported because these substances are complex mixtures. Values for PC properties will be represented as a range of values according to the product's component composition. Although some data for products in this category exist, not all of these endpoints are defined and a consensus database for chemicals that represent products in this category does not exist. Therefore, calculated and measured representative data will be identified and a technical discussion provided where appropriate. The EPIWIN© computer model, as discussed in the US EPA document entitled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program. " is used to calculate physical/chemical properties of representative PONA constituents for selected naphtha streams. The hydrocarbon components in these selected naphtha streams have been identified by detailed hydrocarbon analysis using gas chromatography coupled with flame ionization detection and/or mass spectrometry (GC/FID, GC/MS). Log P_{ow}, atmospheric oxidation half-lives and environmental media partitioning were calculated for these individual hydrocarbon constituents identified by GC/FID or GC/MS in specific naphthas, and the range of these properties are summarized.

Summary: Where measured data does not exist and is impractical to develop, calculated physicochemical and environmental data for selected constituents of gasoline blending streams have been developed using the EPIWIN© computer model**.**

Partition Coefficient: calculated log P_{ow} at 25^{0}C

  (Paraffinic)   Light alkalyte naphtha   3.11-4.54 (C5-C9)
  (Olefinic)   Light catalytic cracked naphtha   2.13-4.54 (C5-C9)
  (Naphthenic) Lt. straight run naphtha (high naphthenic)   2.73-4.85 (C5-C9)
               Lt. straight run naphtha (moderate naphthenic)   2.13-4.76 (C5-C9)
               Lt. straight run naphtha (low naphthenic)   2.13-4.00 (C5-C7)
  (Aromatic)   Light catalytic reformed naphtha   2.13-4.54 (C5-C9)
  Gasoline   2.13-4.50 (C5-C8)

  Summary: Range of partition coefficients for gasoline and blending streams is 2.13-4.85.

Water Solubility: determined from preparations of water accommodated fractions
Calculated and measured water solubilities differ for individual components of complex petroleum substances. At any particular loading rate, aqueous concentrations of each component is a function of relative volume of aqueous and petroleum phases, partition coefficient between phases, amount of component present and the maximum water solubility of each component.

(Paraffinic): Light alkylate naphtha
   freshwater 1.6ppm equilibrium at 24 hrs.
   saltwater   0.9ppm equilibrium at 12 hrs.
   based on chromatographic analysis of combined concentrations of alkyl butanes, alkyl pentanes and dimethyl hexane comprising 68% of test substance.

(Olefinic): Light catalytic cracked naphtha
   freshwater 4.6ppm equilibrium at 24 hrs.
   saltwater   4.3ppm equilibrium at 12 hrs.
   based on analysis of benzene, toluene, ethylbenzene, o-xylene, p-xylene comprising 13% of test substance.

(Naphthenic): Light straight run naphtha (high naphthenic)
   freshwater 5.7-7.9ppm equilibrium at 19 hrs. based on chromatographic analysis of toluene, ethyl benzene and xylene comprising approx. 13% of test substance.
   Light straight run naphtha (low naphthenic) freshwater 4.9ppm as benzene equilibrium at 24 hrs. based on chromatographic analysis of benzene, ethyl benzene, toluene and xylenes.

(Aromatic): Light catalytic reformed naphtha
   freshwater 13.7ppm equilibrium at 24 hrs.
   saltwater   14.0ppm equilibrium at 24 hrs.
   based on total combined concentrations of pentane, 2-methylpentane, benzene, toluene, ethylbenzene, and xylenes comprising 50% of test substance

(Gasoline):
   freshwater   3.1, 3.1, <6.9E-3, 0.92ppm as benzene, toluene, ethyl benzene and xylene respectively

Summary: Solubility in fresh and salt water ranged from 1- 14ppm ranked in order of greatest solubility as LCRN>LSRN>LCCN>gasoline>LAN. Although none of the naphtha streams are appreciably water soluble, streams higher in aromatics and naphthenics demonstrate greater

solubility than other streams or gasoline. There is sufficient data on all four PONA categories for this endpoint. **No additional testing is necessary.**

Environmental Fate Data

Environmental fate endpoints include biodegradation, photodegradation, hydrolysis, and fugacity. Biodegradation data, available for several representative naphthas in this category, show that these products can exhibit a moderate to rapid rate of biodegradation. For the photodegradation endpoint, data is calculated. Products in this category are not subject to hydrolysis at measurable rates, therefore, hydrolysis is not a relevant endpoint for these products. Calculated environmental partitioning behavior (fugacity modeling) for selected constituents of the naphtha streams indicate that these chemicals will partition largely to the air, and therefore their fate in air is of environmental interest.

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. In general, most products in the Gasoline Naphtha 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.

Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model. Indirect photolysis can be estimated using models accepted by the US EPA and other authorities. An estimation method accepted by the US EPA includes the calculation of atmospheric oxidation potential (AOP). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model. Hydrocarbon constituents of Gasoline Naphtha Streams, readily volatilize to air. In air, chemicals may undergo reaction with photosensitized oxygen in the form of hydroxyl radicals (OH^-). The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows), used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances), calculates a chemical half-life based on an overall OH^- reaction rate constant, a 12-hr day, and a given OH^- concentration. This AOPWIN calculation will be performed for those hydrocarbon constituents detected in representative naphtha streams for each of the PONA groupings.

Summary: Insufficient data are available to characterize the atmospheric oxidation potential of chemical components found in products in this category. Therefore, representative components for this category will be identified and their AOP values calculated.

AOPWIN version 1.89 calculates atmospheric oxidation half-lives of hydrocarbons in contact with hydroxyl radicals in the trophosphere, under the influence of sunlight and in contact with O_3, based on a 12-hour day at 25^0C.

(Paraffinic): Light alkylate naphtha, calculated for C5-C9 components
    ½ life range: 1.074 days (2,3,5 trimethylhexane) to 15.985 days (isopentane)

(Olefinic): Light catalytic cracked naphtha, calculated for C5-C9 components
    ½ life range for constituents due to OH reaction: 2.5 hrs (2-methyl-1-butene) to 15.985 days
    (isopentane)

½ life range for olefinic constituents (30% of stream composition): 38.378 min (1-methyl cyclopentene) to 22.950 hrs. (C5 olefins)

(Naphthenic): Light straight run naphtha (high naphthenic), calculated for C5-C9 components
    ½ life range: 0.902 days (toluene) to 2.047 days (m-xylene)
  Light straight run naphtha (moderate naphthenic, 19.8%), calculated for C5-C9 components
    ½ life range: 0.789 days (m-xylene) to 15.985 days (isopentane)
  Light straight run naphtha (low naphthenic), calculated for C5-C9 components
    ½ life range: 1.262 days (isopentane) to 15.985 days (cyclohexane)

(Aromatic): Light catalytic reformed naphtha, calculated for C5-C8 components
    ½ life range: 1.498 days (2,3 dimethyl pentane) to 15.985 days (isopentane).

(Gasoline): calculated for C5-C8 components
    ½ life range: 0.789 days (m-xylene) to 15.985 days (isopentane)

Summary: Calculated atmospheric half-lives for naphtha blending streams and gasoline under conditions of 12 hours of sunlight daily, ranged from a minimum of 38.4 min (1-methylcyclopentene in light catalytic cracked naphtha) to approximately 16 days (isopentane or cyclohexane). This modeling was based on detailed hydrocarbon analyses of each stream constituents and the known half-lives of these constituents. Because naphthas are composed of the same groups of hydrocarbons in varying concentrations, it can be concluded that the gasoline blending streams from all 4 PONA categories degrade in sunlight at a rate of one half the overall content within 16 days. **No additional modeling is necessary**

Stability in Water:
Summary: Hydrolysis is unlikely for gasoline and blending streams (C4-C12). 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. The chemical components that comprise the naphtha category are hydrocarbons, which are not included in these chemical groups, and they are not subject to hydrolysis reactions with water. **No additional testing or modeling is necessary.**

Chemical Transport and Distribution in the Environment (Fugacity Modeling):
Equilibrium models are used to calculate chemical fugacity that 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. Fugacity data can only be calculated. A widely used fugacity model is EQC (Equilibrium Criterion) model. In its guidance document for HPV data development, the US EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. Level I is a steady state, equilibrium model that utilizes the input of basic physicochemical parameters including molecular weight, vapor pressure, and water solubility. Distribution is calculated as percent of chemical partitioned to the 6 environmental compartments (air, soil, water, biota, suspended sediment and sediment) within a unit world. Level I data 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. Values represent the calculated range of distribution to environmental media of C5-C9 hydrocarbon components found in each stream.

(Paraffinic): Light alkylate naphtha – Mobility in aquatic and terrestrial environment is low due to low water solubility and high vapor pressure. Components partition primarily into air: Air: 99.4-100%; soil 0.01-0.27%, water 0.001-0.01%.

(Olefinics): Light catalytic cracked naphtha – Partitions into air >99% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%

(Naphthenics): Light straight run naphtha (High naphthenic) – Partitions into air >97% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%
Light straight run naphtha (Low naphthenic) – Partitions rapidly into air for all components:. Air: 98.9-99.98%; soil 0.01-0.11; water: 0.01-1.0%

(Aromatics): Light catalytic reformed naphtha – Partitions into air >99% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%

(Gasoline): Partitions into air >97% for all components: Air: 97-99.9%; soil 0.00-1.2%; water 0.003-2.7%

Summary: Fugacity modeling for those constituents in gasoline blending streams and gasoline indicate that, at steady-state, these petroleum mixtures components partition >97% to air where hydrocarbons are rapidly oxidized by OH radicals. Partitioning into soil or water does not exceed 1.2% or 2.7%, respectively. Partitioning to sediment or suspended sediment is minimal. These data are adequate to define environmental distribution of naphtha streams and gasoline. **No additional modeling is necessary for this endpoint.**

Biodegradation: Analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
Selected data for products in this category show that they have the potential to biodegrade to a high extent. These data are based on results of carbon dioxide evolution tests for three products; one that is composed primarily of isoparaffinic hydrocarbons, a second that consisted of iso-paraffinic, olefinic, naphthenic and aromatic hydrocarbons, and a third product composed of linear paraffins, iso-paraffins and aromatic hydrocarbons. The procedure used consists of a closed system, which is recommended when assessing the biodegradability of poorly water soluble, volatile materials like those in this category.
These naphtha streams typically contain several different isomers from the PONA hydrocarbon classes mentioned above. This variety of chemical structure can impede achieving a potential maximum extent of biodegradation within a standard testing period because microbial adaptation to a series of differing isomers and chemical classes is likely to occur with numerous stepwise biodegradation lag phases. This can result in a lag period between chemical classes before a maximum degradation rate is once again achieved with the next class. Typically, these data will not clearly exhibit the occurrence of these stepwise events because of the varied metabolic potentials in a mixed bacterial inoculum. As a consequence, the evaluation of data from standard tests performed with these complex products can lead to an underestimation of biodegradation rate. Therefore an acclimation step was employed as reported in the selected data in order to optimize enzymatic activity in the microbial inoculum by pre-adapting the inoculum individually to each of the naphtha product. Following a 14-day acclimation period, ultimate biodegradability as a measure of CO2 evolution was determined in test systems containing the pre-adapted inoculum and fresh naphtha substrate. These data suggest that products in this category can demonstrate relatively high extents of biodegradability and that they are not expected to persist in the environment.

(Paraffinic): Light alkylate naphtha was tested using a mixed adapted inoculum of domestic
activated sludge and soil for 56 days. LAN achieved 42% biodegradation by day 28, slight
increase to 48% by day 42, and a return to 40% by day 56, indicating inherent biodegradability.

(Olefinic): Light catalytically cracked naphtha was tested using a mixed adapted inoculum of
domestic activated sludge and soil for 56 days. LCCN achieved 75% inherent biodegradation by
day 28, which increased slightly to 79% by day 56, indicating inherent biodegradability.

(Napththenic): No biodegradability data is available on any naphthenic sample.

(Aromatic): Light catalytic reformed naphtha was tested using a mixed adapted inoculum of
domestic activated sludge and soil for 56 days. LCRN achieved 96% biodegradation by day 28,
and maintained a level rate to day 56.

Summary: Biodegradation tests of gasoline blending streams high in paraffins, olefins and
aromatics demonstrate inherent biodegradability of 40-96% depending on the stream. Results of
these studies are adequate to characterize the P, O, and A streams. The profile is incomplete
without biodegradation data on a naphthenic enriched sample. **Testing is proposed for a
selected high naphthenic sample using OECD protocol 301F.**

EVALUATION OF EXISTING ECOTOXICITY DATA AND PROPOSED TESTING

The HPV Chemical Test Program includes acute toxicity to a freshwater fish and invertebrate, and
toxicity to a freshwater alga. The products in the Gasoline Naphtha Category are expected to
produce a similar range of toxicity for these three endpoints based on results of comparable
studies using standard test methods and exposure solution preparation procedures since the
aquatic toxicity data for selected products within this category can be predicted based on carbon
number range and constituent composition of those products. (See Appendix 3, discussion of
aquatic toxicity)

Aquatic Toxicity

The following information summarizes reliable representative aquatic toxicity data of selected
naphtha streams prepared as WAFs. Additionally, calculated values for fish and invertebrate
aquatic toxicity are reported for a Full Range Catalytic Reformed Naphtha (CAS # 68955-35-1, API
sample 83-05, high aromatic naphtha). This stream is expected to have a greater aromatic
distribution than the light catalytic reformate naphtha (LCRN, CAS # 64741-63-5) for which reliable
ecotoxicity data have been summarized. In order to evaluate the impact of a higher percentage of
aromatics hydrocarbons, ecotoxicity was estimated (Appendix 3, Calculation Of Acute Toxicity
From Composition) since the hydrocarbon composition and percentage distribution were available
for this sample. Supporting these estimated values is a comparison to ecotoxicity test data for a
LCRN sample developed by Concawe.

(Paraffinic): Light alkylate naphtha, tested as a water accommodated fraction (WAF) induced a 96
hr LL_{50} = 8.2mg/L (95% C.I. 5.2-9.7mg/L) nominal loading rate in fathead minnow and a 48 hr.
EL_{50} = 32mg/L (95% C.I. 18-140mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr
EL_{50} = 45mg/L (95% C.I. 18-70mg/L) in *Selenastrum caprocornutum*.

(Olefinic): Light catalytically cracked naphtha tested as a water accommodated fraction (WAF)
induced a 96 hr LL_{50} = 46 mg/L (95% C.I. 37-74mg/L) nominal loading rate in fathead minnow

and a 48 hr. EL_{50} = 18mg/L (95% C.I. 13-25mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr EL_{50} = 64mg/L (95% C.I. 44-111mg/L) in *Selenastrum caprocornutum*.

(Naphthenic): Ecotoxicity results for two light straight run naphtha (low and high naphthenic content) have been evaluated. Robust summaries for a low naphthenic, Light straight run naphtha (approximately 19.8% naphthenic) tested as a water-accommodated fraction (WAF) have been prepared. Light straight run naphtha (approximately 19.8% naphthenic) tested as a water accommodated fraction (WAF) induced a 96 hr LL_{50} = 15mg/L (95% C.I. 6.3-25mg/L) nominal loading rate in fathead minnow and a 48 hr. EL_{50} = 18mg/L (95% C.I. 12-24mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr EL_{50} = 6.4mg/L (95% C.I. 5.7-7.1mg/L) in *Selenastrum caprocornutum*.

High naphthenic, Light straight run naphtha (Concawe sample W94/809, approximately 34% naphthenic) tested as a water accommodated fraction (WAF), test data reported based on review of gasoline product dossier (Concawe,, Acute, Aquatic Toxicity of Gasolines, report no. 96/57). WAFs of high naphthenic LSRN induced a 96 hr LL50 = 18 mg/L (95% C.I. 15-20 mg/L) based on nominal loading rate in rainbow trout and a 48 hr. EL50 = 4.5 mg/L, nominal loading rate in Daphnia magna. The algal 72 hr EL50 = 3.6 mg/L (95% C.I. 1.7-6.2 mg/L) in Selenastrum capricornutum.

(Aromatic): Light catalytic reformed naphtha tested as a water accommodated fraction (WAF) induced a 96 hr LL_{50} = 34mg/L (95% C.I. 25-50mg/L) nominal loading rate in fathead minnow and a 48 hr. EL_{50} = 10mg/L (95% C.I. 6-12mg/L) nominal loading rate in *Daphnia magna*. The algal 96 hr EL_{50} = 8.5mg/L (95% C.I. 7.3-9.8mg/L) in *Selenastrum caprocornutum*.

High Aromatic (Reformate): Calculated toxicity using hydrocarbon block method and published values:
Full Range Catalytic Reformed Naphtha (CAS # 68955-35-1, API sample 83-05, high aromatic naphtha) Daphnia Acute calculated 48 hr EL50 loading rate =0.9 mg/L; Fish Acute calculated 96 hr LL50 loading rate =2.09 mg/L.
Light Catalytic Reformed Naphtha (CAS # 64741-63-5, Concawe sample W94/812), high aromatic naphtha) Daphnia Acute tested 48 hr EL50 loading rate =8.4 mg/L; Fish Acute tested 96 hr LL50 loading rate =12 mg/L. (Concawe, Acute, Aquatic Toxicity of Gasolines, report no. 96/57.) An analysis of the calculated and reported ecotoxicity for high aromatic reformate naphtha streams having similar composition indicate ecotoxicity between 1 to 10 mg/L WAF loading. The lower measured toxicity of LCRN can likely be attributed to decreased aqueous hydrocarbon concentration resulting from partitioning to vapor headspace, adsorption, and degradation as compared to the more conservative calculated values.

Summary: Aquatic toxicity data is adequate for naphtha streams high in paraffins, olefins, naphthenics and aromatics. Levels of toxicity to different aquatic organisms (the freshwater fish, fathead minnow, aquatic invertebrate, *Daphnia magna*, and alga) varied for each stream and between streams. In general, light alkylate naphtha was the most toxic to fathead minnow, and light straight run naphtha showed greatest toxicity to algae. Sufficient data of good quality were identified to accurately characterize the three aquatic toxicity endpoints in the HPV program for this category. In general, products in this Category have the potential to be moderately toxic to aquatic organisms. **Therefore, no further aquatic testing is proposed.**

Terrestrial Toxicity

Summary: Gasoline blending streams have been demonstrated to be volatile and biodegradable.
No testing is proposed for this endpoint.

Conclusions and Test Proposal

There is sufficient and adequate data to assess the mammalian toxicity and ecotoxicity of naphtha streams high in paraffinic, olefinic, naphthenic and aromatic constituents; data well supported by toxicity studies on the gasoline product (Appendix 3). Modeling for physical properties and environmental endpoints has been completed for all classes of naphthas.

TABLE 4. MATRIX OF AVAILABLE ADEQUATE DATA AND PROPOSED TESTING FOR THE PRIMARY TEST MATERIALS

	P	O	N	A	
	Naphtha, light alkylate 64741-66-8	Naphtha, light catalytic cracked 64741-55-5	Naphtha, heavy straight-run 64741-78-2	Naphtha, catalytic reformed 68955-35-1	Gasoline
Melting Point	N/A	N/A	N/A	N/A	N/A
Boiling Point	Adequate	Adequate	Adequate	Adequate	Adequate
Vapor Pressure	Adequate	Adequate	Adequate	Adequate	Adequate
Partition Coefficient	TD	TD	TD	TD	TD
Water Solubility	Model/TD	Model/TD	Model/TD	Model/TD	Model/TD
Photodegradation	TD	TD	TD	TD	TD
Stability in Water	Model	Model	Model	Model	Model
Transport and Distribution	Model	Model	Model	Model	Model
Biodegradation	Adequate	Adequate	**Test**	Adequate	C
Acute Toxicity to Fish	Adequate	Adequate	Adequate	Adequate	Adequate
Acute Toxicity to Aquatic Invertebrates	Adequate	Adequate	Adequate	Adequate	Adequate
Toxicity to Algae	Adequate	Adequate	Adequate	Adequate	Adequate
Acute Toxicity	Adequate	Adequate	C	Adequate	Adequate
Repeated Dose	Adequate	Adequate	**Test**	Adequate	Adequate
Genotoxicity, in vitro	Adequate	Adequate	C	Adequate	Adequate
Genotoxicity, in vivo	Adequate	Adequate	C	Adequate	Adequate
Repro/Developmental	Adequate	Adequate	**Test**	Adequate	Adequate

Adequate   Indicates adequate existing data.
Test   Indicates proposed testing
Model   Indicates data will be obtained with EPA approved models
C   Indicates category read-across from existing or proposed test data
TD   Indicates technical discussion to define endpoint
N/A   Indicates that evaluation of endpoint is Not Applicable due to physical-chemical state or route of administration.

There is limited data available on naphtha streams high in naphthenes (cycloparaffins). Therefore this study plan proposes a Combined Repeated Dose Toxicity Study with the Reproductive/ Developmental Toxicity Screening Test (OECD protocol 422) and a biodegradation study (OECD protocol 301F) using a selected naphthenic-rich stream, to complete the hazard profile for gasoline blending streams.

REFERENCES

Mammalian Toxicity

Bui, Q.Q., Burnett, D.M., Breglia, R.J., Koschier, F.J., Lapadula, E.S., Podhasky, P.I., Schreiner, C.A., White, R.D., Dalbey, W.E., and Feuston, M.H. 1998. Toxicity evaluation of petroleum blending streams: Reproductive and developmental effects of a distillate from light alkylate naphtha. *J. Toxicol. Environ. Health* 53: 121-133.

Chevron Products Co. 1996. Motor Gasoline Technical Review (FTR-1) San Francisco, CA.

Dalbey, W., and Feuston, M. 1996. Partially vaporized full range catalytic reformed naphtha: Subchronic and developmental toxicity studies in rats. *Inhalation Toxicol*. 8: 271-284.

Dalbey, W.E., Feuston, M.H., Yang, J.J., Kommineni, C.V., and Roy, T.A. 1996. Light catalytically cracked naphtha: Subchronic and developmental toxicity screen in rats. J. *Toxicol. Environ. Health* 47: 77-91.

Halder, C.A., Warne, T.M., and Hatoum, N.S. 1984. Renal toxicity of gasoline and related petroleum naphthas in male rats. Chapter VI in Renal Effects of Petroleum Hydrocarbons, Mehlman et al., Eds. Princeton Scientific Publishers, Princeton, NJ pp. 73-88.

Halder, C.A., Van Gorp, G.S., Hatoum, N.S., and Warne, T.M. 1986. Gasoline vapor exposures Part 1. Characterization of workplace exposures. *Am. Ind. Hyg. Assocs. J.*; 47: 164-172.

Kuna, R.A., and Ulrich, C.E. 1984. Subchronic inhalation toxicity of two motor fuels. *J. Am. Coll. Toxicol*. 3: 217-230.

Lapin, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Podhasky, P., Lapadula, E., Roth, R., Schreiner, C., White, R., Mandella, R., and Hoffman. 2001. Toxicity Evaluation of Petroleum Blending Streams: Inhalation Subchronic Toxicity/Neurotoxicity Study of Light Catalytic Cracked Naphtha Distillate in Rats. *Internat'l J. of Toxicology: in press*

MacFarland, H.N., Ulrich, C.E., Holdsworth, C.E., Kitchen, D.N., Halliwell, W.H., Blum, S.C. A chronic inhalation study with unleaded gasoline vapor. *J. Am. Coll. Toxicol*. 1984; 3: 231-248.

McKee, R.H., Trinmer, G.W., Whitman, F.T., Nessel, C.S., Mackerer, C.R., Hagemann, R., Priston, R.A., Riley, A.J., Cruzan, G., Simpson, B.J., Urbanus, J.H. 2000. Assessment in rats of the reproductive toxicity of gasoline from a gasoline vapor recovery unit. *Reproductive Toxicol.* 14: 337-353.

Roberts, L., White, R., Bui, Q., Daughtrey, W., Koschier, F., Rodney, S., Schreiner, C., Steup, D., Breglia, R., Rhoden., Schroeder., and Newton, P. 2001. Developmental toxicity evaluation of unleaded gasoline vapor in the rat. *Reproductive Toxicol.* 15:

Schreiner, C., Lapadula, E., Breglia, R., Bui, Q., Burnett, D., Koschier, F., Podhasky, P., White, R., Mandella, R., and Hoffman, G.: 1998. Toxicity Evaluations of Petroleum Blending Streams: Inhalation Subchronic Toxicity/ Neurotoxicity Study of a Light Alkylate Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 55: 277-296.

Schreiner, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Lapadula, E., Podhasky, P., White, R., and Schroeder, R.E.: 1999. Toxicity Evaluation of Petroleum Blending Streams: Reproductive and Developmental Effects of Light Catalytic Cracked Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 58:101 –118

Schreiner, C., Bui, Q., Breglia, R., Burnett, D., Koschier, F., Podhasky, P., White, R., Hoffman. G., and Schroeder, R.E.: 2000. Toxicity Evaluation of Petroleum Blending Streams: Reproductive and Developmental Effects of Light Catalytic Reformed Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 60:101 – 116.

Schreiner, C., Breglia, R., Bui, Q., Burnett, D., Koschier, F., Lapadula, E., Podhasky, P., White, R., Hoffman, G. and Mandella, R. 2000. Toxicity Evaluations of Petroleum Blending Streams: Inhalation Subchronic Toxicity/Neurotoxicity Study of a Light Catalytic Reformed Naphtha Distillate in Rats. *J. Toxicol & Environ. Health* 60: 489 – 513

U.S. Environmental Protection Agency. Alpha 2 microglobulin: association with chemically induced renal toxicity and neoplasia in the male rat. 1991. In Risk Assessment Forum. US Government Printing Office, Washington, DC: EPA:85

U.S. Environmental Protection Agency Fuels and Fuels Additives Registration Regulations; Final Rule pursuant to the Clean Air Act 211(b), 211(e). 40 CFR Part 79, FR59, No. 122: 33042-33142 (27 June 1994). Washington, DC:EPA. 1994

U.S. Environmental Protection Agency. Fuels and Fuels Additives Registration Regulations; Final Revised 211(b) Tier2 Testing Requirements. Correspondence to Director of Health and Environmental Sciences Dept., American Petroleum Institute. November 2, 1998.

Ecotoxicity

Cline, P.V. et al. 1991. Partitioning of aromatic constituents into water from gasoline and other complex solvent mixtures. *Environ Sci Technol 25: 914-920*

CONCAWE. 1996. Environmental risk assessment of petroleum substances: the hydrocarbon block method. Report No. 96/52. Brussels: CONCAWE

Deneer, J.W. et al. 1988. The joint toxicity to *Daphnia magna* of industrial organic chemicals at low concentrations. *Aquatic Toxicology* 12: 33-38).

Di Toro, D.M., J.A. McGrath, and D.J. Hansen. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. *Environmental Toxicology and Chemistry* 19: 1951-1970.

Hermens, J.L.M. et al. 1985. Quantitative structure-activity relationships in aquatic toxicity studies of chemicals and complex mixtures. *Environ Toxicol Chem* 4: 273-279.

McCarty, L.S. and D. Mackay. 1993. Enhancing ecotoxicological modeling and assessment. *Environmental Science and Technology* 27:1719-1728.

McCarty, L.S. et al. 1991. Interpreting aquatic toxicity QSARs: the significance of toxic body residues at the pharmacologic endpoint. In: Hermens, J.L.M. and Opperhuizen, A. (Eds). QSAR in environmental toxicology. Volume IV, p. 515-525. Amsterdam: Elsevier

Peterson, D.R. 1994. Calculating the aquatic toxicity of hydrocarbon mixtures. *Chemosphere* 29,(12)*: 2493-2506*

Solano-Serena, F., R. Marchal, D. Blanchet, and J-P. Vandecasteele. 1998. Intrinsic Capacities of Soil Microflorae for Gasoline Degradation. *Biodegradation* 9:319-326.

Solano-Serena, F., R. Marchal, M. Ropars, J.-M. Lebeault, and J.-P. Vandecasteele. 1999. Biodegradation of Gasoline: Kinetics, Mass Balance and Fate of Individual Hydrocarbons. *J. Appl. Microbiol*. 96:1006-1016.)

Van Wezel, A.P. and A. Opperhuizen. 1995. Narcosis Due to Environmental Pollutants in Aquatic Organisms: Residue-Based Toxicity, Mechanisms, and Membrane Burdens. *Critical Reviews in Toxicology* 25:255-279.

Verbruggen et al. 2000. Polyacrylate-Coated SPME Fibers as a Tool To Simulate Body Residues and Target Concentrations of Complex Organic Mixtures for Estimation of Baseline Toxicity. *Environ. Sci. Technol*. 34: 324-331

Appendix 1

Gasoline Category Constituents by CAS

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 87 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	Substance
008006619	Gasoline, natural
008030306	Naphtha from natural gas
008032324	Ligroine
008052413	Stoddard solvent
064741419	Naphtha (petroleum), heavy straight-run
064741420	Naphtha (petroleum), full-range straight-run
064741464	Naphtha (petroleum), light straight-run
064741475	Natural gas condensates (petroleum)
064741486	Natural gas (petroleum), raw liquid mix
064741544	Naphtha (petroleum), heavy catalytic cracked
064741555	Naphtha (petroleum), light catalytic cracked
064741635	Naphtha (petroleum), light catalytic reformed
064741646	Naphtha (petroleum), full-range alkylate
064741657	Naphtha (petroleum), heavy alkylate
064741668	Naphtha (petroleum), light alkylate
064741680	Naphtha (petroleum), heavy catalytic reformed
064741691	Naphtha (petroleum), light hydrocracked
064741704	Naphtha (petroleum), isomerization
064741726	Polymerization naphtha, intermediate C6-C12
064741748	Naphtha (petroleum), light thermal cracked
064741782	Naphtha (petroleum), heavy hydrocracked
064741839	Naphtha (petroleum), heavy thermal cracked
064741840	Naphtha (petroleum), solvent-refined light
064741873	Naphtha (petroleum), sweetened
064741920	Naphtha (petroleum), solvent-refined heavy
064741997	Extracts (petroleum), light naphtha solvent
064742229	Naphtha (petroleum), chemically neutralized heavy
064742230	Naphtha (petroleum), chemically neutralized light
064742489	Naphtha (petroleum), hydrotreated heavy
064742490	Naphtha (petroleum), hydrotreated light
064742730	Naphtha (petroleum), hydrodesulfurized light
064742821	Naphtha (petroleum), hydrodesulfurized heavy

064742898	Solvent naphtha (petroleum), light aliph.
064742956	Solvent naphtha (petroleum), light aromatic
067891796	Distillates (petroleum), heavy arom.
067891809	Distillates (petroleum), light arom.
068333299	Residues (petroleum), light naphtha solvent extracts
068410059	Distillates (petroleum), straight-run light
068410719	Raffinates (petroleum), cat. reformer ethylene glycol-water countercurrent exts.
068410968	Distillates (petroleum), hydrotreated middle, intermediate boiling
068410979	Distillates (petroleum), light distillate hydrotreating process, low-boiling
068410980	Distillates (petroleum), hydrotreated heavy naphtha, deisohexanizer overheads
068425310	Gasoline (natural gas), natural
068475796	Distillates (petroleum), catalytic reformed depentanizer
068476437	Hydrocarbons, C4-C6, C5-rich
068476460	Hydrocarbons, C3-C11 catalytic cracker distillates
068476506	Hydrocarbons, C>=5, C5-C6-rich
060476551	Hydrocarbons, C5-rich
068476562	Hydrocarbons, cyclic C5 and C6
068477349	Distillates (petroleum), C3-C5, 2-methyl-2-butene-rich
068477634	Extracts (petroleum), reformer recycle
068477894	Distillates (petroleum), depentanizer overheads
068478126	Residues (petroleum), butane splitter bottoms
068478159	Residues (petroleum), C6-C8, catalytic reformer
068478160	Residual oils (petroleum), deisobutanizer tower
068513020	Naphtha (petroleum), full-range coker
068513031	Naphtha (petroleum) light catalytic reformed, arom.-free
068513633	Distillates (petroleum), catalytic reformed straight-run naphtha overheads
068514158	Gasoline, vapor recovery
068514385	Hydrocarbons, C4-C10 Unsaturated
068514794	Petroleum products, hydrofiner-powerformer reformats
068526523	Alkenes, C6-rich
068526556	Alkenes, C9-rich
068527219	Clay treated naphtha, full range, C4-C11
068527264	Naphtha (petroleum) light steam-cracked, debenzenized, C4-C12
068527275	Naphtha (petroleum, full-range alkylate), butane contg.
068551166	Alkanes, C9-C11-iso
068551177	Alkanes, C10-C13-iso
068602799	Distillates (petroleum), benzene unit hydrotreater dipentanizer overheads
068603010	Distillates (petroleum), thermal cracked naphtha and gas oil, C5-dimer-contg
068603087	Naphtha (petroleum), arom.-contg.
068606111	Gasoline, straight-run, topping-plant
068783119	Polymerization naphtha, light C5-C11
068783120	Naphtha (petroleum), unsweetened
068783664	Naphtha (petroleum), light, sweetened
068919153	Hydrocarbons, C6-C12, benzene-recovery
068919379	Naphtha (petroleum), full-range reformed
068919391	Natural gas condensates
068920069	Hydrocarbons, C7-9
068921084	Distillates (petroleum), light straight run gasoline fractionation stabilizer overheads
068921095	Distillates (petroleum), naphtha unifiner stripper
068955293	Distillate (petroleum), light thermal cracked, debutanized arom.
068955351	Naphtha (petroleum), catalytic reformed
070024929	Alkanes, C7-C8-iso
070693060	Aromatic hydrocarbons, C9-C11
070955087	Alkanes, C4-C6
092045584	Isomerization naphtha

APPENDIX 2

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
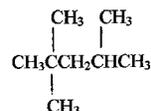
Olefins: C_nC_{2n} are similar to paraffins but have 2 fewer hydrogen atoms and contain at least one double bond (e.g. 2-butene, $CH_3CH=CHCH_3$). Olefins with 4 or more carbons can exist as structural isomers. Cyclic olefins are present in cracked products and are found mostly in motor gasoline, for example:
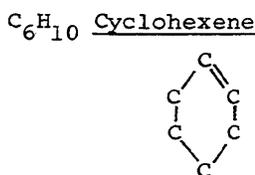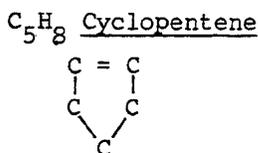
Naphthenes: Cycloparaffins in gasoline have 5 or 6 carbon atoms arranged in a ring and belong to either a cyclopentane or cyclohexane series, for example:
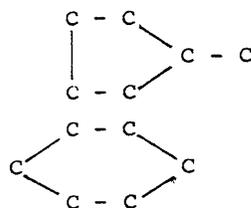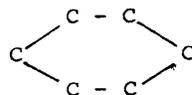
Aromatics: Some carbon atoms are arranged in a ring joined by aromatic bonds. for example:
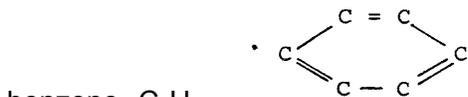
benzene, C_6H_6 .   In polycyclic aromatics, some carbons are shared by 2 or more rings, for example, indane, C_9H_{10}
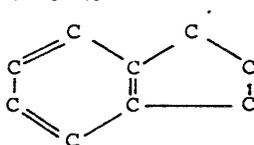
A Short Course in Gasoline Refining

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 id 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 is 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 which 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, 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 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 3

Gasoline Mammalian Toxicity

Acute toxicity

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.

Repeat Dose Toxicity

Thirteen week inhalation toxicity studies were performed with wholly vaporized leaded and unleaded gasoline at concentrations of 0, 100 and 400ppm, or 0, 400, 1500ppm (1493, 5597 mg/m^3) respectively, in Sprague Dawley rats and squirrel monkeys (API, 1976, Kuna and Ulrich, 1984). Light hydrocarbon nephropathy was induced in kidneys of male rats exposed to leaded or unleaded gasoline but not in kidneys of squirrel monkeys. In rats, slight increases in platelet 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 dose of unleaded gasoline, a dose that was 4 times higher than that of leaded gasoline. 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 (API, 1983, McFarland et al, 1984). Mortality rates were unaffected. Rats and mice in the highest dose group had lower body weights throughout the study. Kidney weights of male rats were elevated accompanied by 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). In mice, liver tumors were present in high dose females.
A testing program currently in progress under Clean Air Act 211(b) includes a 13 week rat inhalation study of "industry average" gasoline vapor at concentrations of 2000, 10000 and 20000 mg/m3 (650, 3250 and 6500ppm) which also includes neurotoxicity, immunotoxicity, and cytogenetic endpoints. The vapor condensate of an EPA designated "industry average" gasoline was distilled by a method acceptable to EPA that produce a light end vapor similar in composition to vehicle exposure emissions. This test material induced light hydrocarbon nephropathy with minimal other systemic effects but did not cause neurobehavioral or neuropathologic effects and did not cause immunotoxic responses in spleen cells.

In Vitro Genetic Toxicology

Unleaded gasoline samples, diluted in dimethyl sulfoxide, tested in the Ames *Salmonella* microbial mutation assay and in the mouse lymphoma (L5178Y TK+/ -) forward mutation assay did not induce mutagenic events with or without metabolic activation in either test system. Gasoline was also negative in an Unscheduled DNA synthesis assay in rat hepatocytes. In the CAA 211(b) test program, "industry average" gasoline vapor is being tested in the Ames *Salmonella* assay with and without metabolic activation

In Vivo Genetic Toxicology

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) 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). Gasoline did not induce chromosome

aberrations or disruption of cell cycle kinetics in either regime at any dose level. In the CAA 211(b) test program currently in progress, "industry average" gasoline vapor is being evaluated for sister chromatid exchange in peripheral blood, and chromosome aberrations in bone marrow of rats exposed in a 13 week inhalation study.

In the dominant lethal assay, gasoline was administered by inhalation to male mice 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. At termination of exposure, males were mated with untreated females; females were then sacrificed 14 days after mating (approx. 2/3rd through pregnancy) and uterine contents evaluated. Gasoline exposure of male mice did not cause any significant reduction in fertility index, did not affect the number of total implants or number of dead implants/pregnant female.

Reproductive and Developmental Toxicity

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, 1493, and 5970mg/m^3 (0, 400 and 1600ppm) from day 6-15 of gestation; caesarean sections were performed on day 20. 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 (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 concentrations of 0. 2653, 7960, and 23900mg/m^3 (0, 1000, 3000, and 9000ppm) 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 observed on any reproductive parameter (pregnancy ratio, live litters, implantation sites, litters with resorptions, dead fetuses, litter size, fetal weights) or fetal malformations or variations. NOAEL for maternal and developmental toxicity = 23900mg/m^3. (Roberts et al, 2001).

Vapor recovery gasoline was evaluated by inhalation for reproductive toxicity in a 2 generation reproductive toxicity screen in Sprague Dawley rats at concentrations of 0, 5000, 1000, and 20000mg/m^3 (0, 1850, 3700 and 7400ppm) in accordance with OECD protocol 416 and US EPA OPPTS 870.3800 draft guideline for reproduction and fertility effects (1994). There were no treatment related systemic in parental females and only the species and sex specific hyaline droplet nephropathy was observed in kidneys of male rats of both generations. No reproductive parameters were affected and there were no deleterious effects on offspring survival and growth. Sperm count and quality were comparable in all dose groups. NOAEL reproductive toxicity = 20000mg/m^3 (McKee et al, 2000). In the Clean Air Act 211(b) test program, "industry average" gasoline vapor is being evaluated in both a developmental toxicity assay and a 2-generation reproduction assay (in progress).

Gasoline Environmental Toxicity (Experimental data only)

Aquatic Toxicity-Mode of Action
The aquatic toxicity data for selected products within this category can be predicted based on carbon number range and constituent composition of those products. This is because the constituent chemicals of those products are neutral organic hydrocarbons whose toxic mode of action is non-polar narcosis. The toxic mechanism of short-term toxicity for these chemicals is disruption of biological membrane function (van Wezel and Opperhuizen, 1995), and the 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). The existing fish toxicity database for hydrophobic neutral chemicals supports a critical body residue (CBR, the internal concentration that causes mortality) of between 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). Products in this category are multi-constituent hydrocarbons containing various combinations of isomeric structures (i.e., n-paraffinic, isoparaffinic, cycloparaffinic, olefinic and aromatic) and with carbon (C) numbers ranging primarily between C4 to C12.

Multi-constituent hydrocarbon solvent products with a range of carbon numbers and water solubility as those in this category are expected to exhibit lower toxicity compared to the most toxic constituent alone. This occurs because the aqueous concentration of the constituent is a function of the partitioning of the constituents between the bulk hydrocarbon and water. Within the carbon number range of products in this category, a C9 hydrocarbon alone would be expected to exhibit the greatest toxicity based on the relationship of Kow with aquatic toxicity. However, products in this category are not composed of a single chemical and because two different products with a similar carbon number range can contain varying proportions of those carbon numbers, it is possible that different toxicities are expressed for the same organism. Thus, two products representing low or high carbon number ranges in this category can show different toxicities. Therefore, characterizing the fish, daphnid, and algal toxicity of this category using values from the low and high carbon number ranges is supported.

The endpoint values for the three trophic levels reflect the loading rates of the test substance added to exposure solutions prepared as water accommodated fractions (WAF) in closed test systems. The WAF method is described in the relevant Robust Summaries provided with this test plan. This method is the appropriate procedure for products in this category because these products are multi-constituent hydrocarbons whose constituent hydrocarbons vary in water solubility. The dissolution thermodynamics of a multi-constituent hydrocarbon in an aqueous medium prevent the possibility of achieving consistent proportional concentrations of the constituent hydrocarbons at various test substance loading rates. For this reason:

- exposure solutions are not prepared from dilutions of a stock solution (the relative proportion of hydrocarbon constituents in the dilutions would not accurately reflect the relative concentration of those constituent chemicals in individually prepared, successively lower exposure solutions of the test material);

- separate exposure solutions are prepared at each exposure loading for products that are multi-constituent hydrocarbons; and

- results for multi-constituent hydrocarbons are expressed as lethal loadings (LL) rather than lethal concentrations (LC) as is possible for single, water-soluble chemicals.

CALCULATION OF ACUTE TOXICITY FROM COMPOSITION

There are two situations when it may be necessary to estimate the toxicity of a petroleum substance viz., to validate test results and to predict toxicity when data are lacking. This approach requires that the chemical composition of the petroleum substance should be known. 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) which can be summed to predict the toxicity of the parent material (see below). As previously described, the quantity of any particular component of a petroleum substance detected in the water phase is related to the loading rate. Theoretically, using closed test systems brought to equilibrium, simple equilibrium partitioning and mass balance calculations may be used to estimate the concentration of each hydrocarbon constituent in water. The hydrocarbon/water partition coefficient (Kp) for each of the components is an essential part of the calculation. The details of this calculation approach have been published (Peterson, D.R., 1994). Further simplification is

obtained by combining the concentration calculations for isomers of particular hydrocarbon species (e.g. iso-hexanes), since all of the isomers have essentially the same values of log Kow and Kp. This procedure is the equivalent of the "hydrocarbon block method" used in the risk assessment of petroleum substances (CONCAWE, 1996; Hermens, J.L.M. et al., 1985). Experimental Kp values (Peterson, D.R., 1994; Cline, P.V. et al., 1991) simply related to Kow, for individual hydrocarbons are available in the published literature. In order to calculate the joint toxic action of a mixture of hydrocarbons dissolved in water, the concentrations cannot be added directly. Since each component will have a different toxicity, the concentration of each component must be scaled to its toxicity. This is done by division of the concentration by the toxicity (by the LL_{50} in the case of acute toxicity). The resulting values express the concentrations in equivalent "toxic units." Thus, the sum of TUs for the components of a mixture will equal one at the LL_{50} of the mixture. Considerable experimental support for this conceptual framework has been developed, which confirms that mixtures of substances exerting toxicity via a common mechanism, are additive and further, that hydrocarbons act through a common mechanism of non-polar narcosis (Hermens, J.L.M. et al, 1985; Deneer, J.W. et al., 1988). Toxicity QSARs may be used to provide LL_{50} estimates for hydrocarbons or blocks where acute toxicity data are not available, since these are well established for hydrocarbons (details are included in the EU Technical Guidance Document (TGD) which recommends procedures for risk assessment). Furthermore, the use of QSAR allows for extrapolation of LL_{50} values to hydrocarbons or blocks that are beyond the solubility 'cut-off' and have no measured LC_{50} value. This provides a conservative approach for assessing the partial contribution of hydrocarbons or blocks that are individually not expected to exert toxicity.

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 undissolved 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.

Aquatic Toxicity

(Gasoline): Ecotoxicity results for two blended gasolines (Concawe samples W94/813 and W94/814) have been evaluated and robust summaries for these samples tested as a water-accommodated fraction (WAF) have been prepared.

Concawe sample W94/813, (PONA 48-1-5-46) WAF induced a 96 hr LL_{50} = 11 mg/L (95% C.I. 9-16 mg/L) nominal loading rate in rainbow trout and a 48 hr. EL_{50} = 7.6 mg/L (95% C.I. 6.4-9.3mg/L) nominal loading rate in *Daphnia magna*. The algal 72 hr EL_{50} = 1.4 mg/L (95% C.I. 0-20 mg/L) in *Selenastrum capricornutum*.
Concawe sample W94/814, (PONA 40-12-6-41) WAF induced a 96 hr LL_{50} = 16 mg/L (95% C.I. 10-25mg/L) nominal loading rate in rainbow trout and a 48 hr. EL_{50} = 12 mg/L (95% C.I. 7.3-22 mg/L) nominal loading rate in *Daphnia magna*. The algal 72 hr EL_{50} = 4.2 mg/L (95% C.I. 0-24 mg/L) in *Selenastrum capricornutum*.

Biodegradation
(Gasoline):

Biodegradability of a commerical gasoline in aqueous medium was evaluated by measuring the disappearance of hydrocarbon constituents by gas chromatography with flame ionization detector, O_2 consumption (respirometry), and CO_2 production by gas chromatography with thermal

conductivity detector (Solena-Serena et al., 1999). Activated sludge microorganisms were found to biodegrade unleaded commercial gasoline up to 94% within 25 days. The carbon balance of gasoline degradation showed that 61.7% of gasoline was mineralized to CO2 and that microbial cell production accounted for the remaining carbon of gasoline degraded. For each hydrocarbon class, degradation occurred at different rates. Aromatic compounds were found to be the most readily consumed, although compounds bearing neighboring substituents and those containing longer alkyl groups were consumed at a slower rate than those with no or only one alkyl chain. Likewise, linear alkanes (exception for undecane), alkenes with five to nine carbons, cyclohexane and substituted cyclopentanes were biodegraded. Residual components of gasoline most recalcitrant to biodegradation were found to be branched alkanes, particularly those containing a quaternary carbon and/or alkyl chains on consecutive carbon atoms. The results of this study indicated that under the conditions of this test, the majority of gasoline constituents are rapidly and ultimately biodegraded by aquatic microorganisms.

Solano-Serena et al (1998) also evaluated the biodegradability of a representative gasoline prepared as a composite of 23 typical gasoline hydrocarbons by soil microflora suspended in aqueous media. The method of analysis of parent mixture, individual components, and CO_2 production was made by gas chromatography with flame ionization detector. The gasoline model mixture GM3 was degraded about 89% by a native soil suspension, based on GC/FID analysis of the initial and residual individual hydrocarbon concentrations. The results of this study indicated that the pattern of gasoline degradation was represented as the sum of the degradation of the individual compounds. No marked occurrence of co-metabolism was observed. Inhibitory effects were observed for 1,3,5 trimethylbenzene, 2-ethyltoluene and 1,2,3 trimethylbenzene at 200 mg/L, but were totally degraded at 35 mg/L by non-acclimated soil suspensions. The use of optimized degradative inoculum (soil microbes pre-exposed to cyclohexane and 2,2,4 TMP) in conjunction with non-acclimated soil organisms enhanced both rate and extent of the more structurally complex hydrocarbons that showed little to minimal degradation in non-acclimated soil systems.

APPENDIX 4

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) N0 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 bold-face 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: To be selected

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 5

Robust Summaries: Separate Documents