NAPHTHENIC ACIDS CATEGORY
ANALYSIS AND HAZARD CHARACTERIZATION

Submitted to the US EPA
by
The American Petroleum Institute
Petroleum HPV Testing Group
www.petroleumhpv.org

Consortium Registration # 1100997

May 14, 2012

Table of Contents

Summary

Naphthenic acids are extracted from kerosene and diesel streams in the refinery to improve the performance characteristics and storage properties of the finished products. Accordingly, from a petroleum industry perspective, the naphthenic acid streams are wastes; however, in some cases streams containing naphthenic acids can be treated to produce refined naphthenic acids which can be used as intermediates in the production of other substances. The major uses of substances derived from refined naphthenic acids are oil-soluble metal soaps for driers and other catalysts, wood preservatives, tire cord adhesion promoters, and as amine derivatives for corrosion inhibitors.

Previous data indicated that naphthenic acids have a low to moderate degree of acute mammalian toxicity, and do not produce mutation under *in vitro* conditions. The petroleum industry HPV Testing Group (HPVTG) determined that additional studies were necessary to adequately characterize the potential systemic toxicity and/or reproductive and developmental hazards of naphthenic acids and proposed that a combined repeated-dose, reproductive/developmental toxicity screening test be conducted and that the potential for micronucleus induction also be evaluated in that study to assess the potential for *in vivo* mutational effects. In addition, toxicity testing in fish, aquatic invertebrates, and algae was conducted to address the potential aquatic toxicity of naphthenic acids. There is sufficient data on the physicochemical and environmental fate of naphthenic acids to characterize the potential for physical/chemical hazards of these substances.

Toxicology studies conducted to fulfill the petroleum industry obligation under the HPV program provide evidence that refined naphthenic acids may produce systemic effects in rats although the effects observed are of doubtful toxicological significance to humans. The no effect level for all systemic effects was 100 mg/kg/day. The reproductive toxicity screening test revealed that refined naphthenic acids can be toxic to developing fetuses. The observed effects included an elevated frequency of resorptions, a reduction in live born offspring and reduced fetal weight. The overall no effect level for developmental effects was 100 mg/kg/day. The frequency of micronucleus induction was not increased, providing evidence that naphthenic acids are not mutagenic under *in vivo* conditions.

New studies were conducted to provide sufficient data to characterize the potential for the acute ecotoxicity of naphthenic acids. Toxicological endpoints were addressed experimentally and the results were then compared using loading rates for water accommodated fractions (WAFs) and measured concentrations of total dissolved naphthenic acids in the WAFs. These comparisons revealed that fish were the most sensitive aquatic organisms with 96-hour LL50 and LC50 values of 9.0 mg/L and 5.6 mg/L, respectively. The 48-hour EL50 and EC50 endpoints for the toxicity of naphthenic acids to invertebrates (*Daphnia magna*) were 24 mg/L and 20 mg/L, respectively. The 72- and 96-hour toxicity values reflecting the toxicity of naphthenic acids to *Pseudokirchneriella subcapitata* ranged from 30 mg/L to 43 mg/L when calculated on the basis of growth rate. These same endpoints determined on the basis of biomass yield ranged from 18 mg/L to 25 mg/L. These ranges covered both loading rate-based (EL50 values) and concentration-based endpoints (EC50 values).

Substances in the Category

Naphthenic acids are a naturally occurring, complex mixture of cycloaliphatic carboxylic acids recovered from petroleum distillates. The naphthenic acid-containing chemical substances sponsored by the Testing Group are as follows:

64754-89-8
Naphthenic acids (petroleum), crude
A complex combination of compounds, predominantly naturally occurring organic acids, obtained from petroleum fractions by saponification and acidification. It consists predominantly of compounds which contain carboxylic acid functional groups and five- to six-member naphthenic rings in their molecular structures. Phenolic compounds and acidic sulfur compounds may also be present.

1338-24-5
Naphthenic acids
The term “Naphthenic acids”, as used in the petroleum industry, refers collectively to all of the carboxylic acids present in crude oil. Naphthenic acids (CASRN 1338-24-5 and 64754-89-8) are classified as monobasic carboxylic acids of the general formula RCOOH, in which R represents the naphthene moiety consisting of cyclopentane and cyclohexane derivatives. Naphthenic acids are composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids, with smaller amounts of acyclic aliphatic acids. The cycloaliphatic acids include single and fused multiple cyclopentane and cyclohexane rings. The carboxyl group is usually attached to a side chain rather than directly to the ring. Aromatic, olefinic, hydroxy and dibasic acids are present as minor components (Brient et al, 1995).

A third related substance, Naphthenic acids, sodium salts (CASRN 61790-13-4) is not officially sponsored in the HPV Challenge but is part of the refining process of removing naphthenic acids from petroleum products and therefore discussed in this category assessment document as a supporting chemical.

Naphthenic acids recovered from refinery streams occur naturally in the crude oil and are not formed during the refining process. Heavy crudes have the highest acid content, and paraffinic crudes usually have low acid content. Although the presence of naphthenic acids has been established in almost all types of crude oil, only certain naphthenic and asphalt based crudes contain amounts that are high enough to require treatment in order to meet product specifications.

Naphthenic acids are obtained by caustic extraction of petroleum distillates, primarily kerosene and diesel fractions. In addition to reducing corrosion in the refinery, the caustic wash of the distillates is necessary to improve the technical properties, storage stability, and odor of the finished kerosene and diesel fuels. The commercial production of naphthenic acid from petroleum is based on the formation of sodium naphthenates which occurs when the petroleum distillates are treated with sodium hydroxide caustic. Since this reaction occurs *in situ*, naphthenic acids, sodium salts (CASRN 61790-13-4) is considered an intermediate stream in the production of refined naphthenic acid. The sodium naphthenate-containing solutions contain approximately 5-15% sodium naphthenate, 0-0.5% sodium mercaptide and 3-4% sodium hydroxide in water with a pH > 12. These caustic solutions are typically sent to specialized facilities in which they undergo further processing to recover the naphthenic acids.

The first step in recovery of the naphthenic acid involves “springing” (acidulating) the caustic solutions produced in the refinery to recover the organic acids. The resulting intermediate stream is crude naphthenic acids (petroleum), CASRN 64754-89-8. This is followed by a series of additional refining steps, including distillation, to recover the naphthenic acids.

The major uses of naphthenic acids are as oil-soluble metal soaps for driers and other catalysts, wood preservatives, tire cord adhesion promoters, and in amine derivatives for corrosion inhibitors.

Summary – Naphthenic acids are a naturally occurring component of crude oil that is removed during the manufacture of petroleum products such as diesel fuel, jet fuel and kerosene to improve their technical characteristics. The removal process generates a waste stream that can be reprocessed to obtain refined naphthenic acids that are useful as wood treatments, corrosion inhibitors, emulsifiers, defoamers, paint and ink driers, tire cord adhesives, fuel additives, cutting oils and vinyl stabilizers.

Category Rationale

Of the three substances discussed in the naphthenic acid category, refined naphthenic acids (CASRN 1338-24-5) is the only material sold commercially. The other two CAS numbers represent intermediates in the production of refined naphthenic acids. Since all three of these substances contain the same basic naphthenic acid molecules, information on the health and environmental effects of refined naphthenic acids can be used to assess the potential hazards of the other two intermediate streams. The Testing Group has reviewed the available health, environmental and physicochemical information on refined naphthenic acids and has used this information to characterize the potential hazards of all of the substances in the category.

DESCRIPTION OF TEST SUBSTANCE

Naphthenic acids are naphthenes (cycloparaffins) that contain carboxyl groups and are described by the general formula $C_nH_{2n+z}O_2$ in which n indicates the carbon number and z is zero or a negative integer that specifies the hydrogen deficiency resulting from ring formation. The absolute value of z divided by 2 gives the number of rings in the compounds. The acyclic compounds are highly branched, unlike fatty acids (Clemente and Fedorak, 2005). Examples of proposed structures representing different z groups are shown below.

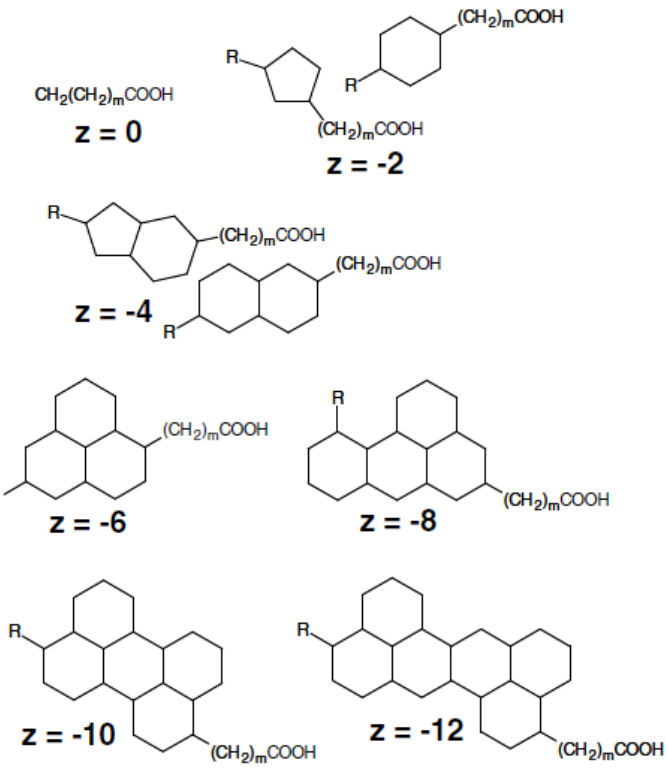


Figure 1. Examples of different naphthenic acids compounds with examples of ring families (Z) and alkyl (R) and carboxyl (m) side chains (taken from Frank et al., 2008).

The test sample (HPV test sample) used in the current program was a blend of naphthenic acids from three sources. The samples were dried under a stream of nitrogen and then re-dissolved in 0.5 mL dichloromethane. The samples were analyzed by GC-MS (Young et al., 2008) and the total ion current mass spectra were collected and tabulated (Holowenko et al., 2002). An analysis of the HPV test sample showed the substance contained constituents with carbon numbers predominantly in the range of C6-C16 (corresponding to a molecular weight range of approximately 116-250) and with a ring distribution of approximately 0 rings (24%), 1 ring (39%), 2 rings (30%), 3 rings (5%) and 4 rings (2%).

Composition of the HPV sample on the basis of Z-family distributions was compared to similar analyses for extracts of naphthenic acids derived from oil sands process water (Rogers et al., 2002a,b,c; Rogers 2003). The sample was described as having a carbon number range of 14-27 (corresponding to a molecular weight range of 220-368) and a ring distribution of 0 rings (20%), 1 ring (23%), 2 rings (20%), 3 rings (20%) and 4 rings (18%) (Rogers, 2003). In other words, the test material used by Rogers was of higher molecular weight than the refined naphthenic acid sample used in the present studies and had greater percentages of constituents with more rings. A comparison of the two sources of naphthenic acids (i.e., HPV test substance and Rogers, 2003) is presented in Figure 2.

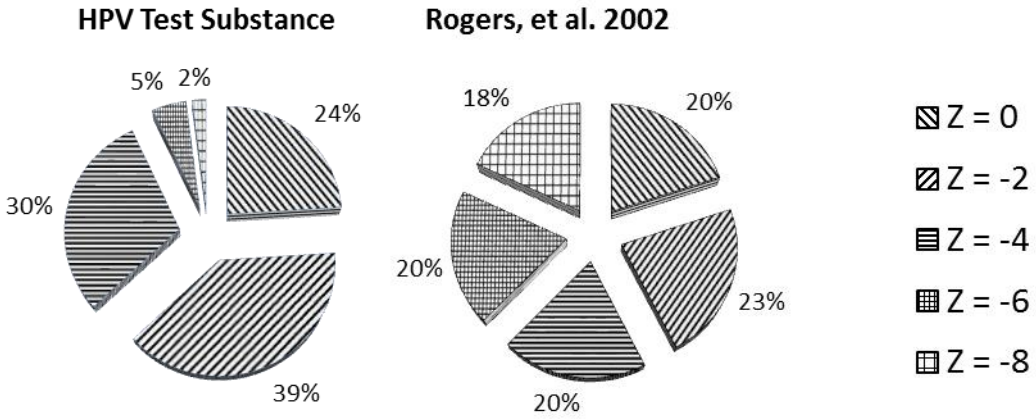


Figure 2. Comparison of Z-family composition of the naphthenic acids used in new testing conducted for the HPV program and that reported by Rogers et al. (2002c) for oil sands tailings pond extract.

The oil sands extracts used in testing described in Rogers et al. (2003) was composed of a greater proportion of higher molecular weight naphthenic acid isomers than the HPV sample. Three and four ring constituents comprised 38% of their test sample whereas they totaled 7% of the HPV test sample. The higher molecular weight naphthenic acid constituents in the oil sands extracts may have implications to the interpretation of effects found in the current studies, as these were qualitatively similar to those reported by Rogers, 2003. Rogers drew attention to the fact that that the material he had isolated from oil sands had properties that differed from those of commercial materials that he was using for standards, and he suggested that these differences translated into differences in toxic properties. In fact, there were differences in toxic properties as discussed in more detail below, but the extent to which these are due to differences in the naphthenic acids *per se*, possible contaminants in the sample isolated by Rogers which were not present in commercial materials, or methodological differences in the

test protocols is not known. Table 1 presents a more in-depth tabulation of isomeric constituents for different carbon number and ring distribution families found in the HPV test sample. A graphical distribution is shown in Figure 3.

Table 1. Mean percentages of naphthenic acid constituents for different C-number and Z-number families measured in the test sample (n=3).

C Number	Z = 0	Z = -2	Z = -4	Z = -6	Z = -8	Z = -10	% C Number
5	0.9	0.0	0.0	0.0	0.0	0.0	0.9
6	0.4	0.0	0.0	0.0	0.0	0.0	0.4
7	0.9	0.8	0.0	0.0	0.0	0.0	1.7
8	0.7	0.5	0.0	0.0	0.0	0.0	1.2
9	0.6	1.0	0.0	0.0	0.0	0.0	1.6
10	1.8	3.3	1.2	0.0	0.0	0.0	6.2
11	3.2	7.0	4.2	0.0	0.0	0.0	14.4
12	4.6	8.5	7.1	0.6	0.0	0.0	20.8
13	3.6	7.4	7.1	1.0	0.1	0.0	19.3
14	3.0	4.9	4.8	1.1	0.3	0.0	14.1
15	1.7	2.6	2.6	0.8	0.3	0.0	8.0
16	0.8	1.4	1.5	0.5	0.2	0.1	4.5
17	0.4	0.7	0.8	0.4	0.1	0.1	2.5
18	0.3	0.4	0.5	0.2	0.1	0.1	1.6
19	0.2	0.3	0.3	0.1	0.1	0.1	1.1
20	0.2	0.2	0.2	0.1	0.1	0.0	0.8
21	0.1	0.1	0.1	0.0	0.0	0.0	0.3
22 - 33	<0.1	<0.1	<0.1	<010	<0.1	<0.1	0.1
Total %	23.5 %	39.0 %	30.2 %	4.9 %	1.3 %	0.4 %	100 %

Based on these data it was determined that there were no significant differences between the naphthenic acids from the three sources (Fedorak, 2009), and they were then combined to produce the test sample.

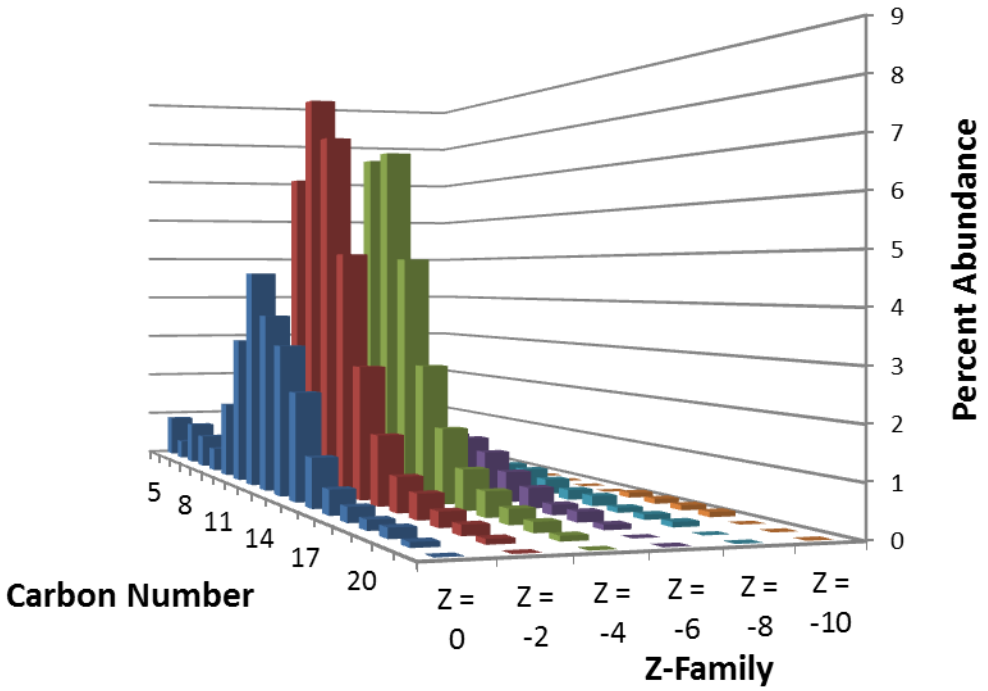


Figure 3. Graphical representation of the distribution of carbon number and ring families (Z) determined for the HPV test sample.

The different molecular weights associated with these groupings are shown in Table 2.

Table 2. Molecular weights for different carbon numbers and z-series of naphthenic acids ($C_nH_{2n+z}O_2$).

Number of Carbon Atoms (n)	Series Z = 0 (open chain)	Series Z = -2 (one ring)	Series Z = -4 (two rings)	Series Z = -6 (three rings)	Series Z = >-6 (more than 3 rings)
5	102	--	--	--	--
6	116	--	--	--	--
7	130	128	--	--	--
8	144	142	--	--	--
9	158	156	--	--	--
10	172	170	168	--	--
11	186	184	182	--	--
12	200	198	196	197	--
13	214	212	210	208	--
14	228	226	224	222	➢ 220
15	242	240	238	236	➢ 234
16	256	254	252	250	➢ 248
17	270	268	266	264	➢ 262
18	284	282	280	278	➢ 276
19	298	296	294	292	➢ 290
20	312	310	308	306	➢ 304

21	326	324	322	320	➢ 318

Health Effects Data

This section addresses the mammalian toxicity endpoints by:

1. Reviewing the literature for relevant studies and then evaluating these reports for suitability for use in a regulatory context;
2. Using data from studies of similar materials for read-across purposes; and
3. Conducting additional testing when necessary to provide additional information.

The toxicological endpoints which require assessment to fulfill the HPV obligations include: acute toxicity, repeated dose toxicity, *in vitro* and *in vivo* mutagenicity, and reproductive/developmental toxicity. When complete studies were not available for review (e.g., technical meeting abstracts or poster presentations), the summary information is provided, and the source citations are provided in the Reference section of this document. In addition, information from studies conducted following relevant non-OECD SIDS/HPV Chemical Program protocols is also cited but comments are including regarding the applicability of the information to the specific substances under study.

Acute Toxicity

The acute toxicity of naphthenic acids has been investigated in experimental animals via the oral and dermal routes of exposure. In initial studies (Rockhold, 1955), two samples of raw naphthenic acids were reported as having oral LD50 values of 3000 and 5200 mg/kg. In another series of studies (Esso Research and Engineering Company, 1963), a test material described as having been isolated from a kerosene cut (i.e., more likely a raw than a refined naphthenic acid preparation) with an average molecular weight of 250 and an acid number of 220/230 was evaluated in an acute toxicity test battery. In an acute oral toxicity study, albino rats were given single doses the naphthenic acid preparation at levels ranging from 0.036 to 10 g/kg. All rats died in the 10000 mg/kg group, and the reported LD50 value was 7280 mg/kg. Signs of central nervous system effects were reported to have been associated with doses $\geq$ 5000 mg/kg. In an acute dermal toxicity study in rabbits, the naphthenic acid preparation produced slight to moderate signs of skin irritation at doses > 50 mg/kg, but as all of the treated animals survived, the dermal LD50 > 3160 mg/kg. In an acute inhalation toxicity study, mice, rats and guinea pigs were exposed for 6 hours to air saturated with the naphthenic acids preparation. All animals survived the exposure period. The exposure levels were not determined analytically, but the nominal concentration was estimated to have been about 0.63 mg/l (60 ppm). The naphthenic acid preparation also caused “moderate” eye irritation in a Draize eye irritation test.

In another series of studies, the acute oral LD_{50} of a naphthenic acids sample was determined to be 5880 g/kg body weight (Exxon, 1979a;b). In rabbits, the same material had a dermal LD_{50} of >31606 g/kg (Exxon, 1979c). The naphthenic acid test substance was also determined to be moderately irritating to the eyes of rabbits (Exxon, 1979d). The oral LD_{50} of naphthenic acids was reported to be 3550 mg/kg in young white male mice (Pennisi and Lynch, 1977). However, the data from Pennisi and Lynch (1977) are available only in abstract form. Thus, the quality of this study cannot be verified since the report does not provide complete experimental details.

In a study using a non-OECD SIDS/HPV Chemical Program protocol, adult female rats received a single oral dose of naphthenic acids of 3, 30, or 300 mg/kg body weight while adult male rats were treated with 300 mg/kg only. All of the rats survived the 14 day post-treatment observation period although a few were described as lethargic and mildly ataxic immediately after dosing. It should be noted that the test material used in this study was a naphthenic acid preparation made from Canadian oil sands tailings. The test substance used in this study had a higher average molecular weight and a higher average number of rings than is typical for naphthenic acids obtained during petroleum refining. Thus the direct relevance of these data to the HPV program is unclear. (Rogers, 2003).

Summary: The data indicate that naphthenic acids have a low order of toxicity, with acute oral toxicity (LD50) values $\geq$ 3550 mg/kg; acute dermal toxicity (LD50) values > 3160 mg/kg; and acutely lethal airborne concentrations (LC50) > saturated vapor concentrations. In addition, naphthenic acids have been shown to be moderate skin and eye irritants.

Repeated-Dose Toxicity

A 90-day oral gavage study (non-OECD SIDS/HPV Chemical Program protocol) in female rats of naphthenic acids at doses of 0.6, 6, and 60 mg/kg/day resulted in a number of apparent treatment-related effects (Rogers et al, 2002a; Rogers, 2003). These included the following: body weight decreases, increases in relative liver, brain and kidney weights, and plasma biochemical differences indicating the liver as a target organ. All of these effects occurred in the high dose groups. In addition, the authors reported severe seizure activity in some high and mid-dose animals after day 40. The limited number of organs examined and the absence of male data limit the usefulness of this study in estimating repeated-dose toxicity. (As above, it should also be noted that the test material used had a composition that differed from the naphthenic acids that are more typical of petroleum-derived materials).

Male mice given 1000 mg/kg/day oral doses of naphthenic acid for 30 days were reported as showing signs of CNS depression and exhibiting hematological changes, weight loss (leading eventually to death due to respiratory arrest), gross morphological changes in the liver and stomach, and histological changes in a few unidentified, selected organs (Pennisi and Lynch, 1977). However, the data were reported only in abstract form, and quality of this study cannot be evaluated since complete experimental details are not available.

In part because of the incomplete reporting of previous studies and also because of the uncertain relevance of the studies of naphthenic acids derived from oil sands tailings to those from petroleum refining, a sample of refined naphthenic acids was tested in a Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening test (OECD, 1996) to assess the potential for systemic and/or reproductive and developmental effects (WIL Research, 2012).

In the repeated dose part of the study, male and female Sprague-Dawley rats were given daily oral doses of refined naphthenic acids (CAS number 1338-24-5) for approximately 30 days at dose levels of 100, 300 or 900 mg/kg/day. Animals were examined daily, and body weight and food consumption were determined on a weekly basis. Prior to the initiation of dosing and then again on day 28 the animals were given neurological evaluations including a functional observation battery and an assessment of locomotor activity. On the day of scheduled termination, blood samples were taken for hematological and serum chemistry measurements,

and the animals were then euthanized. After a gross necropsy, selected organs were weighed and then preserved for pathologic examination.

All of the animals scheduled for systemic evaluation survived to scheduled termination. There was a statistically significant reduction in body weight gain in high dose group animals (table 3). There were no significant differences in the neurological examination, and, although there were a few statistically significant findings in the clinical chemistry examination, the differences were small and within normal ranges and were judged to have not been toxicologically important. In the hematological evaluation there were small but statistically significant reductions in red blood cell parameters and a statistically significant increase in white blood cells (table 4). The investigation of organ weights revealed statistically significant increases in liver weights in rats of both genders and elevated kidney weights in male rats. In the female rats the lung and uterine weights were significantly reduced but this may have been related to reductions in body weight gain as the differences were not significant when expressed on a body weight basis, and there were no pathological changes in these organs.

The results of the pathological investigation are summarized in Table 5. Kidney changes, reported in male rats only, were consistent with hyaline-droplet nephropathy (α2u-globulin-mediated nephropathy). The liver changes, found in organs from both male and female rats from the high dose group, were described as hepatocellular hypertrophy. Other changes included cortical lymphoid depletion of the thymus in females, primarily in rats from the high dose group. Epithelial hypertrophy and cytoplasmic vacuolation of the thyroid gland was noted in all treated animals, and cytoplasmic vacuolation of the *zona fasciculate* in the adrenal cortex was reported in males from all treatment groups and in high dose group females. There were no pathological changes in the reproductive organs of rats of either gender.

Table 3. Statistically significant changes in terminal body weights and organ weights. Data are given as mean ± SD.

Parameter	Sham Control	Corn Oil Control	100 mg/kg/day	300 mg/kg/day	900 mg/kg/day
Males					
Final Body Weight	467 ± 27	454 ± 45	448 ± 45	439 ± 34	412 ± 28
Liver	15.61 ± 1.43	13.46 ± 2.01	13.98 ± 2.04	15.69 ± 1.83*	19.94 ± 2.08**
Kidney	3.51 ± 0.25	3.21 ± 0.20*	3.38 ± 0.39	3.53 ± 0.33	3.77 ± 0.46**
Thyroid/parathyroid	0.019 ± 0.002	0.019 ± 0.001	0.020 ± 0.002	0.020 ± 0.002	0.020 ± 0.002
Heart	1.46 ± 0.09	1.46 ± 0.21	1.41 ± 0.14	1.43 ± 0.13	1.32 ± 0.13
Epididymis (LT)	0.57 ± 0.14	0.60 ± 0.05	0.60 ± 0.04	0.66 ± 0.05*	0.63 ± 0.06
Epididymis (RT)	0.62 ± 0.04	0.62 ± 0.06	0.61 ± 0.03	0.66 ± 0.04	0.65 ± 0.06
Females					
Final body Weight	335 ± 25	313 ± 23	301 ± 30	294 ± 24	289 ± 24
Liver	13.6 ± 2.0	11.7 ± 1.5	12.1 ± 1.1	13.3 ± 1.5	17.9 ± 2.4 **
Kidney	2.39 ± 0.17	2.07 ± 0.18*	2.11 ± 0.15	2.05 ± 0.25	2.17 ± 0.19
Heart	1.21 ± 0.23	1.10 ± 0.10	1.08 ± 0.10	1.07 ± 0.11	1.01 ± 0.13
Lungs	1.36 ± 0.13	1.40 ± 0.13	1.26 ± 0.12*	1.20 ± 0.12**	1.20 ± 0.07**
Uterus/Vagina	1.07 ± 0.19	1.00 ± 0.14	0.86 ± 0.08*	0.88 ± 0.11*	0.85 ± 0.12*

* = $P < 0.05$, ** = $P < 0.01$ by comparison to the corn oil controls

Table 4. Results of Assessments of hematology and clinical chemistry parameters which were statistically different from control values. [1]

Parameter Measured	Corn Oil Control	100 mg/kg/day	300 mg/kg/day	900 mg/kg/day
Males, data taken at terminal sacrifice				
Red Blood Cell Count (10^6/ul) [2]	9.22 ± 0.54	9.28 ± 0.28	8.91 ± 0.34	8.78 ± 0.22*
Hemoglobin (g/dL)[2]	15.7 ± 0.72	15.8 ± 0.48	15.2 ± 0.54	14.7 ± 0.44*
Hematocrit (%)[2]	48.1 ± 2.4	48.6 ± 1.6	46.5 ± 1.5	45.0 ± 1.8**
Platelet (10^3/ul)[2]	854 ± 151	885 ± 84	803 ± 144	976 ± 87**
Leukocytes, absolute ((10^3/ul)[2]	0.02 ± 0.02	0.03 ± 0.02	0.02 ± 0.02	0.04 ± 0.03*
RDW (%)[2]	11.4 ± 0.4	11.5 ± 0.4	11.6 ± 0.4	12.5 ± 0.6**
HDW (g/dL)[2]	2.58 ± 0.10	2.68 ± 0.12	2.76 ± 0.16*	2.77 ± 0.27*
Females, data taken at termination (lactation day 4)				
White blood cell count[2]	5.15 ± 1.30	6.89 ± 1.58	7.68 ± 2.24*	7.59 ± 1.85*
APTT (seconds)[2]	16.8 ± 1.9	15.9 ± 2.3	15.8 ± 3.1	13.9 ± 1.4 *
Lymphocytes, absolute (10^3/ul),	3.32 ± 0.61	4.50 ± 1.42	5.11 ± 1.75*	4.96 ± 1.60*
Monocytes, absolute (10^3/ul)	0.11 ± 0.10	0.24 ± 0.21	0.21 ± 0.12	0.35 ± 0.23*

1. Parameters not affected by treatment included:
    a. Males – white blood cell count, mean corpuscular volume (fL), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin content (g/dL), prothrombin time (sec), APTT (sec), reticulocytes (%), reticulocytes, absolute (10^3/ul), MPV (fL), neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%), basophils (%), leucocytes(%), neutrophils, absolute (10^3/ul), lymphocytes, absolute (10^3/ul), monocytes, absolute (10^3/ul), eosinophils, absolute (10^3/ul), basophils, absolute (10^3/ul).
    b. Females – red blood cell count (10^6/ul), Hemoglobin content (g/dL), hematocrit (%), mean corpuscular volume (fL), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin content (g/dL), platelet count (10^3/ul), prothrombin time (sec), reticulocytes (%), reticulocytes, absolute (10^3/ul),), MPV (fL), neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%), basophils (%), leucocytes(%), neutrophils, absolute (10^3/ul), eosinophils, absolute (10^3/ul), basophils, absolute (10^3/ul), Leukocytes absolute (10^3/ul), RDW (%), HDW (g/dL)

2. Data given as mean ± SD

* = $p < 0.05$, ** = $p < 0.01$

Table 5. Summary of microscopic findings from rats treated with refined naphthenic acids.

	Males				Females			
Doses, mg/kg/day	Corn Oil	100	300	900	Corn Oil	100	300	900
N	12	12	12	12	9	12	10	10
Kidney								
Hyaline Droplets	0	3	10**	11**	0	0	0	0
Minimal	0	3	9**	9**	0	0	0	0
Mild	0	0	1	2				
Nephropathy	0	0	2	9**	0	0	0	0
Minimal	0	0	2	5*	0	0	0	0
Mild	0	0	0	4				
Liver								
Hypertrophy, hepatocellular, centrilobular	0	0	0	8**	0	0	0	10**
Minimal	0	0	0	8**	0	0	0	10**
Vacuolation, hepatocellular	2	1	2	0	0	1	0	2
Minimal	1	1	2	0	0	1	0	2
Mild	1	0	0	0	0	0	0	0
Thymus								
Depletion, lymphoid, cortex	0	0	0	0	0	1	0	5
Minimal	0	0	0	0	0	1	0	4
Mild	0	0	0	0	0	0	0	1
Thyroid								
Hypertrophy, epithelial	0	6	9*	11**	0	3	4	8**
Minimal	0	6	9**	11**	0	3	4	6*
Mild	0	0	0	0	0	0	0	2
Vacuolation, cytoplasmic	0	6	9**	10**	0	3	4	8**
Minimal	0	6	9**	10**	0	3	4	8**
Adrenal Cortex								
Vacuolation, cytoplasmic	0	2	3	2	0	0	0	2
Minimal	0	2	3	2	0	0	0	1
Mild	0	0	0	0	0	0	0	1
Heart								

Cardiomyopathy								
Minimal	4	7	8*	8*	3	3	2	5
Mild	2	0	0	0	0	0	0	0

- * = P < 0.05, ** = p < 0.01

The systemic findings were not remarkable. There were no significant findings in the neurological assessments. There were a number of statistically significant findings in the clinical chemistry and hematology evaluations, but these differences were small, not consistent between the genders and, for the most part, unrelated to microscopic changes. Further, all of these differences were within the historical control data range of the testing facility and were not considered to have been adverse outcomes.
Overall, the gross and pathological assessments did reveal some differences that were treatment-related but were unlikely to have been toxicologically important. Liver weights were significantly increased in high dose groups of both male and female rats, and there was also a statistically significant increase in liver weight in the 300 mg/kg/day dose group in the males. The histological findings were essentially limited to minimal evidence of hepatocellular hypertrophy in the high dose group animals. As none of the liver enzyme markers was increased, this was most likely evidence of enhanced metabolic capacity and adaptive rather than adverse (Cattley and Popp, 2002). Kidney weights were significantly elevated in the male rats from the high dose group, but not in the female rats. The histological evidence revealed the presence of hyaline droplets, mostly judged to have been of minimal severity, which increased in frequency in the male rats in a dose-dependent manner. As kidney effects were not found in female rats, the histological findings and gender-specificity, suggest the kidney changes were the consequence of an α-2u-globulin-related process which is male rat specific and not relevant to humans (Hard et al., 2008; Baetcke et al., 1991; Swenberg and McKeeman, 1998).
Other changes included higher mean thyroid/parathyroid weights with corresponding epithelial hypertrophy and cytoplasmic vacuolation. The histologic changes were mostly judged as minimal. It is plausible that these changes reflected a compensatory response related to the increased metabolic capacity of the liver and more rapid turnover of thyroid hormones (Curran and De Groot, 1991; Capen, 1997). Lymphoid depletion of the thymus was observed in the high dose females and microscopic findings of cytoplasmic vacuolation of the adrenal cortex were noted in the males and high dose group females. The lymphoid cortical depletion of the thymus and adrenal cortex vacuolation were considered to have been stress responses (Greaves, 2007a) although cytoplasmic vacuolation of the adrenal cortex can also occur spontaneously (Frith et al., 2000) or as the result of pharmacological effects (Greaves, 2007a). Minimal cardiomyopathy was observed with a significantly increased incidence in the 300 and 900 mg/kg/day males. Cardiomyopathy is a common finding in rats (Greaves, 2007b). The cardiomyopathy observed in the naphthenic-acid-treated rats was similar to or of lesser severity than that observed in the control group rats. Further, the cardiomyopathy was not associated with any gross observations, organ weight changes, or alterations in clinical pathology parameters. The overall no effect level for all systemic effects was 100 mg/kg/day.

Summary: Because the Testing Group believed that the previous repeat-dose toxicity studies on naphthenic acids were either not of sufficient quality or not directly relevant to adequately characterize this endpoint, an OECD 422 study was conducted. Male and female Sprague-Dawley rats were given daily doses of refined naphthenic acids by gavage at levels of 100, 300, or 900 mg/kg/day. There were few findings that were of human relevance, and the overall no effect level for systemic effects was 100 mg/kg/day.

In Vitro Mutagenicity

Although no studies were available on the *in vitro* genotoxicity of naphthenic acid, there are data on the calcium and sodium salts. NTP studies indicate that neither calcium naphthenate nor sodium naphthenate were mutagenic in *S. typhimurium* with or without S9 (NTP, 2012a,b). Sodium naphthenate did not produce chromosomal aberrations in Chinese hamster ovary cells, but was positive in a sister chromatid exchange assay.

Summary: The NTP data on the sodium and calcium salts of naphthenic acid provide sufficient data to demonstrate that naphthenic acids are not genotoxic under *in vitro* conditions.

In Vivo Mutagenicity

Because there had previously been no *in* vivo mutagenicity data for naphthenic acids, the testing group conducted an assessment of the potential for refined naphthenic acids to induce micronucleus formation in rat bone marrow. To provide the necessary data, bone marrow was taken from rats in the repeated dose study described above and evaluated for micronucleus formation (WIL Research, 2012). The micronucleus test was consistent with the US EPA guidelines for studies of this type (OPPTS 870.5395).

A total of 1000 erythrocytes/slide were evaluated (both polychromatic (PCE) and normochromatic erythrocytes (NCE)), and the PCE/NCE ratio was calculated. The number of micronucleated PCEs from a total of 2000 PCEs was then determined for each animal.
As shown in table 6, the frequencies of micronuclei in in bone marrow from rats treated with refined naphthenic acids did not differ statistically from those in the sham and vehicle control groups. A significant increase in micronucleus frequency was found in material harvested from rats treated with the positive control, cyclophosphamide.

Table 6. Summary of results of micronucleus data from rats treated with refined naphthenic acids.

Treatment	Gender	Total MN PCEs/2000 PCEs (N=5)	% MN PCEs	Total MN NCEs/2000 NCEs (N=5)	Ratio of PCEs to Total Erythrocytes
Corn Oil	Males	8	0.08 ± 0.08	3	0.54 ± 0.07
	Females	8	0.08 ± 0.12	4	0.69 ± 0.11
Sham Control	Males	6	0.06 ± 0.04	3	0.52 ± 0.11
	Females	8	0.08 ± 0.08	2	0.55 ± 0.17
Naphthenic Acid					
100 mg/kg/day	Males	7	0.07 ± 0.07	1	0.53 ± 0.09
	Females	4	0.04 ± 0.04	7	0.65 ± 0.16
300 mg/kg/day	Males	4	0.04 ± 0.04	3	0.49 ± 0.67
	Females	5	0.06 ± 0.05	5	0.67 ± 0.13
900 mg/kg/day	Males	8	0.08 ± 0.08	5	0.61 ± 0.11
	Females	5	0.06 ± 0.05	5	0.75 ± 0.19
Cyclophosphamide	Males	128	1.28 ± 0.14*	13	0.40 ± 0.21

60 mg/kg/day					
	Females	97	0.97 ± 0.19*	16	0.51 ± 0.12*

- * = $p < 0.05$ by comparison to the corn oil treated group.

Summary: A micronucleus test has provided evidence that naphthenic acids are not mutagenic under *in vivo* conditions.

Reproductive/Developmental Toxicity

Rogers (2003) reported a reproductive toxicity/developmental toxicity test of naphthenic acids derived from oil sands. In this study male and female rats were exposed by oral administration starting two weeks prior to mating. Dosing of females was continued through the mating and gestation periods. The pregnant rats were allowed to deliver normally. The pups were euthanized immediately after parturition and examined for abnormalities. The dams were euthanized after delivery and examined. The most striking outcome was related to fetal survival. In the high dose (60 mg/kg/day) group, only 1/14 mated dams produced offspring. There were no apparent malformations, but only 7 pups in the high dose group were available for examination.

In part because of the limitations of the study design and also because of questions about the relevance of the sample tested to naphthenic acids from petroleum refining, the testing group conducted a Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening (WIL Research, 2012). As in the repeated dose portion of this study described above, the rats were given the test material by gavage at doses of 100, 300, or 900 mg/kg/day.

As shown in Table 7, there were no apparent effects on mating. A single female in the 300 mg/kg/day group had a pre-coital interval of 13 days, resulting in a statistically significant increase in pre-coital incidence in this group. Otherwise all of the pairs productively mated and pre-coital intervals were within the historical control range for the laboratory. Further, the length of the gestational period was similar across the groups. There were reductions in the numbers of *corpora lutea* and implantation sites in the high dose group, but the differences were not statistically significant (Table 7). However, there was a significant reduction in the number of offspring born/litter in the high dose group (Table 8). There was also a significant reduction in survival in offspring in the high dose group, and those pups that did survive had significantly lower body weights than the offspring in the control groups. The number of pups found dead or euthanized *in extremis* during the period PND 0-4 was: control = 1(1), 100 mg/kg/day = 0(0), 300 mg/kg/day = 12(5), and 900 mg/kg/day = 38(8).
The most profound effects were seen in the high dose group, but there were also significant effects in the 300 mg/kg/day group. The overall no effect level for developmental effects was 100 mg/kg/day.

Table 7. Summary of reproductive parameters assessed in the study of refined naphthenic acids

Dose (mg/kg/day)	Corn Oil Control	100 mg/kg/day	300 mg/kg/day	900 mg/kg/day
Number of females paired	12	12	12	12

Number of female mated	12	12	10	11
Number of females pregnant [a]	9	12	10	11
Number of females with litters	9	12	10	11
Pre-coital interval (days) [b]	1.4 ± 0.7	2.3 ± 1.1	4.2 ± 3.3*	3.8 ± 3.5
Gestation length (days)	21.4 ± 0.6	21.9 ± 0.3	22.0 ± 0.5	22.1 ± 0.5
Corpora lutea	15.6 ± 2.3	14.0 ± 1.4	15.1 ±3.0	13.8 ± 2.1
Implantation sites	15.0 ± 2.4	13.6 ± 1.1	13.0 ± 1.2	12.2 ± 3.7
Number born	14.1 ± 1.9	12.9 ± 1.1	12.0 ± 1.6	10.8 ± 3.8*
Post-Implantation loss (%) [c]	6.0	5.1	7.7	11.5

a. Pregnant = uterine implantation sites.
b. Data summarized as mean ± standard deviation.
c. Post-implantation loss =
[(number of implantation sites – number of pups born)/number of implantation sites] x 100

- * = $p < 0.05$ by comparison to the corn oil control group.

Table 8. Survival, Viability and Growth of Offspring following *in utero* exposure to refined naphthenic acids. Data given as mean ± SD.

Dose (mg/kg/day)	Corn Oil Control	100 mg/kg/day	300 mg/kg/day	900 mg/kg/day
Number of viable litters	9	12	10	11
Number of pups born alive/litter	13.9 ± 1.9	12.9 ± 1.1	10.1 ± 4.0*	9.6 ± 4.0**
Percentage of pups surviving from birth to termination (PND 4)	98.1 ± 3.8%	100.0 ± 0.0	88.0 ± 24.5	67.7 ± 40.6
Pups (litters) found dead or euthanized *in extremis*	1(1)	0(0)	12(5)	38(8)
Sex ratio (% males/litter)	58.9 ± 9.6	53.9 ± 9.6	55.2 ± 19.1	58.1 ± 22.7
Pup weight PND 1 – males	7.0 ± 0.5	6.7 ± 0.7	6.7 ± 0.5	5.7 ± 0.8*
Pup weight PND 1 – females	6.6 ± 0.6	6.5 ± 0.6	6.4 ± 0.4	5.6 ± 1.1
Pup weight PND 4 – males	9.7 ± 1.1	9.4 ± 1.2	9.4 ± 0.9	7.2 ± 1.5**
Pup weight PND 4 – females	9.1 ± 1.0	9.0 ± 1.0	8.8 ± 0.7	7.3 ± 1.5**

= P , 0.05, ** = p < 0.01

Summary – The reproductive toxicity screening test showed that refined naphthenic acids have no apparent effects on fertility but can produce effects on developing organisms. There were no dose-related effects on mating behavior, mating success, gestation length, number of corpora lutea or number of implantation sites. However, there were significant increases in pre-implantation loss and significant reductions in live pups/litter and pup weight in the 900 mg/kg/day group and there was also a small but statistically significant reduction in number of live pups/litter in the 300 mg/kg/day dose group. The lowest dose (100 mg/kg/day) was a no effect level for all effects. It should also be noted that, as discussed previously, there was a pathological examination of the reproductive organs from both male and female rats in the repeated dose portion of this study. There was a significant reduction in absolute uterine weights, but this was most likely a consequence of reduced body weight gain since there were no pathological findings in these or in other reproductive organs at doses up to 900 mg/kg/day.

Physicochemical Data

Although some data for products in this subcategory exist, not all of the physicochemical SIDS endpoints are defined and a consensus database for chemicals that represent products in this subcategory does not exist. Therefore, calculated and measured representative data have been identified and a technical discussion provided, where appropriate. The EPIWIN© computer model (U.S. EPA, 2000), as discussed in the US EPA document entitled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program" (U.S. EPA, 1999a) has been used to calculate physical-chemical properties of representative constituents of naphthenic acids. Because of the diversity of compounds encompassing naphthenic acids, it is not feasible to model the physicochemical endpoints for each potential compound. Modeling efforts were directed towards those constituents of naphthenic acids covering representative molecular weights and isomeric structures most likely to exist in the streams defined in this category. Appendix A provides the structures and names of the individual naphthenic acids used in physical-chemical modeling.

Melting Point

Because naphthenic acids are not pure chemicals, the melting point characteristics of these complex substances vary with the hydrocarbon composition of their make-up. Based on data available in commercial product specifications and Material Safety Data Sheets (MSDS), substances produced for commercial use have melting points that fall in the range from –35 °C to +2 °C.

Summary: Melting points of complex streams containing naphthenic acids are in the range of -35°C – +2 ^{o}C.

Boiling Point

Because these substances are not pure chemicals, the boiling point characteristics of naphthenic acids and their salts vary according to the hydrocarbon component make-up of the complex substances in which they are found. Based on data available in commercial product specifications and Material Safety Data Sheets (MSDS), substances produced for commercial use have boiling points that fall in the range from 140 °C to 370 °C.

Summary: Naphthenic acids distill in the range of 140 – 370 ^{o}C.

Vapor Pressure

Commercial product data typically provided narrative comments such as “negligible”, “very low”, or “not applicable” for vapor pressures of those substances (SocTech 2003; AGS Chemicals Limited 2003; Mallinckrodt Baker Inc. 1997). Because naphthenic acids are complex substances, the vapor pressures of the substances overall is a function of the sum of the vapor pressures of the individual components in their pure state times their mole fraction in the mixture (Raoult’s Law). Estimates of the vapor pressures of C10 to C21 naphthenic acid compounds described by Brient (1995) were made using EPIWIN (U.S. EPA 2000). A technical discussion of those estimates and predicted vapor pressures for complex naphthenic acids was developed

in the robust summary. Based upon modeled estimates of representative constituent naphthenic acid structures, vapor pressures ranged from 0.32 Pa to 1.9×10^{-5} Pa.

Summary: Estimated vapor pressures of individual naphthenic acid structures containing C10 to C21 carbon atoms range from 0.32 to 1.9×10^{-5} Pa.

Partition Coefficient

Due to their complex composition, unequivocal determination of the log K_{ow} of naphthenic acid mixtures cannot be made. To gain an understanding of the partitioning potential of these substances, partition coefficients of selected molecular weights and naphthenic ring structures were modeled using EPIWIN© (U.S. EPA, 2000). Structures were selected that have been reported to represent molecular weights and ring constituents of naphthenic acids found in crude oil extracts (Brient, 1995). Estimates demonstrated that molecules containing longer carboxyl side chains tend to have higher partition coefficients. Also, multi-ring compounds tend to have lower partition coefficients than single-ring compounds of equal molecular weight. Partition coefficients for the modeled naphthenic acid structures ranged from 3.8 to >6. This range of values is based on structures known to predominate in some naphthenic acid extracts; however, lower partition coefficients would be predicted for structures having lower molecular weights.

Summary: Estimated partition coefficients for the modeled naphthenic acid structures range from 3.8 to >6.

Water Solubility

Naphthenic acids are weak acids having pKa values of approximately 5 to 6 (Brient, 1995; CEATAG, 1998; Havre, 2002). As the pH of a solution of naphthenic acids increases above the pKa value, a greater proportion of the constituents are ionized and exist in the dissolved phase of the aqueous medium (Havre, 2002). Therefore, alkaline solutions increase a naphthenic acid's solubility, and acid solutions decrease solubility (Havre 2002). Product literature references have cited narrative statements such as "very low water solubility" (SocTech S.A., 2003), or "only slightly soluble in water" (AGS Chemicals Limited, 2003). CETAG (1998) provided solubility limits at 25 °C of 70 mg/L and 5040 mg/L for pH 0.91 and pH 9.16, respectively. However, no details of the methodology were provided in that report. In support of new ecotoxicity testing of naphthenic acids (CAS 1338-024-5) for the HPV program, water-accommodated fractions of naphthenic acids were prepared and analyzed using Fourier transform infrared (FTIR) spectroscopy (Jivraj, et al. 1991). When a 100 mg/L loading rate solution of naphthenic acids was stirred and sampled over 72 hours, the dissolved naphthenic acids concentration peaked at 88.1 mg/L (ABC Laboratories, 2009). Water pH in that experiment was controlled at 7.5 and temperature was ambient laboratory conditions (approximately 20°C).

Summary: Naphthenic acids are acyclic and cyclic carboxylic acids having pKa values of 5 – 6. Consequently the pH of aqueous media will influence the solubility characteristics of these mixtures. Over a wide pH range (0.91 – 9.17) solubility was measured at 70 mg/L and 4520 mg/L. For the key study cited here, the measured water solubility of a solution of commercial naphthenic acids was 88.1 mg/L at pH 7.5.

Environmental Fate Data

Photodegradation

Atmospheric oxidation as a result of hydroxyl radical attack is indirect photodegradation. Substances in this subcategory have low vapor pressures and therefore do not have a tendency to volatilize to air where they can undergo reactions with photosensitized oxygen in the form of hydroxyl radicals (OH^-). Therefore, these reactions are not expected to be an important environmental fate process. The potential to undergo indirect photodegradation was estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.90) in EPIWIN© (U.S.EPA, 2000), which calculates a chemical half-life and an overall OH reaction rate constant based on a 12-hour day and a given OH concentration. Atmospheric oxidation rates and half-lives were thus calculated for a range of molecular weight and naphthenic ring structures covering one to four-ring cycloalkyl carboxylic acids having molecular weights from 254 to 325. These structures were considered appropriate because they have been found to be present in naphthenic acid extracts from Athabasca oil sands, a source considered high in naphthenic acid content (Rogers et al., 2002c). AOP half-life estimates for these compounds ranged from 0.3 to 0.6 days and show a lack of persistence in the atmosphere. However, with vapor pressures of $1.8x10^{-3}$ to $1.4x10^{-5}$ Pa, there is low potential for these substances to partition to the atmosphere where indirect photodegradation would occur.

Summary: The atmospheric oxidation potential of representative components in naphthenic acids has been estimated to be in the range of 0.3 to 0.6 days.

Stability in Water

Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). Because naphthenic acids do not contain significant levels of these functional groups, components in the naphthenic acid subcategory are not subject to hydrolysis.

Summary: Components in the naphthenic acids subcategory do not undergo hydrolysis.

Chemical Transport and Distribution (Fugacity Modeling)

Fugacity-based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (e.g., air, water, soil, sediment, suspended sediment and biota). The U.S.EPA has agreed that computer modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model (Mackay 1991). The EQC model is a Level 1 (i.e., steady state, equilibrium, closed system and no degradation) model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment. EPA cites the use of this model in its document "Determining the Adequacy of Existing Data" that was prepared as guidance for the HPV chemicals program (U.S. EPA, 1999b).

To gain an understanding of the potential transport and distribution of naphthenic acids, the EQC model was used to characterize the environmental distribution of different molecular

weights and structural conformations of naphthenic acid molecules. These constituents were selected because they had been shown to be present in extracts of Athabasca oil sands (Rogers et al., 2002c). Modeling results show that when naphthenic acids are released to the environment, they would bind to soil/sediments, with negligible fractions partitioning to air, water or biota. However, not all naphthenic acids are composed of identical chemical species as reported by Rogers et al. (2002c), and low molecular weight naphthenic acid constituents, if present, would be expected to partition to some degree to water depending on their pKa characteristics and the pH of the water.

Summary: Fugacity modeling which has been done to provide an estimate of the percent distribution in environmental media of various molecular weight and ring-structured naphthenic acids shows that the naphthenic acids would mostly bind to soil/sediments.

Biodegradation

Although no standardized ready or inherent biodegradation studies were available for naphthenic acids, research has shown these materials to be amenable to microbial utilization similar to other hydrocarbon compounds. Studies have demonstrated that microorganisms indigenous to oil sands tailings were capable of degrading complex mixtures of commercial sodium salts of naphthenic acids as well as mixtures of organic acids extracted from oil sands tailings (Herman et al., 1993, 1994; Clemente et al., 2004; Clemente and Fedorak, 2005). Although rates of biodegradation may be affected by steric factors related to the numbers of cycloalkane rings or the alkyl constituents on the ring structure, microbial populations respond to naphthenic acid substrates through increased CO_2 production, O_2 consumption, and enhancement of metabolism with the addition of nutrients. With single-ring naphthenic acids, biodegradation of both the ring and side-chain acid has been shown to occur (Herman et al., 1993, 1994). As the number of cycloalkane rings increase, it may be inferred from what is known about degradation of multi-ring naphthenes that biodegradation rates may slow, but these substances will degrade given time (Bartha and Atlas 1977).

Biodegradation (as percent of organic carbon converted to CO_2) of model naphthenic acid compounds, cyclohexane carboxylic acid, cyclohexane pentanoic acid, 2-methyl cyclohexane carboxylic acid, and trans-4-pentylcyclohexane carboxylic acid ranged from 6 to 67% depending on the micro-organism culture and the presence of nitrogen and phosphorus nutrients in the medium (Herman et al., 1994).

The aerobic biodegradation of two commercial naphthenic acids were studied in detail using enriched microbial cultures originating from oil sands tailings pond water (Clemente et al. 2004). In a system similar to the headspace test design but with pre-adapted inoculum, commercial naphthenic acids sodium salts and commercial refined naphthenic acids were incubated in 125-mL serum bottles using the enriched culture. Headspace gas and the liquid culture medium were sampled using a syringe, without removing the serum stoppers. Both commercial naphthenic acid sodim salts and commercial refined naphthenic acids were aerobically degraded in a similar pattern. Beginning with 109 mg NAs/L of refined naphthenic acids, the concentration declined to 25 mg NAs/L by day 7 and to 8 mg NAs/L by day 10. Based on the decrease in concentration of naphthenic acids, primary biodegradation attained 77% and 93% by days 7 and 10, respectively. Approximately 60% of the naphthenic acids was measured as CO_2 after 17 days of incubation. This value did not change during incubation to 45 days. Analysis of the liquid cultures at the beginning showed 78% of the acids fell into the C5-C13 group, while by day 7 only 41% fell into this group, and nearly all C5-C10 acids were removed.

This work showed that two commercial NA preparations can be biodegraded extensively under laboratory conditions. Furthermore, the change in composition indicated that the lower molecular weight acids (C5-C13) were degraded more readily than the high molecular weight acids.

Summary: Studies show that commercial naphthenic acids mixtures can be utilized as an energy source by microbial organisms and that these substances can be inherently biodegraded.

Environmental Health Effects Data

The HPV ecotoxicity dataset developed for naphthenic acids (CAS 1338-24-5) includes acute toxicity to a freshwater fish (*Pimephales promelas*), an invertebrate (*Daphnia magna*), and an alga (*Pseudokirchneriella subcapitata*). The data presented are new key studies for the three trophic levels, and were developed for the HPV program. All tests were conducted as static renewal (fish and invertebrate) or static (alga) tests with exposure solutions created using the water accommodated fraction (WAF) technique (Girling et al, 1994). WAF preparations are the recommended method of preparing exposure solutions for aquatic toxicity testing of complex substances having low water solubility (OECD, 2000). Independent WAFs were prepared on a loading rate basis, which is the amount of test substance equilibrated with the aqueous test medium, and the WAF is the aqueous phase at equilibrium with the loading rate.
The test endpoints cited in the robust summaries and described below for the three test species are presented on the basis of the 50% lethal or effective loading rate (LL50, EL50) and the 50% lethal or effective concentration (LC50, EC50). Loading rate endpoints were calculated using the nominal WAF loading rates, while the concentration-based endpoints were calculated using the mean concentrations of total dissolved naphthenic acids in the WAFs (LC50, EC50) measured by Fourier transformed infrared (FTIR) analysis (Jivraj, et al 1991).

Acute Toxicity to Aquatic Vertebrates

Table 9 presents the results of new test data of a key study on the acute toxicity of commercial naphthenic acids to fathead minnows (*P. promelas*).

Table 9. Acute toxicity of the water accommodated faction of naphthenic acids to fathead minnow.

Test Substance	Test Species	Toxicity Endpoint	Endpoint Value, mg/L (95% confidence interval)	Reference
CAS No. 1338-24-5, Naphthenic acids	fathead minnow (*P. promelas*)	96-h LL50 96-h LC50	9.0 (6.6 – 12) 5.6 (2.5 – 11)	ABC Laboratories (2010a)

Other data on the toxicity of naphthenic acids to fish can be found in the scientific literature, and selected studies are reviewed in the robust summaries. While some studies were considered reliable, all cited tests either reported only nominal concentrations, contained insufficient description of the test substance, or lacked documentation of testing procedures. Although these published data did not include extensive details, toxicity values were reasonably similar to the new data presented here. For example, Cairns et al (1965) determined a 96-hour TL_m of

16.3 mg/L for a non-specific naphthenic acids substance to adult zebrafish (*Brachydanio rerio*) and a 48-hour TL_m of 3.5 mg/L for zebrafish embryos. Dorn (1992) estimated the 96-hour LC50 to three-spined stickleback (*Gasterosteus aculeatus*) to lie between 2.5 and 5 mg/L when commercial naphthenic acids were spiked into nontoxic refinery effluent.
Growth and developmental effects in yellow perch (*Perca flavescens*) and Japanese medaka (*Orizias latipes*) embryos exposed to sodium naphthenate solutions were described by Peters et al. (2007). For both species, exposure to the treatments began soon after fertilization and continued until the hatching stage was met. Embryos that survived were measured for body length at hatch. Predominant deformities in perch included optic-cephalic irregularities and dorso-lateral curvatures of the spine. For medaka, pericardial edema and tube-heart led to systemic circulatory problems, and optic-cephalic abnormalities were present. Deformity and growth threshold concentrations (defined as the geometric mean of the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC)) were calculated for each species. For perch the threshold effect concentration for deformities was 1.67 mg/L. The threshold effect concentration for larval length at hatch was 0.88 mg/L. For medaka, deformity and length threshold concentrations were 1.51 mg/L and 1.44 mg/L, respectively. Concentrations were based on measurements taken using Fourier transform infrared spectroscopy (FTIR).
Other sublethal responses of perch to exposure of commercial naphthenic acids were reported by Nero et al. (2006). While a concentration of 3.6 mg/L was lethal to all fish, sublethal responses were observed at 0.9 mg/L naphthenic acids. The predominant sublethal response of fish gills exposed to a commercial naphthenic acids preparation were proliferation of gill epithelial, chloride, and mucous cells. However, no significant changes in liver pathology indices were found.

Summary: The 96-hour LL50 for the acute toxicity of naphthenic acids (CAS no. 1338-24-5) to fish was 9.0 mg/L based on the WAF nominal loading rate, and the 96-hour LC50 was 5.6 mg/L based on the measured total dissolved naphthenic acids concentrations.

Acute Toxicity to Aquatic Invertebrates

Table 10 presents the results of new test data of a key study on the acute toxicity of commercial naphthenic acids to a freshwater invertebrate (*D. magna*).

Table 10. Acute toxicity of the water accommodated fraction of naphthenic acids to *Daphnia magna*.

Test Substance	Test Species	Toxicity Endpoint	Endpoint Value, mg/L (95% confidence interval)	Reference
CAS No. 1338-24-5, Naphthenic acids	cladoceran (*D. magna*)	48-h EL50 48-h EC50	24 (21 – 27) 20 (17 – 23)	ABC Laboratories (2010b)

No studies were found in the literature describing the acute toxicity of commercial naphthenic acids to *D. magna*. Therefore, the data developed for the HPV program are new data for this species. Frank et al. (2009) measured the acute toxicity of eight individual carboxylic acid compounds to *D. magna* in standard 48-hour tests. Four compounds were mono-carboxylic acids and four were di-carboxylic acids. The data showed that the toxicity of these eight

individual acids to *D. magna* was considerably less than what was demonstrated by the new test data for the commercial mixture (CAS 1338-24-5). Mono-carboxylic acids expressed greater toxicity to *D. magna* than di-carboxylic acids, and for both groups of acids, toxicity of the individual compounds increased with increasing molecular weight. For the mono-carboxylic acids, EC50 values ranged from 109 mg/L (cyclohexanepentanoic acid) to 1166 mg/L (hexanoic acid), while for di-carboxylic acids EC50 values ranged from 1344 mg/L (cyclohexylsuccinic acid) to 3223 mg/L (succinic acid). Other data cited in the European Chemicals Agency (ECHA) database for CAS 1335-24-5 included a study by Linden et al. (1984) that cited a 96-hour LC50 of 4.8 mg/L for the marine copepod *Nitroca spinipes* exposed to calcium naphthenate. This endpoint was used as read-across to the registered substance, CAS 1335-24-5. These data indicate that acid type, molecular weight, and Z-family parameters may dictate bioavailability and subsequently toxicity.

Summary: The 48-hour EL50 for the acute toxicity of naphthenic acids (CAS no. 1338-24-5) to *D. magna* was 24 mg/L based on the WAF nominal loading rate, and the 48-hour EC50 was 20 mg/L based on the measured total dissolved naphthenic acids concentrations.

Toxicity to Freshwater Algae

Table 11 presents the results of new test data of a key study on the toxicity of commercial naphthenic acids to a freshwater algae (*P .subcapitata*).

Table 11. Toxicity of the water accommodated faction of naphthenic acids to *Pseudokirchneriella subcapitata*.

Test Substance	Test Species	Endpoint Basis[1]	Toxicity Endpoint	Endpoint Value, mg/L (95% confidence interval)	Reference
CAS No. 1338-24-5, Naphthenic acids	Algae (*P. subcapitata*)	growth rate	72-h E_rL50 72-h E_rC50 96-h E_rL50 96-h E_rC50	 41 (40 – 42) 30 (29 – 30) 43 (42 – 45) 30 (29 – 31)	ABC Laboratories (2010c)
		biomass yield	72-h E_yL50 72-h E_yC50 96-h E_yL50 96-h E_yC50	 24 (23 – 25) 18 (17 – 19) 25 (24 – 26) 18 (17 – 19)	

[1] Endpoints were calculated based on growth rate (E_rL50/E_rL50) and net algal biomass yield (E_yL50/ E_yC50) in accordance with the OECD 201 guideline.

No studies were found in the literature describing the toxicity of commercial naphthenic acids to *P. subcapitata*. Therefore, the data developed for the HPV program are new data for this species. Toxicity endpoints for the tested substance were always lower for the yield-based calculation than for the rate determinations, but there were no substantial differences between the 72-hour and 96-hour endpoints. Other data cited in the ECHA database for CAS 1335-24-5 included a range of 96-hour EC50 values of 26.0 to 80.5 mg/L for the freshwater diatom *Navicula seminulum*. The data were originally cited in EPA's ECOTOX database, but the original paper for these data could not be obtained by ECHA.

Summary: All toxicity endpoints based on biomass yield were more conservative than the corresponding endpoints for growth rate. On the basis of yield and nominal loading rates, the 72- and 96-hour EL50 values were 24 mg/L and 25 mg/L, respectively. On the basis of yield and mean measured concentrations, the 72- and 96-hour EC50 values were both 18 mg/L.

Data Summary

Endpoint	Result	Source of Information
Physical Chemical Properties		
Melting Point	-35 ^{o}C - + 2^{o}C	Safety Data Sheets
Boiling Point	140 – 370 ^{o}C	Safety Data Sheets
Vapor Pressure	0.32 – 1.9 x 10^{-5} Pa	Modeled Data
Partition Coefficient	3.8 - > 6	Modeled Data
Water Solubility	88.1mg/l @ pH 7.5	Present report
Environmental Fate		
Photodegradation	0.3 – 0.6 days	Modeled Data
Stability in Water	Stable	No functional groups subject to hydrolysis
Chemical Transport and Distribution (Fugacity Modeling)	Predominantly bound to soil and/or sediment	Modeled Data
Biodegredation	Inherently biodegradable	Published Literature
Environmental Effects		
Algae Growth Inhibition		
Growth rate	72 hour ErL 50 – 41 mg/l (40-42) 72 hour ErC50 – 30 mg/l (29-30) 96 hour ErL 50 – 43 mg/l (42-45) 96 hour ErC50 – 30 mg/l (29-31)	Present report
Biomass yield	72 hour EyL 50 – 24 mg/l (23-25) 72 hour EyC50 – 18 mg/l (17-19) 96 hour EyL50 – 25 mg/l (24-26) 96 hour EyC50 – 18 mg/l (17-19)	Present report
Acute Freshwater Invertebrate (Daphnia)	48 hour EL50 – 24 mg/l (21-27) 48 hour EC50 – 20 mg/l (17-23)	Present report

Acute Freshwater Fish (Fathead minnow)	96 hour LL50 = 9.0 mg/l (6.6 – 12) 96 hour LC50 = 5.6 mg/l (2.5 – 11)	Present report
Mammalian Toxicity		
Acute Oral	➢ 3550 mg/kg	Published Literature
Acute Dermal	➢ 3160 mg/kg	Published Literature
Acute Inhalation	➢ 0.63 mg/l (saturated vapor concentration)	Published Literature
Skin Irritation	Moderate	Published Literature
Eye Irritation	Moderate	Published Literature
Repeat Dose	LOAEL = 300 mg/kg/day NOAEL = 100 mg/kg/day	Present report
Genetic Toxicity		
In vitro	Not mutagenic	Published Literature
In vivo	Not mutagenic	Present Report
Reproductive Toxicity	No effects on fertility or reproductive organs	Present Report
Developmental Toxicity	Maternal effects – NOAEL = > 900 mg/kg/day Effects on Offspring LOAEL = 300 mg/kg NOAEL = 100 mg/kg	Present Report

References

ABC Laboratories Inc. (2010b). Acute toxicity of water accommodated fractions of naphthenic acids to the water flea, Daphnia magna, determined under static-renewal conditions. ABC study no. 64404, Analytical Bio-Chemistry Laboratories, Columbia, Missouri.

ABC Laboratories Inc. 2009. Validation of test solution preparations and analytical methods for use in the determination of naphthenic acids in various media used in environmental toxicity studies. ABC study no. 64403, Analytical Bio-Chemistry Laboratories, Columbia, Missouri.

ABC Laboratories, Inc. (2010a). Acute toxicity of water accommodated fractions of naphthenic acids to the fathead minnow, *Pimephales promelas*, determined under static-renewal test conditions using a step-down approach. ABC Study no. 64406, Analytical Bio-Chemistry Laboratories, Columbia, Missouri.

ABC Laboratories, Inc. (2010c). Growth inhibition test of water accommodated fractions of naphthenic acids to the unicellual green alga, Pseudokirchneriella subcapitata. ABC Report no. 64405, Analytical Bio-Chemistry Laboratories, Columbia, Missouri.

AGS Chemicals Limited, (2003). Material Safety Data Sheet, Naphthenic Acids. Web Version URL: http://www.amtrade.co.uk/prodinfo.htm.

Baetcke K, Hard G, Rodgers I, McGaughy R, Tahan L. *Alpha 2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat.* EPA/625/3-91/019F., 1991.

Bartha, R. and R.M. Atlas. (1977). The microbiology of aquatic oil spills. Adv Appl Microbiol. 22:225-266.

Brient, J., Wessner, P., and Doyle, M. (1995). Naphthenic acids In: *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th edition, Volume 16, pp 1017-1029.

Cairns, J. Scheier, A., and Loos, J. (1965). A comparison of the sensitivity to certain chemicals of adult zebra danios *Brachydanio rerio* (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish *Lepomis macrochirus* Raf. Notulae Naturae. No. 381:1-9, Acad. Nat. Sci. Philadelphia.

Capen C. Toxic responses of the thyroid gland. In: *Comprehensive Toxicology* I. G. Sipes *et al*., eds. Elsevier Science Inc., New York, NY. 1997:pp 683-700.

Cattley R, Popp J. Liver. In: *Handbook of Toxicologic Pathology*. W. Haschek, C. Rousseaux, and M. Wallig, eds. 2nd edition. Academic Press, New York, NY. 2002:p. 202.

CETAG (CONRAD Environmental Aquatics Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, Alberta, Canada. 65 pp.

Clemente, J.S. and P.M. Fedorak. (2005). A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere 60:585-600.

Clemente, J.S., M.D. MacKinnon, and P.M. Fedorak. (2004). Aerobic biodegradation of two commercial naphthenic acids preparations. Environ Sci Technol 38:1009-1016.

Curran P, De Groot L. The effect of hepatic enzyme-inducing drugs on thyroid hormones and thyroid gland. *Endocrine Reviews* 1991;12:135-150.

Dorn, P. (1992). The petroleum refining industry. In: Ford, D. (ed.). Water Quality Management Library, Volume 3, Toxicity Reduction Evaluation and Control. Technomic Publishing Company, Lancaster, PA. pp. 183-223.

Esso Research and Engineering Company (1963). Acute toxicity of naphthenic acids. MRD-2-63.

Exxon (1979a). Test for oral toxicity in rats. M.B. Research Laboratories, Inc., 79 MRR 457.

Exxon (1979b). Test for oral toxicity in rats. M.B. Research Laboratories, Inc. 79 MRR 781.
Exxon (1979c). Acute dermal toxicity in albino rabbits. M.B. Research Laboratories, Inc., 79 MRR 456.
Exxon (1979d). Eye irritation in albino rabbits. M.B. Research Laboratories, Inc., 79 MRR 455.
Fedorak, P. (2009). Report of the results of GC-MS analyses of naphthenic acids from API. Personal communication, January 29, 2009.
Frank, R.A., R. Kavanagh, B.K. Burnison, G. Arsenault, J.V. Headley, K.M. Peru, G. Van Der Kraak, and K.R. Solomon. (2008). Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere 72:1309-1314.
Frank, R.A., K Fischer, R. Kavanagh, B.K. Burnison, G. Arsenault, J.V. Headley, K.M. Peru, G. Van Der Kraak, and K.R. Solomon. 2009. Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. Environ Sci Technol 43(2):266-271.

Frith C, Botts S, Jokinen M, Eighmy J, Hailey J, Morgan S, Chandra M. Non-proliferative lesions of the endocrine system in rats. In: *Guides for Toxicologic Pathology,* STP/ARP/AFIP, Washington D.C. 2000.
Girling, A.E., G.F. Whale, and D.M.M. Adema. (1994). A guideline supplement for determining the aquatic toxicity of poorly water-soluble complex mixtures using water-accommodated fractions. Chemosphere 29(12):2645-2649.
Greaves P. Haematopoietic and lymphatic systems. In: *Histopathology of Preclinical Toxicity Studies.* 3rd edition, Associated Press, New York, NY 2007a;600-601, 615.
Greaves, P. (2007b). Cardiovascular System. In: *Histopathology of Preclinical Toxicity Studies.* 3rd edition, Associated Press, New York, NY, 276-277.
Hard G. Some aids to histologic recognition of hyaline droplet nephropathy in ninety-day toxicity studies. Toxicologic Pathology 2008;36:1014-1017.
Harris, J. (1982). Rate of Hydrolysis. In: *Handbook of Chemical Property Estimation Methods.* W. Lyman, W. Reehl and D. Rosenblatt, eds. Mcgraw-Hill Book Company, New York, NY.
Havre, T. (2002). Formation of calcium naphthenate in water/oil systems, naphthenic acid chemistry and emulsion stability. Ph. D. Thesis. Department of Chemical Engineering, Norwegian University of Science and Technology Trondheim, Norway, October, 2002.
Herman, D., Fedorak, P., and Costerton, J. (1993). Biodegredation of cycloalkane carboxylic acids in oil sands tailings. Canadian Journal of Microbiology 39:576-580.
Herman, D., Fedorak, P., MacKinnon, M., and Costerton, J. (1994). Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can J Microbiol 40:467-477.
Holowenko, F., MacKinnon, M., and Fedorak, P. (2002). Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Research 36:2843-2855.
Linden, E., B.E. Bengtsson, O. Svanberg, and G. Sundstrom. 1983. The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the beak (*Alburnus alburnus*) and the harpacticoid copepod (*Nitroca spinipes*). Chemosphere 8:843-851.

Mackay, D. (1991). Multimedia environmental models; The fugacity approach. Lewis Publishers., CRC Press, Boca Raton, Fl.
Malinckrodt Baker, Inc. (1997). Material Safety Data Sheet No. NO310, Naphthenic Acids (CAS No. 1338-24-5). Malinkrodt Baker, Inc. Phillipsburg, New Jersey.
National Toxicology Program (2012a). Calcium naphthenate. M90010. Site Accessed March 23, 2012.

National Toxicology Program (2012b). Sodium naphthenate. M90021. Site Accessed March 23, 2012.

Nero, V., A. Farwell, L.E.J. Lee, T. Van Meer, M.D. MacKinnon, and D.G. Dixon. (2006). The effects of salinity on naphthenic acid toxicity to yellow perch: gill and liver histopathology. Ecotoxicol Environ Safety 65(2):252-264.

OECD. Guideline for testing of chemicals, guideline 422. Combined repeated dose toxicity study with reproduction/developmental toxicity screening. 1996.

OECD. (2000). Guidance document on aquatic toxicity testing of difficult substances and mixtures. OECD Series on Testing and Assessment, No. 23, ENV/JM/MONO(2000)6. Paris, France.

Jivraj MN, MacKinnon MD, Fung B. 1991. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. Syncrude Canada Ltd. Research Department. Edmonton, AB. September 1991.

Pennisi, S. and Lynch, V. (1977). Pharmacologist 19:181 (meeting abstract).

Peters, L.E., M. MacKinnon, T. Fan Meer, M.R. van den Heuvel, and D.G. Dixon. (2007). Effects of oil sands process-affected waters and naphthenic acids on yellow perch (*Perca flavescens*) and Japanese medaka (*Orizias latipes*) embryonic development. Chemosphere 67:2177-2183.

Rockhold, W. (1955). Toxicity of naphthenic acids and their metal salts. American Medical Association Archives of Industrial Health 12:477-482.Rogers V, Wickstrom M, Liber K, MacKinnon M. (2002a). Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. *Toxicological Sciences* 2002a;66:347-355.

Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon (2002a). Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.

Rogers V, Wickstrom M, Liber K, MacKinnon, M. (2002b). Mammalian toxicity of naphthenic acids derived from Athabasca oil sands (AOS). The Toxicologist 2002b;66:645 [meeting abstract].

Rogers V, Liber K, and MacKinnon MD. (2002c). Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere 48:519-527.

Rogers, V. (2003). Mammalian toxicity of naphthenic acids derived from the Athabasca oil sands. Ph.D. thesis. University of Saskatchewan, Saskatoon, Canada.

SocTech (2003). Product data sheet, naphthenic acids. Web Version URL: http://www.soctech.ro/English/Produse /1acizinaft.htm.

Swenberg J, Lehman-McKeeman L. α2u-Globulin associated nephropathy as a mechanism of renal tubular cell carcinogenesis in male rats. In: *Species differences in thyroid, kidney and urinary bladder carcinogenesis.* IARC Scientific Publication no. 147. International Agency for Research on Cancer, Lyon, France, 1998.

U.S. EPA (1999a). High production volume (HPV) challenge program: *Determining the adequacy of existing data,* 2/10/99 rev. URL: http://www.epa.gov/chemrtk/datadfin.htm.

U.S. EPA (1999b). Chemical right to know initiative. *The use of structure-activity relationships (SAR) in the high production volume chemicals challenge program.* URL: http://www.epa.gov/chemrtk/sarfin/1.htm.

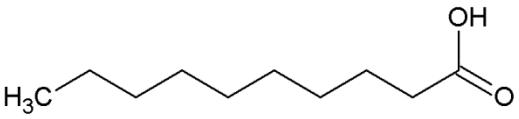
U.S. EPA (2000). EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, D.C.

WIL Research (2012). WIL-402011. A Combined 28-Day Repeated Dose Oral (Gavage) Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Naphthenic Acid with a Mammalian Erythrocyte Micronucleus Test in Rats. WIL Research Laboratories, LLC, Ashland, OH

Young, R., Wismer, W., and Fedorak, P. (2008). Estimating naphthenic acids in laboratory-exposed fish and in fish from the wild. Chemosphere 73:498-505.

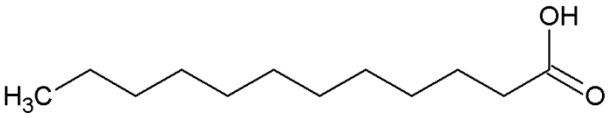
APPENDIX A. Naphthenic Acid Structures used in EPIWIN© for Physical-Chemical Modeling

#1



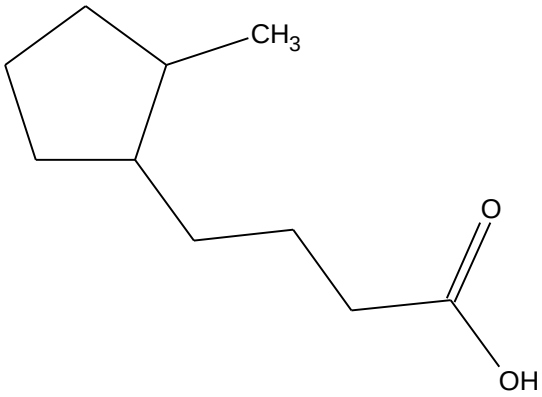
decanoic acid

#2



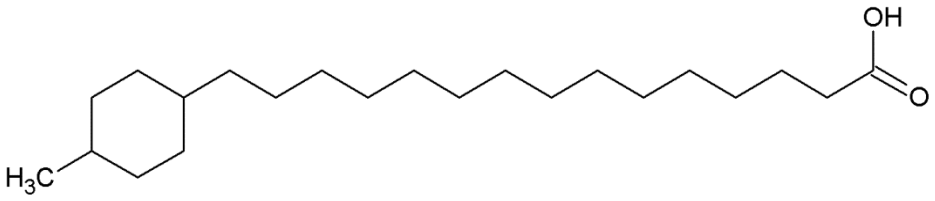
dodecanoic acid

#3



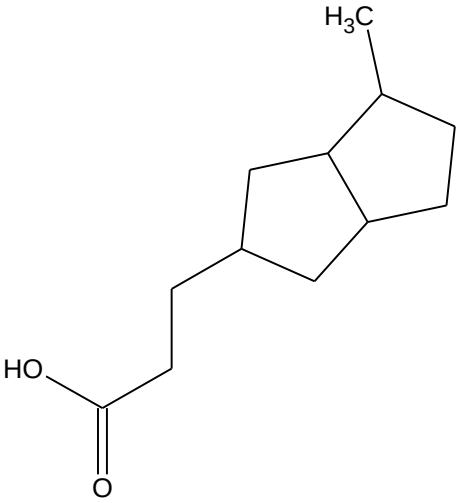
4-(2-methylcyclopentyl)butanoic acid

#4



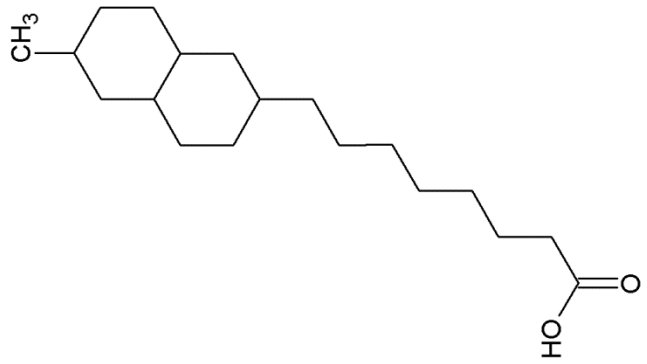
15-(4-methylcyclohexyl)pentadecanoic acid

#5



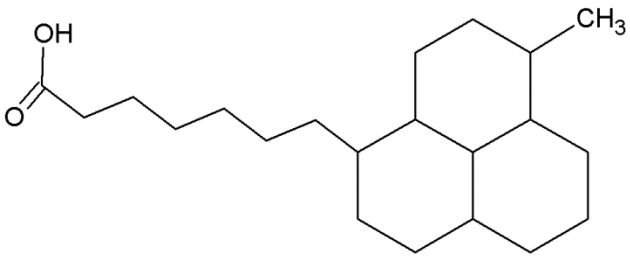
3-(1-methyloctahydropentalen-5-yl-)propanoic acid

#6



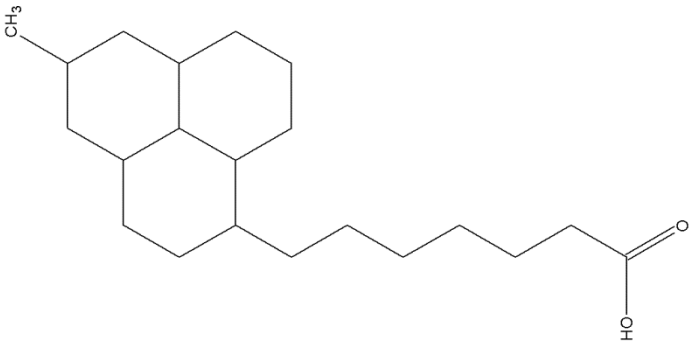
8-(2-methyldecalin-6-yl)octanoic acid

#7



7-(1-methyl-dodecahydrophenalen-4-yl)heptanoic acid

#8



7-(5-methyl-dodecahydrophenalen-1-yl)heptanoic acid

#9

3-(3-methyl-octadecahydrocyclopenta[a]phenanthren-17-yl)propanoic acid)