Naphthenic Acids Category
Robust Summaries

American Petroleum Institute Petroleum HPV Testing Group

Consortium # 1100997

I U C L I D
Data Set

Existing Chemical	:	Naphthenic Acids Category
CAS No.	:	1338-24-5, 64754-89-8 (and supporting chemical 61790-13-4)
EINECS Name	:	

Producer related part

Company	:	
Creation date	:	

Substance related part

Company	:	American Petroleum Institute
Creation date	:	May 15, 2012
Status	:	
Memo	:	Robust summary
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Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	
Substance type	:	
Physical status	:	
Purity	:	
Colour	:	
Odour	:	
Remark	:	Naphthenic acid fractions are oily liquids. The salts may be liquid or solid. Naphthenic acids (CASRN 1338-24-5, 64754-89-8, and 61790-13-4) are classified as monobasic carboxylic acids of the general formula RCOOH, where 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. 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. Naphthenic acids are obtained by caustic extraction of petroleum distillates, primarily kerosene and diesel fractions.
Reference		

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Sublimation	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: Naphthenic acids, commercial mixtures
Remark	:	Values cited represent ranges of melting points cited in product literature data and Material Safety Data Sheet for commercial naphthenic acid products.
Result	:	-35 °C to + 0 °C (Soc Tech, 2003) -35 °C to + 2 °C (AGS Chemicals, 2003) +30 °C (Mallincrodt Baker, 1997)
Reliability	:	(4) not assignable Original source data were not available for review.
Reference		(2) (23) (34)

2.2 BOILING POINT

Decomposition	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)
Remark	:	Values reported vary widely due to varied composition of the hydrocarbon mixture in naphthenic acids. Values given represent various commercial preparations of naphthenic acids.
Result	:	250 °C to 350 °C (Soc. Tech., 20031) 140 °C to 200 °C (AGS Chemicals, 20032) 200 °C to 370 °C (Brient et al., 1995)
Reliability	:	(4) not assignable
Reference		(3) (5) (35)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Decomposition	:	
Method	:	other (calculated): EPIWIN, MPBPWIN V1.40 (US EPA 2000)
Year	:	
GLP	:	
Test substance	:	other TS: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)
Remark	:	A search for pressure values of naphthenic acids failed to

uncover reliable information. Product literature data provided narrative phrases such as "very low" or "not applicable" when describing the vapor pressure characteristic for commercial products (SocTech, S.A., 2003; AGS Chemicals Limited. 2003). To gain an understanding of vapor pressure characteristics of naphthenic acids, various naphthenic acid structures described by Brient et al. (1995) were estimated for vapor pressure using the EPIWIN computer model (U.S. EPA 2000).

The vapor pressure of complex mixtures is equal to the sum of the vapor pressures of the individual constituents in their pure form times their mole fraction in the mixture (Raoult's Law). Therefore, the total vapor pressure of a complex mixture of naphthenic acids will depend on the proportion of different molecular weight constituents making up the mixture. It is estimated from vapor pressure modeling that commercial products will have vapor pressure values near or below the measurable limits cited in standard reference guidelines (OECD Guideline 104, Vapor Pressure; OECD, 1995). Hence, based on Raoult's Law, the total vapor pressure of naphthenic acids is expected to be exceedingly low.

Result :

Naphthenic Acid	C Z-No.	Mole. No.	Vapor Wt.	Pressure, Pa
decanoic acid	0	10	172	0.049
dodecanoic acid	0	12	200	0.0021
2-methyl, 1-cyclopentyl propanoic acid	-2	10	170	0.32
4-methyl, 1-cyclohexyl decabutanoic acid	-2	21	325	0.000020
3-methyl, bicyclooctyl-[3.3]-7-propanoic acid	-4	12	196	0.042
3-methyl, bicyclodecyl-[4.4]-8-decanoic acid	-4	21	323	0.000019
3-methyl, tricyclodecapropyl-[3.3.3]-11-propanoic acid	-6	17	264	0.00056
3-methyl, tricyclodecapropyl-[3.3.3]-11-Heptanoic acid	-6	21	321	0.000019
3-methyl, tetracyclodecaheptyl-[4.2.2.2]-11 propanoic acid	-8	21	319	0.000021

Test condition : Not applicable, vapor pressures were calculated by MPBPWIN, V1.40, EPIWIN V3.10

Reliability : (2) valid with restrictions
Estimated vapor pressures were obtained from a validated computer program.

Reference (1) (25) (29) (33) (38)

2.5 PARTITION COEFFICIENT

Method : other (calculated): EPIWIN, KOWWIN V1.66 (US EPA 2000)
Year : 2000
GLP :
Test substance : other TS: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Remark : No partition coefficient measurements were found for naphthenic acids. Therefore, partition coefficients for a range of molecular weight naphthenic acids were estimated

using the EPIWIN computer model (U.S. EPA 2000). The partition coefficients reported here span the molecular weights and numbers of cycloalkane rings reported to exist in Athabasca oil sands extracts and commercial products (Brient et al. 1995). It may be expected, however, that the lowest molecular weight structures will have the lowest partition coefficients of the compounds in the complex mixtures.

Result :

Naphthenic Acid	C Z-No.	Mole. No.	Log Wt.	Kow
decanoic acid	0	10	170	4.1
dodecanoic acid	0	12	200	4.6
2-methyl, 1-cyclopentyl propanoic acid	-2	10	170	3.8
4-methyl, 1-cyclohexyl decabutanoic acid	-2	21	325	9.2
3-methyl, bicyclooctyl-[3.3]-7-propanoic acid	-4	12	196	3.8
3-methyl, bicyclodecyl-[4.4]-8-decanoic acid	-4	21	323	8.2
3-methyl, tricyclodecapropyl-[3.3.3]-11-propanoic acid	-6	17	264	6.0
3-methyl, tricyclodecapropyl-[3.3.3]-11-Heptanoic acid	-6	21	321	8.0
3-methyl, tetracyclodecaheptyl-[4.2.2.2]-11 propanoic acid	-8	21	319	6.3

Test condition : Not applicable, partition coefficients were calculated by KOWWIN, V1.66, EPIWIN V3.10

Reliability : (2) valid with restrictions
Estimated partition coefficients were obtained from a validated computer program.

Reference (5) (38)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

PHYS CHEM WATER SOLUBILITY	
Category Chemical :	Naphthenic acids, CAS no. 1338-24-5
Test Substance :	Naphthenic acids, CAS no. 1338-24-5
Test Substance Purity/Composition and Other Test Substance Comments:	Specific analyses of the test substance: Acid number: 235 mg KOH/gm Unsaponifiables (total): 4.9% Viscosity @40°C: 32 cst Specific gravity @20°C: 0.969 Color (Garner), GI 4.5 Water content: 0.07% Phenolic content (acid): 0.31% Total sulfur: 0.34 CP – Flash point °F (COC): 343
Category Chemical Result Type :	Measured
Test Substance Result Type :	Measured
RESULTS	
Water Solubility Indicator :	

Water Solubility Input type:	Value or Range?
Water Solubility Value/Range : **Solubility**: = 88.1 mg/L @ **Temperature**: approximately 20°C	
pH Value :	**Value or Lower Range**: 7.5 **Upper Range** :
pKa - Protein Kinase:	
pH Value at Saturation :	
Results Remarks :	The solubility value represented the measured concentration of total dissolved naphthenic acids in the water accommodated fraction of freshwater algal nutrient medium (pH 7.5) using a loading rate of 100 mg/L. Higher solubility concentrations may be achieved using higher loading rates.
STUDY/METHOD	
Key Study Sponsor Indicator :	Key
Year Study Performed :	2009
Method/Guideline Followed :	Other, similar to OECD 105 flask method
Method/Guideline and Test Condition Remarks:	A 100 mg/L loading rate solution of naphthenic acids in freshwater algal nutrient medium was prepared in an aspirator bottle containing a Teflon stir bar. Triplicate bottles were prepared in this manner. The bottles were placed on magnetic stir plates and stirred at a rate to maintain a vortex of approximately 30-50% of the static solution depth. One of the aspirator bottles was removed from the stir pates at 18, 24, and 72 hours and allowed to settle for one hour. After settling, solutions were drained from the bottom outlet of the aspirator bottle into a sample bottle. The first 100 mL was sent to waste and care was taken to ensure that no insoluble fraction was carried over into the sample bottle. Test solutions were analyzed for the concentration of naphthenic acid, using Fourier transform infrared spectroscopy (FTIR). Analysis was accomplished based on a method developed at ABC Laboratories following Jivraj et al. 1991.
GLP :	Yes
Study Reference :	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.
RELIABILITY/DATA QUALITY	
Reliability :	1 (reliable without restrictions)
Reliability Remarks :	comparable to a guideline study

PHYS CHEM **WATER SOLUBILITY**	
Category Chemical :	1338-24-5
Test Substance :	1338-24-5
Test Substance Purity/Composition and Other Test Substance Comments:	
Category Chemical Result Type :	

Test Substance Result Type :	
RESULTS	
Water Solubility Indicator :	
Water Solubility Input type:	Value or Range? RANGE
Water Solubility Value/Range :	**Solubility**: 70 mg/L to 5040 mg/L @ **Temperature**: 25°C
pH Value :	**Value or Lower Range**: 0.91 **Upper Range** : 9.16
pKa - Protein Kinase:	
pH Value at Saturation :	
Results Remarks :	The solubility values were provided in a general background report on naphthenic acids. The report cited the solubility data orginated from a commercial standard liquid formulation obtained from Baker Chemical Co. The original data were taken by CEATAG (1998) from Kharrat (1996).
STUDY/METHOD	
Key Study Sponsor Indicator :	
Year Study Performed :	
Method/Guideline Followed :	
Method/Guideline and Test Condition Remarks:	
GLP :	
Study Reference :	CEATAG (CONRAD Environmental Aquatics Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, Alberta, Canada. 65 pp. Kharrat, A. 1996. Physico-chemical properties of naphthenic acids. Alberta Environmental Centre Progress Report October 1, 1005 – March 31, 1996. XD952287.RPT/6/4/96/PS.
RELIABILITY/DATA QUALITY	
Reliability :	4 (not assignable)
Reliability Remarks :	Data retrieved from a secondary reference. The original report that contained details of the methods and results was not available for review.

Memo : Water solubility of naphthenic acids

Remark : Values of water solubility reported in product literature data have varied widely. CEATAG (1998) reported water solubility values of one commercial product to range from 70 mg/l at pH 0.91 to 5040 mg/l at pH 9.16. Other product data sources for water solubility report narrative phrases such as "very low water solubility" (SocTech S.A., 2003), "not applicable" (Mallinckrodt Baker Inc., 1997), or "only slightly soluble in water" (AGS Chemicals Limited, 2003).

Reliability : (4) not assignable
Data were obtained from secondary literature sources.

04.01.2005 (1) (8) (22) (33)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Deg. product :
Method : other (calculated): EPIWIN V3.10; subroutine AOPWIN V1.90
Year :
GLP :
Test substance : other TS: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Remark : AOPWIN V1.90 calculates atmospheric oxidation rate constants between photochemically produced hydroxyl radicals and organic chemicals. These rate constants are then used to calculate half lives for those compounds based on average atmospheric concentrations of hydroxyl radicals and ozone. Atmospheric oxidation rates were calculated for a range of molecular structures covering a range of molecular weights and ring structures that were reported to exist in Athabasca oil sands extracts and commercial products (Rogers et al., 2002; Brient et al. 1995).

Although the low vapor pressures of these base oils indicate that volatilization will not be a very significant fate process, oxidation half-lives indicate that any vapors emitted to the troposphere would be rapidly oxidized and not persist in the atmosphere.

Result :

Naphthenic Acid	C Z-No.	Mole. No.	Half Wt.	Life, days
2-methyl, 1-cyclopentyl propanoic acid	-2	10	170	0.9
4-methyl, 1-cyclohexyl decabutanoic acid	-2	21	325	0.3
3-methyl, bicyclooctyl-[3.3]-7-propanoic acid	-4	12	196	0.8
3-methyl, bicyclodecyl-[4.4]-8-decanoic acid	-4	21	323	0.3
3-methyl, tricyclodecapropyl-[3.3.3]-11-propanoic acid	-6	17	264	0.3
3-methyl, tricyclodecapropyl-[3.3.3]-11-Heptanoic acid	-6	21	321	0.3
3-methyl, tetracyclodecaheptyl-[4.2.2.2]-11 propanoic acid	-8	21	319	0.3

Test condition : Not applicable, photodegradation potential was calculated by AOPWIN, V1.90, EPIWIN V3.10
Reliability : (2) valid with restrictions
Estimated water solubility values were obtained from a validated computer program.
Reference (5) (30) (38)

3.1.2 STABILITY IN WATER

Remark : 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 alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982).

The chemical components found in the materials that comprise the gas oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

Reference (15)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type :
Media :
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Level 1 Fugacity-Based Environmental Equilibrium Partitioning Model (Version 2.11)
Year :

Remark : Multimedia distribution was calculated for a range of naphthenic acids covering molecular weight and ring structures of such constituents found in Athabasca oil sands extracts and commercial products (Rogers et al., 2002; Brient et al., 1995).

Result : Air / Water / Soil / Sediment / Suspended Sediment / Biota

Naphthenic Acid Type (Z-number)(C-number)(Molecular Weight)

Air	Water	Soil	Sed	Susp Sed	Biota
2-methyl,1-cyclopentyl propanoic acid (-2)(10)(170)					
2	16	81	1.8	<0.1	<0.1
4-methyl,1-cyclohexyl decabutanoic acid (-2)(21)(325)					
<0.1	<0.1	98	2	<0.1	<0.1
3-methyl, bicyclooctyl-[3.3]-7-propanoic acid (-4)(12)(196)					
0.4	15	83	2	<0.1	<0.1
3-methyl, bicyclodecyl-[4.4]-8-decanoic acid (-4)(21)(323)					
<0.1	<0.1	98	2	<0.1	<0.1
3-methyl, tricyclodecapropyl-[3.3.3]-11- propanoic acid (-6)(17)(264)					
<0.1	0.1	98	2	<0.1	<0.1

3-methyl, tricyclodecapropyl-[3.3.3]- 11 heptanoic acid (-6)(21)(321)

<0.1 <0.1 98 2 <0.1 <0.1

3-methyl, tetracyclodecaheptyl-[4.2.2.2]-11 propanoic acid (-8)(21)(319)

<0.1 <0.1 98 2 <0.1 <0.1

Test condition : The EQC Level I is a steady state, equilibrium 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.

Reliability : (2) valid with restrictions
Estimated environmental distribution was obtained from a validated computer program.

Reference (5) (30) (21)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Remark : No standardized testing for ready or inherent biodegradation was found for naphthenic acids. Results of relevant scientific journal articles on the biodegradability of naphthenic acids are reviewed in Section 3.8

Reference

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

Memo : Biodegradation of cycloalkane carboxylic acids in oil sand tailings

Remark : Herman et al. (1994) investigated the ability of microbial populations indigenous to oil sands tailings to biodegrade solutions of natural naphthenic acids from oil sands tailings and commercial naphthenic acid sodium salts (Kodak Chemicals).

Four experiments were run:
1) Evaluation of mineralizaton of naphthenic acids sodium salts (NAS) and oil sands tailings extracts of naphthenic acids (TEX),
2) Evaluation of mineralization of four model naphthenic

acid compounds, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA) 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and trans-4-pentylcyclohexane carboxylic acid (4PCCA),
3) Gas chromatographic analysis of NAS and TEX biodegradation, and
4) Respirometry measurements of cyclohexane pentanoic acid, NAS, and TEX in tailings microcosms.

Test Substances: Test substances used in the four experiments included the following materials: 1) Tailings water extract (TEX), 2) commercial sodium naphthenate mixture (NAS), and 3) pure compound naphthenic acids, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA), 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and trans-4-pentylcyclohexane carboxylic acid (4PCCA).

Inoculum: Inoculum used in the biodegradation experiments was NAS- and TEX- degrading enrichment cultures derived from oil sands tailings water. These cultures were created by diluting a 10-ml sample of oil sands tailing into 90 ml of mineral salts medium that contained either NAS (100 mg/l) or TEX (1:50 dilution). The mineral salts medium was modified Bushnell-Haas medium. Successive transfers 1% v/v) of the enrichment culture into fresh NAS- to TEX-containing medium were on monthly basis and incubated at room temperature on a gyratory shaker (100 rpm). The viable cell number within each enrichment culture was estimated using the plate count technique.

Experiment No. 1. A measurement of CO2 production was used to evaluate the ability of the enrichment cultures to mineralize components within both the NAS and TEX mixtures. Mineralization experiments were performed using 60-ml serum bottles containing 15 ml of growth medium. The growth medium consisted of sterilized mineral salts medium with NAS (100 mg/l) or TEX (1:20 and 1:50 dilutions) as the sole carbon source. Dissolve organic carbon analyses showed that 100 mg/l of NAS contained 60 mg C/l, while 1:20 and 1:50 dilutions of TEX contained 50 and 21 mg C/l, respectively. The serum bottles were inoculated with 0.15 ml of either the NAS-degrading or the TEX-degrading enrichment culture, sealed with rubber stoppers, and incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 6-day intervals over 24 to 30 days, three inoculated bottles and one control (inoculated but lacking NAS or TEX) were acidified to pH <2 using 1 ml of 1M H2SO4 to convert all forms of inorganic carbon into CO2. A 0.5 ml headspace sample from each bottle was analyzed for CO2 content by gas chromatography. Mineralization of the organic substrate was first corrected for the amount of CO2 in the control bottles, then expressed either as the total amount of CO2 produced within the bottle or as the percentage of organic carbon converted to CO2.

Results of Experiment No. 1. The mineralization studies showed that the NAS- and TEX-degrading enrichment culture was capable of mineralizing components within both the NAS and TEX mixtures. The percentage of organic carbon converted to CO2 by the NAS-degrading culture was 48% (day 24) in the NAS bottles and 20% (day 20) in the TEX bottles. The percentage of organic carbon converted to CO2 by the

TEX-degrading culture was 34% (day 30) for the TEX bottles and 20% (day 25) for the NAS bottles.

Experiment No. 2. Mineralization of the four model naphthenic acid compounds was measured as the amount of CO2 evolved from incubating solutions of the compounds dissolved in nutrient medium and inoculated with enrichment cultures of NAS-degrading microorganisms, TEX-degraders, or oil sands tailings pond water (TPW). Fifteen milliliters of 1 mM solutions of the compounds dissolved in mineral salts medium were placed in 60-ml serum bottles and inoculated (1% v/v) with the different sources of microbes then sealed with robber stoppers. Bottles were incubated at room temperature on a gyratory shaker (100 rpm). After 3, 6, 12, and 24 days, duplicate bottles were acidified and headspace CO2 determined by GC. The level of CO2 production was corrected for the amount of CO2 within the control bottles and expressed as the percentage of organic substrate converted to CO2.

Results of Experiment No. 2. The following results were obtained:.

Mineralization by day 24, % organic C converted to CO2:

Substrate	NAS-degraders	TEX-degraders	TPW
CCA	41	56	57
CPA	45	57	58
2MCCA	47	7	67
4PCCA	6	24	24

Experiment No. 3. A 1% (v/v) inoculum of the NAS-degrading enrichment culture was placed in 125-ml Erlenmeyer flasks containing 50 ml of either NAS (30 mg/l) or TEX (1:50 dilution) in mineral salts medium. Control flasks received inoculum of heat-killed cells. The flasks were incubated at room temperature on a gyratory shaker (100 rpm). After an incubation period of 4, 8, and 16 days for NAS and 6, 12, and 24 days for TEX, the contents of two flasks and two control flasks were extracted for GC analysis. Samples were extracted and the carboxylic acids were derivatized to methyl esters prior to analysis. Derivatized extracts were analyzed by GC with a capillary column and flame ionization detector.

Results of Experiment No. 3. Chromatographic analysis of solution from the control flasks revealed an unresolved series of many overlapping peaks that created a hump in the GC profile. When the mixture that was inoculated with NAS-enrichment culture, a reduction in the size of the hump was evident within 4 days, indicating that components within the naphthenic acid mixture were being degraded. Chromatographic analysis of the TEX samples revealed a similar hump of many overlapping peaks that appeared in the NAS GC profile. Biodegradation of TEX by the NAS-degrading culture did not result in a noticeable reduction in the size of the hump associated with TEX, despite evidence of mineralization of components within the mixture.

Experiment No. 4. A measurement of CO2 production and O2 utilization within sealed microcosms was used to monitor microbial activity in samples of TPW, and to determine the

effect of nutrient addition (N and P) or carbon substrate addition (cyclohexane pentanoic acid (CPA), sodium salts of naphthenic acids (NAS), or tailings pond extracts of carboxylic acids (TEX)) on the level of microbial activity within TPW.

60 ml of TPW was placed into sterile 125-ml Erlenmeyer flasks, sealed with rubber stoppers in which a sampling port had been drilled and then sealed with clear silicone. Nutrients in the form of N and P were added. Carbon substrates (CPA, NAS or TEX) were added as a filter-sterilized solution to crate a final concentration of 60 mg organic carbon/l. All flasks were incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 80day intervals, 0.5 ml of headspace was sampled and analyzed for CO2 and O2 using GC. Following 5 weeks of incubation, the contents of the flasks containing CPA were extracted and analyzed using the procedure described for the GC analysis in experiment 3.

Results of Experiment No. 4. The addition of CPA to TPW resulted in increased microbial activity, as indicated by greater levels of CO2 production and O2 utilization when compared with TPW alone. Sterilized TPW demonstrated no CO2 production or O2 utilization. Even greater levels of microbial activity were evident when N and P were added in addition to CPA, indicating that mineralization could be enhanced by the addition of mineral nutrients. GC analysis of CPA in TPW microcosms after 35 d of incubation revealed that the concentration of CPA was below the level of detection in 2/3 microcosms and reduced 10-fold in the third microcosm. There was no detectable CPA in the three N and P-amended microcosms.

Similarly, NAS and TEX additions to microcosms increased microbial activity in TPW, although microbial activity was enhanced by the addition of N and P. Increases in both CO2 evolution and O2 utilization were seen.

Conclusions. This investigation showed that naphthenic acids, either as a commercial preparation of sodium salt (NAS) or natural extracts from oil sands tailing water (TEX) are capable of being utilized by natural assemblages of microorganisms. Addition of nitrogen and phosphorus enhances the utilization of these substrates by the microbes.

Reliability : (2) valid with restrictions
The report was a well-documented study that meets basic scientific principles.

Reference (19)

Memo : Biodegradation of naphthenic acids

Remark : Herman et al. (1993) conducted four experiments on the biodegradation of specific cycloalkane carboxylic acids:

Experiment No. 1. Biodegradation of four naphthenic acid compounds (cyclopentane carboxylic acid, CCP; cyclohexane carboxylic acid, CCH; 1-methyl-1-cyclohexane carboxylic acid, 1MCCH; and 2-methyl-1-cyclohexane carboxylic acid, 2MCCH) was measured in pore water from Athabasca oil sands tailings ponds. The purpose of the tailings ponds was to

serve as a settling basin to separate solids from liquid generated during the extraction of acidic compounds from bitumen. Therefore, the tailings ponds were considered to harbor indigenous microorganisms adapted to naphthenic acids. The collected pore water was centrifuged and filtered and served as the nutrient medium. Inoculum was 0.5 ml of the original oil sands tailings sample. Duplicate flasks containing 30 ml of medium were spiked with 1-ml aliquots of stock solutions of the different naphthenic acids to achieve a final concentration of 1000 mg/l. Test flasks received the inoculum and control flasks received inoculum in which the microbes had been heat-killed. One set of duplicate flasks received a nutrient addition in the form of NH4NO3, K2HPO4, and KH2PO4 to a final concentration of 0.2 g/l of each compound. The flasks were incubated at room temperature on a rotary shaker. After 0, 3, 6, 9, 16, 26, and 40 days, a 3-ml sample was removed, centrifuged, and filtered through a 0.2 micron syringe filter. The samples were analyzed for the test compounds by gas chromatography equipped with a flame ionization detector. Peak areas were converted to concentration using a calibration curve for each compound.

Results of Experiment 1. The bacterial populations of oil sands tailings was shown to have the metabolic capability of degrading carboxylated cycloalkanes as shown in the following table of results.

	Percent Remaining							
	CCP		CCH		MCCH		2MCCH	
Day	NP-	NP+	NP-	NP+	NP-	NP+	NP-	NP+
0	100	42	100	68	100	100	100	100
6	100	5	100	12	100	100	100	100
10	100	0	100	1	100	100	100	100
16	100	0	100	0	100	100	100	100
26	100	0	100	0	100	100	100	49
40	100	0	100	0	100	100	100	0

Using tailings pond water as a growth medium, degradation of CCP, CCH, and 2MCCH was achieved only if nutrients were added to the medium. CCP and CCH were degraded rapidly, within one week, while methylated carboxylic acids were more resistant to biodegradation. 2MCCH was degraded within 40 days, but no degradation was observed for 1MCCH.

Experiment No. 2. Triplicate tailings pond microcosms were created using 200 ml of the tailings sample (as inoculum and medium) in 500-ml Erlenmeyer flasks closed with cotton stoppers. A filter-sterilized solution of CCP and 1MCCH was added to each microcosm for a final concentration of 1000 mg/l. Sterile controls were autoclaved and also spiked with the test compounds. Microcosms were incubated at room temperature on a rotary shaker. After 1, 2, 3, 4, 6, and 9 weeks, samples were removed and analyzed for CCP and 1MCCH by GC.

Results of Experiment No. 2. Biodegradation of CCP was complete within the first week. No biodegradation of 1MCCH was evident after six weeks. At the six-week period, nitrogen and phosphorus was added whereby complete biodegradation of 1MCCH was noted following between the 6 and 9-week sampling. No 1MCCH was measured at 9 weeks.

Neither CCP nor 1MCCH was degraded in the control microcosms.

Experiment No. 3: Tailings pond bacteria were isolated on agar plates and colony types were examined for their ability to utilize carboxylated cycloalkanes as their sole carbon source. Individual colonies were inoculated into a solution of carboxylated cycloalkanes (1000 mg/l) in modified Bushnell and Haas (MGH) minimal salts medium. The ability of the isolate to metabolize the carbon source was monitored by GC analysis. In a second part to this experiment, a carboxylate-degrading mixed bacterial culture was enriched from the tailings pond sample using standard procedures. The mixed culture was maintained on a mixture of CCP, 1MCCH, and 2MCCH (500 mg/l each) in MBH with yeast extract (1000 mg/l) added as a supplemental carbon source.

Results of Experiment No. 3. Of 10 separate colony types isolated from oil sands tailings, one colony type was found to utilize CCP and CCH as its sole carbon source. The isolate was a Gram negative, non-motile, catalase positive, oxidase negative, non-fermenting, aerobic rod, and was identified as an Acinetobacter sp. The isolate rapidly degraded CCP and CCH, with complete loss of substrate from the medium within 2 weeks of incubation. However, this isolate was unable to degrade methyl-substituted cyclohexane carboxylic acids. The mixed bacterial culture enriched from the tailings pond sample on a mixture of carboxylated cycloalkanes was found to degrade 1MCCH and 2MCCH, but only when the medium was supplemented with yeast extract. After a 2-week incubation period, the mixed culture had degraded 100% of the 1MCCH and 67% of the 2MCCH.

Experiment No. 4. Radiolabeled hexadecane was spiked into the maltene fraction of pure bitumen. Hexadecane mineralization experiments were performed using 5 ml of oil sands tailings in 60-ml serum vials and inoculated with 10 ul of spiked maltene. One set of vials received nutrient addition as described before. Sterile controls were autoclaved before the addition of the labeled hydrocarbon. Mineralization was determined from triplicate vials after 5, 10, 16, 27, and 40 days using the closed-loop trapping system. Radioactivity was measured using a scintillation cocktail and a Beckman LS8000 scintillation counter.

Results of Experiment No. 4. The results of hexadecane mineralization within oil sands tailings showed that the biodegradation of an n-alkane was nutrient limited. Percent biodegradation reached 50% by day 16 and maintained a plateau through day 40.

Conclusions. This study showed the potential for biodegradation of naphthenic acids by investigating the biodegradation of both carboxylated cycloalkanes and hexadecane. Although natural naphthenic acids present in oil sands tailings have greater structural complexity than the compounds examined in this study, the results show the potential for both for biodegradation of the alkyl side chain and the carboxylated cycloalkane ring components of naphthenic acids. Biodegradation potential was reduced by methyl substitution on the cycloalkane ring, although these compounds could be degraded with the addition of mineral

nutrients.

Reliability : (2) valid with restrictions
The report was a well-documented study that meets basic scientific principles.

Reference (18)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	
Limit test	:	
Analytical monitoring	:	no
Method	:	
Year	:	1965
GLP	:	no
Test substance	:	other TS: Naphthenic Acids

Method : Statistical Method:
Graphical interpolation for determining the LC50.

Result : 96-hour TLm = 16.3 ppm

The following dose-response data were provided:

Concentration of Naphthenic acids, ppm	Number Tested	% Dead at 96 hours
0 (control #1)	10	0
0 (control #2)	10	0
7.5	10	0
8.7	10	40
10	10	20
11.5	10	0
13.5	10	20
15.5	10	30
18	10	80
21	10	100
24	10	100

The article reported that pH and dissolved oxygen concentrations were taken during the test, but these data were not reported.

Test condition : Test containers were 2.5 gallon aquariums, each fitted with an air stone through which compressed air was bubbled to maintain a 5-9 ppm dissolved oxygen concentration in the dilution water. The aquariums were maintained at a temperature of 24 +/- 1 C. Dilution water was synthetic soft water prepared with distilled water and ACS grade chemicals.

The lot of test fish displayed no visible disease. The average size was 3.2 cm total length. Before testing the fish were acclimated to the dilution water for 5 days. During the acclimation period they were fed Daphnia and white worms, but were not fed for 36 hours before or during the testing.

Test concentrations were prepared by direct addition of the test substance to the test chambers followed by mixing. Test concentrations were control, 7.5, 8.7, 10, 11.5, 13.5, 15.5, 18.0, 21.0, and 24.0 ppm naphthenic acids. After the test solutions were prepared, ten fish were placed in each test container. Controls were run in duplicate, while test levels were run singly. Mortality was evaluated at 24, 48, and 96 hours, and the criteria for death was a cessation of

gill movement and failure to respond to mechanical stimulus.

Following the 96 hour test period the TLm (median tolerance limit) was determined from visual observation of the dose-response pattern. Where no exact TLm response resulted, the TLm was interpolated from a plot of the concentration and survival data on semi-log paper.

Reliability : (2) valid with restrictions
The test was conducted under referenced test conditions current for the period in which the study was run. The report provided sufficient details for assessment.

Reference (6) (10) (16)

Type : static
Species : Gasterosteus aculeatus (Fish, estuary, marine)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test :
Analytical monitoring : no
Method :
Year :
GLP : no data
Test substance : other TS: Naphthenic acid mixture (commercially available from Eastman Chemicals)

Result : LC50 estimated to be in the range of 5 mg/l.

The following dose response data were reported:

Concentration (mg/l)	% Survival
0 (control)	100
2.5	60
5	10
10	0
15	0
30	0

Although an LC50 could have been calculated using contemporary methods, the author elected to estimate its value. The report stated that water chemistry data were collected but no data were reported.

Test condition : Summary of Test Conditions

Organism age:	juvenile
Test Temperature:	20 °C ± 2 °C
Photoperiod:	16 h light/8 h dark
Light intensity:	10 - 50 micro-einsteins
Light quality:	wide spectrum fluorescent
Test container:	5 gallon aquaria
Dilution water:	Carquinex Strait
Test Volume:	15 liters
Animals per container:	10
Replicate containers:	2
Number of concentrations:	6 (5 concentrations and a control)
Food:	none
Test duration:	96 h
Test endpoint:	mortality
Salinity	15 parts per thousand
Test pH:	ambient
Test article:	Martinez Refinery effluent (non-toxic)

with added naphthenic acids

Test solutions were prepared by creating a 1 percent solution using non-toxic effluent pH adjusted to 12 with sodium hydroxide. The stock solution was mixed overnight prior to use. The stock solution was used to spike non-toxic treated effluent to nominal naphthenic acid concentrations from 2.5 to 30 mg/l.

Test organisms were held at least seven days prior to testing in dilution water. During testing at 24-h intervals, the salinity, temperature, pH, and dissolved oxygen were measured in all control and test tanks. Survival data were taken at 24-h intervals and dead individuals were removed when observed.

Reliability : (2) valid with restrictions
A statistically-defined LC50 was not calculated. Water chemistry data were not reported.

Reference (9) (26) (36)

Acute Toxicity to Aquatic Vertebrates

Category Name: RECLAIMED SUBSTANCES – NAPHTHENIC ACIDS

Category Chemical :	Naphthenic acids, CAS no. 1338-24-5
Test Substance :	Naphthenic acids, CAS no. 1338-24-5
Test Substance Purity/Composition and Other Test Substance Comments :	Specific analyses of the test substance: Acid number: 235 mg KOH/gm Unsaponifiables (total): 4.9% Viscosity @40°C: 32 cst Specific gravity @20°C: 0.969 Color (Garner), GI 4.5 Water content: 0.07% Phenolic content (acid): 0.31% Total sulfur: 0.34 CP – Flash point °F (COC): 343
Category Chemical Result Type :	Measured
Test Substance Result Type:	Measured

Method

Year Study Performed :	2010
Method/Guideline Followed:	OECD 203
Deviations from Method/Guideline :	There was a brief temperature excursion outside the boundaries of 22±1°C.
Species:	Pimephales promelas (fathead minnow)

GLP:	Yes
Analytical Monitoring :	Yes
Test Type:	Renewal
Test Vessel:	3.8-L glass jars
Water Media Type:	Modified well water
Test Concentrations:	0 (control), 1.3, 2.5, 5.0, 10, and 20 mg naphthenic acids/L
Nominal and Measured Concentrations:	Nominal WAF loading rates: 0 (control), 1.3, 2.5, 5.0, 10, and 20 mg naphthenic acids/L Mean measured: 0 (control), 0.90, 2.08, 3.22, 604, 13.8 mg naphthenic acids/L
Total Exposure Period:	96 hours

Vehicle Used:	None
Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	148 mg L
Dissolved Oxygen:	7.1 to 9.3 mg/L
pH Value:	Value or Lower Range : 8.0 Upper Range : 8.4
Test Temperature and Units:	Value or Lower Range : 21.6 °C Upper Range : 23.6 °C
Photo (Light/Dark):	16 h light / 8 h dark Light intensity: 523 lux
Salinity:	Freshwater
TOC:	
Water Hardness:	Value or Lower Range: 134 mg/L Upper Range:

Method/Guideline **Test Conditions Remarks**:	Exposure solutions were prepared as water accommodated fractions (WAF). Each WAF was prepared independently based on the selected loading rates used for the test. The WAFs were prepared by adding the appropriate amount of test substance to 4 L of dilution water in clean 5-L glass carboys. Each carboy contained a 2-inch Teflon-coated stir bar and was sealed with a screw cap. The mixtures were stirred for 24 hours at a speed that created a vortex of 30 – 50% of the solution depth. After the stirring period, the solutions were permitted to settle for approximately 1 hour. WAFs were siphoned from the bottom of the mixing vessels, with the first ~100 mL being discarded. This procedure was repeated three times in order to prepare renewal solutions for the 24, 48, and 72-h time points of the test. The different exposure levels were established in single 3.8-L glass jars holding approximately 2.0 L of solution. At the beginning of the test, fish were impartially added one at a time to each test vessel until each vessel held its complement of 7 fish. During renewal periods, fish were transferred to freshly-prepared exposure solutions. Observations for mortality, moribundity, and sublethal responses were made every 24 hours (±1 hour). Measurements of the concentrations of dissolved naphthenic acids in the WAFs were made on samples taken at 0 hours (fresh), 24 hours (old), 72 hours (fresh), and 96 hours (old). The method of analysis included aqueous sample extraction by methylene chloride with detection by Fourier transformed Infrared spectroscopy. The minimum quantifiable limit (MQL) for the method was 0.6 mg naphthenic acids/L. Additional characterization of the exposure solutions included analysis by gas chromatography-mass spectroscopy. This method allowed the proportion of dissolved naphthenic acids to be resolved into families of naphthenic acids having similar carbon numbers and ring numbers.
Limit Test:	No

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:	96	Hours	=	3.22		mg/L	arithmetic mean measured
LOEC:	96	Hours	=	6.04		mg/L	arithmetic mean measured
NOELR:	96	Hours	=	5.0		mg/L	Nominal
LOELR:	96	Hours	=	10		mg/L	Nominal

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:
24	Hours	LL	50	>	20		mg/L	Mortality	Nominal
48	Hours	LL	50	=	11		mg/L	Mortality	Nominal
72	Hours	LL	50	=	11		mg/L	Mortality	Nominal
96	Hours	LL	50	=	9.0		mg/L	Mortality	Nominal
24	Hours	LC	50	>	13.8		mg/L	Mortality	arithmetic mean measured
48	Hours	LC	50	=	7.22		mg/L	Mortality	arithmetic mean measured
72	Hours	LC	50	=	7.22		mg/L	Mortality	arithmetic mean measured
96	Hours	LC	50	=	5.62		mg/L	Mortality	arithmetic mean measured

Results Remarks:	The control and test solutions were clear and colorless with no visible signs of un-dissolved test substance, precipitate, or surface film throughout the study. The LC/LR50 values of the test were based on the percentage of dead fish found in each treatment level. The NOEC/LR values were determined based on scientific judgment of the dose-response pattern. Concentrations of dissolved naphthenic acids in the fresh and old test solutions remained stable over the renewal periods. The measured concentrations in the old solutions were at least 89% of the initial concentrations. Analysis by GC-MS for carbon number and ring distribution indicated 82 – 90% of the dissolved naphthenic acids contained 10 to 16 carbon atoms with a prevalence of one and two ring naphthenic acid isomers. Other than a brief temperature excursion beyond the guideline requirements, this study met the method guideline acceptability criteria.

Reliability/Data Quality

Reliability:	1
Reliability Remarks:	Reliable without restrictions
Key Study Sponsor Indicator:	Key

Reference

Reference:	Gerke, A. 2010. 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.

Acute Toxicity to Aquatic Vertebrates

Category Name: RECLAIMED SUBSTANCES – Naphthenic Acids

Category Chemical :	61790-13-4
Test Substance :	61790-13-4

Test Substance Purity/Composition and Other Test Substance Comments :	Commercial naphthenic acids (sodium salt) was a 50% (w/v) aqueous solution supplied by Pfaltz-Bauer Inc.
Category Chemical Result Type :	Estimated by supporting chemical
Test Substance Result Type:	measured

Method

Year Study Performed :	
Method/Guideline Followed:	
Deviations from Method/Guideline :	
Species:	
GLP:	
Analytical Monitoring :	
Test Type:	
Test Vessel:	
Water Media Type:	
Test Concentrations:	
Nominal and Measured Concentrations:	
Total Exposure Period:	

Vehicle Used:	
Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	
pH Value:	Value or Lower Range : Upper Range :

Test Temperature and Units:	Value or Lower Range : Upper Range :
Photo (Light/Dark):	
Salinity:	
TOC:	
Water Hardness:	Value or Lower Range: Upper Range:

Method/Guideline Test Conditions Remarks:	
Limit Test:	

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:							
LOEC:							
NOELR:							

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:

Results Remarks:	Growth and developmental effects in yellow perch (*Perca flavescens*) and Japanese medaka (*Orizias latipes*) embryos exposed to sodium naphthenate solutions were evaluated over a range of naphthenic acids concentrations. 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).

Reliability/Data Quality	
Reliability:	
Reliability Remarks:	
Key Study Sponsor Indicator:	
Reference	
Reference:	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.

Acute Toxicity to Aquatic Vertebrates

Category Name:

Category Chemical :	61790-13-4
Test Substance :	61790-13-4
Test Substance Purity/Composition and Other Test Substance Comments :	The test substance was a dense, amber-colored mass of naphthenic acids – sodium salts (8-10% sodium) purchased from Acros Organics.
Category Chemical Result Type :	measured
Test Substance Result Type:	measured
Method	
Year Study Performed :	
Method/Guideline Followed:	
Deviations from Method/Guideline :	
Species:	yellow perch (*Perca flavescens*)
GLP:	no data
Analytical Monitoring :	no
Test Type:	semi-static
Test Vessel:	no data
Water Media Type:	freshwater
Test Concentrations:	nominal
Nominal and Measured Concentrations:	0 (control), 0.9, 1.8, and 3.6 mg/L

Total Exposure Period:	21 days

Vehicle Used:	
Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	8.92
pH Value:	Value or Lower Range : 8.38 Upper Range :
Test Temperature and Units:	Value or Lower Range : 18.4 Upper Range :
Photo (Light/Dark):	16/8 hours
Salinity:	0.3 ppt
TOC:	
Water Hardness:	Value or Lower Range: Upper Range:

Method/Guideline Test Conditions Remarks:	
Limit Test:	no

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:							
LOEC:							
NOELR:							

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:
96	hours	LC	100	=	3.6		mg/L	mortality	nominal

Results Remarks:	Wild young of year yellow perch were captured and held for two days prior to initiating experiments. Groups of perch were exposed for three weeks in a static-renewal designed system to control, 0.9, 1.8, and 3.6 mg/L naphthenic acids. The commercial naphthenic acids-sodium salt (8-10% sodium) was obtained from Acros Organics. Total naphthenic acid concentrations were measured in the exposure solutions by Fourier transform infrared spectroscopy (FTIR). Following the 3-week exposure, perch were sacrificed by a sharp blow to the head and severing the spinal cord behind the skull. Fork length, body weight, and liver weight were recorded. Condition factor (K) and liver somatic index were calculated for each fish. Slides of gill and liver tissue were prepared and stained with hemtoxylin and eosin. Histopathological alterations were classified into one of five categories, proliferative, degenerative, inflammatory, structural, and cytoplasmic, each representing a general tissue response by an organism to a particular stressor. Complete fish mortality occurred within 96-hours in the 3.6 mg/L treatment. Consequently gill and liver histopathology comparisons were limited to fish exposed to 0.9 mg/L NAs and control fish. 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.
Reliability/Data Quality	
Reliability:	2 (reliable with restrictions)
Reliability Remarks:	Test concentrations were not measured and a complete description of the dose-response pattern was not provided.
Key Study Sponsor Indicator:	no
Reference	
Reference:	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.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Acute Toxicity to Aquatic Invertebrates

Category Name: RECLAIMED SUBSTANCES – NAPHTHENIC ACIDS

Category Chemical :	Naphthenic acids, CAS no. 1338-24-5
Test Substance :	Naphthenic acids, CAS no. 1338-24-5
Test Substance Purity/Composition and Other Test Substance Comments :	Specific analyses of the test substance: Acid number: 235 mg KOH/gm Unsaponifiables (total): 4.9% Viscosity @40°C: 32 cst Specific gravity @20°C: 0.969 Color (Garner), GI 4.5 Water content: 0.07% Phenolic content (acid): 0.31% Total sulfur: 0.34 CP – Flash point °F (COC): 343
Category Chemical Result Type :	Measured
Test Substance Result Type:	Measured
Method	
Year Study Performed :	2010
Method/Guideline Followed:	OECD 202
Deviations from Method/Guideline :	None noted
Species:	Daphnia magna
GLP:	Yes
Analytical Monitoring :	Yes
Test Type:	Renewal
Test Vessel:	250-mL glass jars
Water Media Type:	Aged laboratory well water
Test Concentrations:	0 (control), 5.0, 10, 20, 40, and 80 mg naphthenic acids/L (test concentrations expressed as loading rate)
Nominal and Measured Concentrations:	Nominal WAF loading rates: 0 (control), 5.0, 10, 20, 40, and 80 mg naphthenic acids/L Mean measured: <MQL (control), 3.90, 7.68, 17.0, 33.3, and 69.0 mg naphthenic acids/L
Total Exposure Period:	48 hours

Vehicle Used:	None
Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	148 mg/L
Dissolved Oxygen:	6.8 to 8.8 mg/L
pH Value:	Value or Lower Range : 7.5 Upper Range : 8.6
Test Temperature and Units:	Value or Lower Range : 20.6 Upper Range : 22.0
Photo (Light/Dark):	16 h light / 8 h dark Light intensity: 521 lux
Salinity:	N/A (freshwater)
TOC:	
Water Hardness:	Value or Lower Range: 150 mg/L Upper Range:

Method/Guideline Test Conditions Remarks:	Exposure solutions were prepared as water accommodated fractions (WAF). Each WAF was prepared independently based on the selected loading rates used for the test. Each WAF was prepared by adding the appropriate amount of test substance to 4 L of dilution water in a clean 4-L glass carboy. Each carboy contained a 2-inch Teflon-coated stir bar and was sealed with parafilm. The WAF preparations were stirred for 24±1 hours at a speed that created a vortex of 30 – 50% of the solution depth. After the stirring period, the solutions were permitted to settle for approximately 1 hour. The WAF was siphoned from the bottom of the mixing vessel, with the first ~100 mL being discarded. Enough WAF was collected to prepare four replicate test chambers per treatment. This procedure was repeated in order to prepare renewal solutions for the 24-h time point of the test. Each replicate test vessel contained 200-mL of the WAF or control solution. At the beginning of the test, five neonate daphnids (<24-h old) were added to each test vessel in a random process. At 24-hours into the test, the daphnids were transferred to fresh WAF solutions. Observations for immobile daphnids and sub-lethal responses were made every 24 hours (±1 hour). Measurements of the concentrations of dissolved naphthenic acids in the WAFs were made on samples taken at 0 hours (fresh), 24 hours (fresh and old), and 48 hours (old). The method of analysis included aqueous sample extraction by methylene chloride with detection by Fourier transformed Infrared spectroscopy. The minimum quantifiable limit (MQL) for the method was 0.6 mg naphthenic acids/L. Additional characterization of the exposure solutions included analysis by gas chromatography-mass spectroscopy. This method allowed the proportion of dissolved naphthenic acids to be resolved into families of naphthenic acids having similar carbon numbers and ring numbers.

Limit Test:	No

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:	48	Hour	=	7.68		mg/L	arithmetic mean measured
LOEC:	48	Hour	=	17.0		mg/L	arithmetic mean measured
NOELR:	48	Hour	=	10		mg/L	nominal
LOELR:	48	Hour	=	20		mg/L	nominal

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:
24	Hours	EC	50	=	23.8		mg/L	immobile	arithmetic mean measured
48	Hours	EC	50	=	20.0		mg/L	Immobile	arithmetic mean measured
24	Hours	EL	50	=	28.3		mg/L	Immobile	nominal
48	Hours	EL	50	=	24.0		mg/L	immobile	nominal

Results Remarks:	The control and test solutions were clear and colorless with no visible signs of un-dissolved test substance, precipitate, or surface film throughout the study. The endpoints of the test were based on the percentage of immobile daphnids found in each treatment level. The observation of "floating daphnids" was observed at 24 and 48 hours in the solutions prepared at the 20 mg/L loading rate. This effect was considered a sublethal effect by the testing laboratory and was used to define the NOEC(LR)/LOEC(LR), but was not included in the calculation of the study endpoints. Concentrations of dissolved naphthenic acids in the fresh and old test solutions remained stable over the renewal period. The measured concentrations in the old solutions were at least 87% of the initial concentrations. Analysis by GC-MS for carbon number and ring distribution indicated 85 – 91% of the dissolved naphthenic acids contained 10 to 16 carbon atoms with a prevalence of one and two ring naphthenic acid isomers. This study met all guideline requirements of acceptability criteria.

Reliability/Data Quality

Reliability:	1
Reliability Remarks:	Reliable without restriction
Key Study Sponsor Indicator:	Key

Reference

Reference:	Rebstock, M. 2010. 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.

Acute Toxicity to Aquatic Invertebrates

Category Name: **RECLAIMED SUBSTANCES: Naphthenic acids**

Category Chemical :	1338-24-5
Test Substance :	1338-24-5
Test Substance Purity/Composition and Other Test Substance Comments :	calcium naphthenate
Category Chemical Result Type :	estimated by supporting chemical
Test Substance Result Type:	Measured
Method	
Year Study Performed :	
Method/Guideline Followed:	
Deviations from Method/Guideline :	
Species:	Nitocra spinipes
GLP:	no data
Analytical Monitoring :	
Test Type:	
Test Vessel:	
Water Media Type:	brackish water
Test Concentrations:	
Nominal and Measured Concentrations:	
Total Exposure Period:	96 hours

Vehicle Used:	
Vehicle Name:	

Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	
pH Value:	Value or Lower Range : Upper Range :
Test Temperature and Units:	Value or Lower Range Upper Range :
Photo (Light/Dark):	
Salinity:	7 parts per thousand
TOC:	
Water Hardness:	Value or Lower Range: Upper Range:

Method/Guideline Test Conditions Remarks:	Details on testing procedures have been published by 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.
Limit Test:	No

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:							
LOEC:							
NOELR:							
LOELR:							

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:
96	hours	LC	50	=	4.8		mg/L	mortality	Nominal

Results Remarks:	This data endpoint value was reported in the ECHA registration dossier for naphthenic acids, CAS 1338-24-5. The ECHA dossier concludes that the LC50 for the naphthenate ion of the calcium salt should be valid for naphthenic acid since the naphthenate ion is the ionized form of the acid.

Reliability/Data Quality	
Reliability:	2 (reliable with restrictions)
Reliability Remarks:	The endpoint was determined for a supporting substance (structural analog or surrogate of the test substance).
Key Study Sponsor Indicator:	no
Reference	
Reference:	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.

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Acute Toxicity to Aquatic Plants	
Category Name	**RECLAIMED SUBSTANCES – NAPHTHENIC ACIDS**
Category Chemical :	Naphthenic acids, CAS no. 1338-24-5
Test Substance :	Naphthenic acids, CAS no. 1338-24-5
Test Substance Purity/Composition and Other Test Substance Comments :	Specific analyses of the test substance: Acid number: 235 mg KOH/gm Unsaponifiables (total): 4.9% Viscosity @40°C: 32 cst Specific gravity @20°C: 0.969 Color (Garner), GI 4.5 Water content: 0.07% Phenolic content (acid): 0.31% Total sulfur: 0.34 CP – Flash point °F (COC): 343
Category Chemical Result Type :	Measured
Test Substance Result Type:	Measured
Method	
Year Study Performed :	2010
Method/Guideline Followed:	OECD 201 and OPPTS 850.5400
Deviations from Method/Guideline :	None noted
Species:	Pseudokirchneriella subcapitata
GLP:	Yes

Analytical Monitoring :	Yes
Test Type:	Static
Test Vessel:	250-mL Erlenmeyer flasks
Water Media Type:	Algal nutrient medium prepared to ASTM E1217-97a recipe
Test Concentrations:	0 (control), 2.5, 5.0, 10, 20, and 80 mg naphthenic acids/L
Nominal and Measured Concentrations:	Nominal WAF loading rates: 0 (control), 2.5, 5.0, 10, 20, and 80 mg naphthenic acids/L 72-h Mean measured: <MQL (control), 1.69, 3.48, 7.38, 15.0, 28.9, and 44.9 mg naphthenic acids/L 96-h Mean measured: <MQL (control), 1.64, 3.51, 7.41, 14.8, 28.4, and 44.8 mg naphthenic acids/L
Total Exposure Period:	96 hours

Vehicle Used:	None
Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	
pH Value:	Value or Lower Range : 6.8 Upper Range : 8.9
Test Temperature and Units:	Value or Lower Range : 23.2 °C Upper Range : 24.2 °C
Photo (Light/Dark):	Continuous lighting Intensity: 4357 to 4527 lux
Salinity:	
TOC:	
Water Hardness:	Value or Lower Range: Upper Range:

Method/Guideline Test Conditions Remarks:	Exposure solutions were prepared as water accommodated fractions (WAF). Each WAF was prepared independently based on the selected loading rates used for the test. Each WAF was prepared by adding the appropriate amount of test substance to 4 L of nutrient medium in a clean, autoclaved 4-L glass carboy. Each carboy contained a 2-inch Teflon-coated stir bar and was sealed with a screw cap. The WAF preparations were stirred for 24±1 hours at a speed that created a vortex of 30 – 50% of the solution depth. After the stirring period, the solutions were permitted to settle for approximately 1 hour. The WAF was siphoned from the bottom of the mixing vessel, with the first ~100 mL being discarded. Enough WAF was created to prepare 7 replicate test vessels per treatment. Replicates designated A, B, C, D, E,

	and F were 250-mL Erlenmeyer flasks filled with 100 mL of WAF or control solution. Replicate G was a 1-L Erlenmeyer flask containing 600 mL of WAF or control solution. This replicate was placed beside the other replicates during the test, but served only to provide sufficient volume of solutions for analytical measurements at 72 hours. At 96 hours, replicates D, E, and F were pooled to provide the volume needed for analytical measurements at the end of the test. At the beginning of the test, replicates A-F were inoculated with 1.0 mL of algal concentrate containing approximately 1.0×10^6 cells/mL. This provided approximately 1.0×10^4 cells/mL at initiation. Replicate G also received an aliquot of the algal concentrate to achieve an initial cell density of 1.0×10^4 cells/mL. Flasks were placed on an orbital shaker table (100 rpm) in a temperature controlled environmental chamber (24±2°C) under continuous cool-white fluorescent lighting. Positions were established by random assignment, and were re-randomized on a daily basis throughout the 4-day test. At 24, 48, 72, and 96 hours, cell density was measured in each treatment group by direct microscopic counting using a hemacytometer. For the control group, samples from replicates A – F were counted. For all naphthenic acid WAF treatments, samples from replicates A – C were counted. Temperature and pH were measured in all parent solutions prior to distribution of the solutions to the test flasks. At 72 hours, temperature and pH were measured in all replicate G vessels. At 96 hours, temperature and pH were measured in all replicate A vessels. Measurements of the concentrations of dissolved naphthenic acids in the WAFs were made on samples taken at 0, 72, and 96 hours. The method of analysis included aqueous sample extraction by methylene chloride with detection by Fourier transformed Infrared spectroscopy. The minimum quantifiable limit (MQL) for the method was 0.6 mg naphthenic acids/L. Additional characterization of the exposure solutions included analysis by gas chromatography-mass spectroscopy. This method allowed the proportion of dissolved naphthenic acids to be resolved into families of naphthenic acids having similar carbon numbers and ring numbers.
Limit Test:	No

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOELR:	72	Hours	=	10		mg/L	nominal
LOELR	72	Hours	=	20		mg/L	nominal
NOEC	72	Hours	=	7.38		mg/L	arithmetic mean measured
LOEC	72	Hours	=	15.0		mg/L	arithmetic mean measured
NOELR	96	Hours	=	10		mg/L	nominal
LOELR:	96	Hours	=	20		mg/L	nominal
NOEC:	96	Hours	=	7.41		mg/L	arithmetic mean measured
LOEC:	96	Hours	=	14.8		mg/L	arithmetic mean measured

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:
72	Hours	EL	50	=	41.3		mg/L	Growth Rate	nominal
72	Hours	EL	50	=	23.8		mg/L	Cell Yield	nominal
72	Hours	EC	50	=	29.6		mg/L	Growth Rate	arithmetic mean measured
72	Hours	EC	50	=	17.7		mg/L	Cell Yield	arithmetic mean measured
96	Hours	EL	50	=	43.3		mg/L	Growth Rate	nominal
96	Hours	EL	50	=	24.8		mg/L	Cell Yield	nominal
96	Hours	EC	50	=	29.9		mg/L	Growth Rate	arithmetic mean measured
96	Hours	EC	50	=	18.1		mg/L	Cell Yield	arithmetic mean measured

Results Remarks:	The NOELR/LOELR and NOEC/LOEC at 72 and 96 hours were the same values when based on growth rate or cell yield. Algal cells appeared normal with no unusual cell shapes, color differences, flocculation, adherence of algae to the test chambers, or aggregations of algal cells. Concentrations of dissolved naphthenic acids in the test solutions remained stable over the renewal period. The measured concentrations in the solutions at 72 hours were at least 80% of the initial measured concentrations. At 96 hours, the measured concentrations were at least 85% of the initial measured concentrations. Analysis by GC-MS for carbon number and ring distribution indicated 81 – 94% of the dissolved naphthenic acids contained 10 to 16 carbon atoms with a prevalence of one and two ring naphthenic acid isomers.

Reliability/Data Quality

Reliability:	1
Reliability Remarks:	Reliable without restrictions
Key Study Sponsor Indicator:	Key

Reference

Reference:	Rebstock, M. 2010. 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.

Acute Toxicity to Aquatic Plants

Category Name	**RECLAIMED SUBSTANCES: Naphthenic acids**
Category Chemical :	1338-24-5
Test Substance :	1338-24-5
Test Substance Purity/Composition and Other Test	

Substance Comments :	
Category Chemical Result Type :	unknown
Test Substance Result Type:	measured
Method	
Year Study Performed :	1966
Method/Guideline Followed:	unknown
Deviations from Method/Guideline :	
Species:	Navicula seminulum
GLP:	no data
Analytical Monitoring :	no data
Test Type:	no data
Test Vessel:	no data
Water Media Type:	freshwater
Test Concentrations:	nominal
Nominal and Measured Concentrations:	
Total Exposure Period:	96 hours

Vehicle Used:	
Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	
pH Value:	Value or Lower Range : Upper Range :
Test Temperature and Units:	Value or Lower Range :

	Upper Range :	
Photo (Light/Dark):		
Salinity:		
TOC:		
Water Hardness:	Value or Lower Range: Upper Range:	

Method/Guideline Test Conditions Remarks:	
Limit Test:	

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	**Exposure Units**:	**Value Description**:	**Value or Lower Range**:	**Upper Range**:	**Units**:	**Basis for Concentration**:
NOEC:							
LOEC:							
NOELR:							
LOELR:							

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	**Exposure Units**:	Type	**%**:	**Value Description**:	**Mean Value or Lower Mean Value**:	**Upper Mean Value**:	**Units**:	**Basis for Effect**:	**Basis for Concentration**:

Results Remarks:	These data endpoint values were reported in the ECHA registration dossier for naphthenic acids, CAS 1338-24-5. The original source of data could not be obtained but was gathered from the US EPA ECOTOX database (secondary source). A total of 12 endpoints were reported in the ECOTOX database. All were based on 96-hour tests evaluated on the basis of population growth rate. The ECHA dossier concludes that the toxicity of naphthenic acids to populations of the freshwater diatom, Navicula seminulum, was measured. The 96-hour EC50 for growth ranged from 26.0 to 80.5 mg/L.

Reliability/Data Quality

Reliability:	4 (not assignable)
Reliability Remarks:	The data was reported in a secondary literature source, and the original report could not be obtained to evaluate the test substance or test methods used.
Key Study Sponsor Indicator:	

Reference

Reference:	The sensitivity of aquatic life to certain chemicals commonly found in industrial wastes. Final Report No. RG-3965(C2R1), US Public Health Service Grant, Acad. of Nat. Sci., Philadelphia, PA. 89 p.

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

Memo : Effect of naphthenic acids on survival of bluegill (Lepomis macrochirus)

Remark : The value was reported in a summarized journal article (Cairns et al., 1965) as originating in Cairns and Scheier (1962).

Result : 48-hour TLm = 5.6 mg/l naphthenic acids

Reliability : (3) invalid
The endpoint was cited in the text of a journal article without details of the test.

Reference (6) (7)

Memo : Effect of naphthenic acids on survival of bluegill (Lepomis macrochirus)

Remark : Test chambers were 30x60x30 cm all-glass vessels. Dilution water was well water. Testing was performed at a temperature of 22 ± 1°C under a 16-h light/8-h dark photoperiod.

The test included five concentrations of the test substance and a dilution water control. Each test level included 20 fish distributed 10 each to two replicate chambers per

treatment.

Dissolved oxygen ranged from 4.3 to 8.1 mg/l, pH ranged from 7.4 to 8.0, and temperature ranged from 22 to 24 °C when measured daily during the test. Specific conductance between the test solutions remained constant at 550 (no units given) when measured at the beginning of the test.

The report stated that serial dilutions of the test product were created for testing, although no details were given as to how the serial dilutions or the original solution was created. The raw data indicated that concentrations were expressed as a percent, while the LC50 and confidence interval was reported as parts per million. There was no explanation how the values for percent were related to parts per million.

Critical details of testing procedures and animal culture were omitted from the report.

Result : 96-hour LC50 = 0.0026 mg/l

Reference (14)

Memo : Effect of naphthenic acids on survival of zebra fish (Brachydanio rerio) embryos

Remark : Zebra fish embryos were exposed for 48 hours to a range of naphthenic acids concentrations to determine the TLm (median tolerance limit) for embryo survival. Embryos were collected from a culture unit once they attained Stage 21 as designated by Hisaoka and Battle (1958). Ten embryos were exposed to each test solution and control in petri dishes holding 45 ml of the exposure solutions. Exposure solutions were prepared by diluting a stock solution of naphthenic acids (100 mg naphthenic acids in 50 ml acetone) with water. In addition to a control group, nine concentrations of naphthenic acids were prepared at 2.4, 3.2, 4.2, 6.5, 10, 15.5, 24, 32, and 42 ppm naphthenic acids. Mortality was assessed at 24 and 48 hours of exposure. The embryo was considered dead if it had an opaque appearance.

A TLm of 3.5 ppm was obtained by plotting the survival versus concentration on semilog paper and interpolating the 50% survival concentration. The following dose response was given:

Test Concentration, ppm	Percent Dead
0 (control)	0
2.4	0
3.2	40
4.2	70
6.5	100
10	100
15.5	100
24	100
32	100
42	100

Reliability : (2) valid with restrictions
Although the test was conducted prior to the time of standardized test methods, the report provided sufficient information on the dose-response pattern for the test substance.

Reference	(6) (20)

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 5880 mg/kg bw
Species	:	Rat
Strain	:	Wistar
Sex	:	Male
Number of animals	:	5
Vehicle	:	other: None, administered undiluted
Doses	:	1, 1.47, 2.15, 3.16, 4.64, 6.81 & 10 g/kg
Method	:	
Year	:	1979
GLP	:	no data
Test substance	:	other TS: MRD-79-10 (Raw naphthenic acid derived from kerosene) [CAS number 1338-24-8]
Method	:	Seven groups of 5 male rats were dosed at 1.0, 1.47, 2.15, 3.16, 4.64, 6.81, and 10 g/kg of body weights. Food and water were freely available except for the 16-20 hours prior to dosing. The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.
Result	:	Deaths occurred at the four highest dose levels: 3.26, 4.64, 6.81, and 10 g/kg bw. 8/10 animals died at the two highest dose levels. Significant predeath toxic signs included tremors, lethargy, ptosis, ataxia, prostration, negative righting reflex, flaccid muscle tone, piloerection, diarrhea, chromodacryorrhea, dyspnea and chromorhinorrhea. Body weight changes were noted in the survivors. Significant necropsy findings in the animals that died during the study included dilated hearts and gastrointestinal irregularities. The LD50 was determined to be 5.88 (4.31-8.02) g/kg bw
Reliability	:	(2) valid with restrictions Appears to be comparable to a guideline study with adequate experimental details provided; although the investigators used male rats only, there is sufficient experimental detail to make a conclusion on the study's validity, and the results can be used to assess the potential acute toxicity of naphthenic acid.
Reference		(12)

Type	:	LD50
Value	:	
Species	:	Rat
Strain	:	other: No information
Sex	:	no data
Number of animals	:	
Vehicle	:	other: None - administered undiluted
Doses	:	
Method	:	

Year	:	1955
GLP	:	no data
Test substance	:	
Method	:	"The LD50 ..was determined in rats by use of screening test procedures similar to those of Smyth and Carpenter." (Smyth, H.F., and C.P. Carpenter. 1944. Place of the range finding test in the industrial toxicology laboratory. J. Indust. Hyg. & Tox. 26: 269. Number of animals: "Sufficient animals …so the the LD50 dose could be computed by either the Weil or the Litchfield and Wilcoxon method"
Result	:	Death appears to result from gastrointestinal disturbances, with the mortality peak occurring on the third to fourth day after administration. The animals exhibited anorexia, inanition, diarrhea, and asthenia. The LD50s were determined to be 3.0 g/kg bw (fraction from crude kerosene acids) and 5.2 g/kg bw (fraction from mixed crude oils)
Test substance	:	No CAS number identified 1) 7-93% Naphthenic acid fraction from crude kerosene acids 2) 65-69% Naphthenic acid fraction from mixed crude oils
Reliability	:	(2) valid with restrictions Although not a guideline or GLP study, and some of the experimental details are not available, the study does appear to be well-conducted, and cites that the investigators followed published methodologies for conducting a statistically valid LD50. The data are supportive of other acute toxicity studies reported by Exxon and Pennisi.
Reference		(28)

Type	:	LD50
Value	:	= 3550 mg/kg bw
Species	:	Mouse
Strain	:	other: White - no other information
Sex	:	Male
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	1977
GLP	:	No
Test substance	:	other TS: Naphthenic acid - no further information [Assocoiated with CAS number 1338-24-5 in Toxline search]
Result	:	Oral administration resulted in 1) CNS depression without analgesia and no loss of corneal reflex, 2) corneal eye opacity, 3) dryness of mouth, 4) convulsions, 5) diarrhea, and 6) death due to respiratory arrest.
Reliability	:	(4) not assignable This information is taken from a published, meeting abstract. The level of experimental details provided is not sufficient to verify the conclusions.
Reference		(27)

Type	:	other: Acute oral toxicity study (Not LD50)
Value	:	
Species	:	Rat
Strain	:	Wistar
Sex	:	male/female

Number of animals	:	10
Vehicle	:	Water
Doses	:	
Method	:	
Year	:	2002
GLP	:	no data
Test substance	:	

Method : Female rats were given a single oral dose of naphthenic acids at 3, 30 or 300 mg/kg bw, while male rats received 300 mg/kg. Control animals were given tap water. All animals were monitored continuously for 12 hr after dosing, and thereafter daily. Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination.
Statistical analysis was performed by using a one-way ANOVA to compare means of female dose and control groups with respect to consumption, body weights, and organ weights. A pair wise multiple comparison test was then used in cases where statistical significance was reached. For the male dose and control groups, a Student's t-test was used to compare group means. Probability values of $p < 0.05$ was considered statistically significant.

Result : The following effects were seen in the high dose groups:

Decreased food consumption immediately following dosing.

Lethargy and mild ataxia (2/10 females, 3/10 males)

Statistically significant increase relative organ weights: ovaries, spleen in females- testes, heart in males

7/10 females and 6/10 males exhibiting eosinophilic pericholangitis

6/10 males and 2/10 females with brain hemorrhage.

The following effects were seen in the mid dose group:

7/10 females and 4/10 males with heart lesions.

Test substance : Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 55,080, 5508 or 550.0 mg/l naphthenic acids - derived from athabasca sands sands tailings. [Associated with CAS number 1338-24-5 in Toxline search]

Reliability : (2) valid with restrictions
The study is not an acute toxicity study as defined by OECD SIDS/HPV, however it appears to be well conducted and provides additional information regarding potential acute, non-lethal effects of naphthenic acids following oral exposure.

Reference (30)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	other: LD50 with irritation
Value	:	> 31600 mg/kg bw

Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	2
Vehicle	:	other: None - administered undiluted
Doses	:	3.16 mg/kg
Method	:	
Year	:	1979
GLP	:	no data
Test substance	:	other TS: MRD-79-10 (Raw naphthenic acid derived from kerosene) [CAS number 1338-24-8]

Method :

3.16 g/kg naphthenic acid was applied dermally to the clipped abraded abdomens of each animal. The area was covered with gauze and secured by a thick plastic binder, which was removed after 24 hours, and the skin washed with water or corn oil.
According to experimental protocol, no deaths occurred at the initial level, no addition animals were dosed. If one animal died, the experiment was to be repeated using 3 more groups of animals dosed at varying levels.
Following the skin wash, animals were observed for mortality and toxic effects at 2 hr and 4 hr, and once daily thereafter. Body weights were recorded pretest and at termination. Dermal irritation was recorded at 24 hr, 3, 7, 10 and 14 days.
The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.

Result :

No deaths occurred at the 3.16 mg/kg dose level. Most of the animals (3/4) appeared normal during the first 2 to 4 hours of dosing, after which symptoms of toxicity were observed. 3 out of 4 animals (1 male, 2 female) showed signs of toxicity until day 12 or 13. During the first 5 days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces.
The LD50 was determined to be greater than 3.16 g/kg bw
Redness and irritation scores were recorded at 24 hr, 3, 7, 10 and 14 days post-washing.
4 Hour occluded sites (DOT, OECD methods)
Mean values (24, 48 & 72 hours) for erythema and edema at the intact sites were 1.69 and 1.3 respectively.
The initial response of the skin to the test material was slight, with little difference in response between intact or abraded sites.

The material was judged to be moderately to severely irritating to the occluded skin.

Actual scores were:

Erythema/Eschar Scores

Animal Number		1 day	3 day	7 day	10 day	14 day
1M	2	2	4	4	1	
2M	1	2	4	4	1	
3F	2	4	4	4	0	
4F	2	3	4	4	0	

Note: All animals showed signs of scar formation after 14 days.

Edema Animal Number		1 day	3 day	7 day	10 day	14 day
1M	3	2	2	2	1	
2M	2	3	2	2	0	
3F	3	3	2	2	0	
4F	3	3	2	2	0	

Reliability : (1) valid without restriction
Although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.

Reference (11)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : .1 ml
Exposure time :
Comment :
Number of animals : 3
Vehicle : None
Result : Irritating
Classification :
Method :
Year : 1979
GLP : no data
Test substance : other TS: MRD-79-10 (Raw naphthenic acid derived from kerosene) [CAS number 1338-24-8]

Method : 0.1 ml naphthenic acid was placed into the conjunctival sac of eye of each of the six rabbits. The lids were held together briefly to insure adequate distribution. The untreated eye served as a control.
The rabbits were observed at 1 and 4 hours, and on days 1, 2, 3, 4, and day 7. If a positive score (any score for iritis or opacity, or a score of 2 or more for redness or chemosis) was noted on day 7, ocular reactions were scored on day 10. Likewise readings on day 14 were given if there was a positive reaction on day 10. Fluorescein was used in examining ocular reactions on day 3 and after. The Draize technique was used as the scoring system.

Result : The following is a summary of data taken from the report: One animal had a positive corneal score that was noted on days 1 and 2. One animal had a positive iris score which was noted during hours 1 and 4. All animals exhibited positive conjunctival scores at some pint during the first three days of observation. By day 4, no animals showed positive scores.

The material was judged to be an irritant. (According to

Draize chart, 4 to 6 rabbits with positive scores observed at 24, 48 or 72 hours). In a later Exxon summary report, eye irritation was judged to be moderate (Exxon, 1980).

Reliability : (1) valid without restriction
Although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.

Reference (13)

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	Rat
Sex	:	Female
Strain	:	Wistar
Route of admin.	:	Gavage
Exposure period	:	90 Days
Frequency of treatm.	:	daily, 5 days/week for 90 days
Post exposure period	:	
Doses	:	0.6, 6 & 60 mg/kg
Control group	:	yes, concurrent vehicle
Method	:	
Year	:	2002
GLP	:	no data
Test substance	:	

Method : Female rats were administered naphthenic acid (orally) at doses of 0.6, 6, or 60 mg/kg/day, 5 days per week for 90 days. Control animals were given 7 ml tap water. All animals were monitored daily . Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Blood samples were collected from the ventral tail vein on day 45 of dosing and analyzed for plasma biochemical and hematological effects. Similarly, blood samples taken via cardiac puncture on day 91 were analyzed. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination.
Statistical analysis was performed by using a one-way ANOVA to compare group means for consumption, plasma biochemical/ hematological parameters , and organ weights, while a one-way repeated measure ANOVA was used to compare body weight trends. Probability values of $p < 0.05$ was considered statistically significant.

Result : The following significant effects were seen in the high dose groups:
Decreased food consumption immediately following dosing.
Severe, clonic seizures lasting 20 sec (25%) of animals, observed after day 40 - after which all animals, except one that died, resumed normal activity.*

Lower mean body weight throughout the exposure period.

Increased relative organ weights: liver, kidney and brain

Reduction in plasma cholesterol on days 45 and 91 (41

and 43%), Increase in amylase activity on day 45 and 91 (33 and 30%)

Less pronounced differences in total protein concentration (increased) and albumin/globulin ratio (decreased)

5/12 rats with increased glycogen storage.

The following effects were seen in the mid-dose group:

Severe, clonic seizures lasting 20 sec (17%) of animals, observed after day 40 - after which all animals except one that died, resumed normal activity.*

3/12 rats with increased glycogen accumulation

The following effects were seen in the low-dose group:

2/12 rats with increased glycogen accumulation

*Note: Rats in the low-dose (8%) and control (17%) demonstrated milder episodes, characterized primarily by muscle twitching.

Dose-related changes in liver tissue with respect to glycogen accumulation.

Test substance : Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 8549, 845.9 or 84.50 mg/l naphthenic acids derived from Athabasca sands tailings. [Associated with CAS number 1338-24-5 in Toxline search]

Reliability : (2) valid with restrictions
The study is not a typical subchronic toxicity study as defined by OECD SIDS/HPV, i.e., the study was conducted with female rats only and examined a limited number of organs. However, it is well-conducted and provides limited information regarding potential subchronic effects of naphthenic acids following oral exposure.

Reference (30)

Type : Sub-chronic
Species : Rat
Sex : Male
Strain : Wistar
Route of admin. : Gavage
Exposure period : 30 days
Frequency of treatm. : Daily
Post exposure period :
Doses : 1000 mg/kg bw (no information on number of animals per dose)
Control group : no data specified
Method :
Year : 1977
GLP : No
Test substance : other TS: Naphthenic acid - no further information [Associated with CAS number 1338-24-5 in Toxline search]

Method : Male rats were given daily oral doses of 1000 mg/kg naphthenic acids. No other experimental details provided in abstract.

Result : The following statements appeared in the abstract:

Repeated daily administration (30 days) of naphthenic acid

at doses of 1000 mg/kg orally .. revealed a few cases of (1) CNS depression without analgesia and no loss of the corneal reflex (2) hematological changes, (3) weight loss leading eventually to death due to respiratory arrest, (4) gross morphological changes in the liver and stomach, and (5) histomorphological changes in a few selected organs.

Reliability : (4) not assignable
This information is taken from an abstract. The protocol of the study does not appear to be comparable to a guideline study, and the level of detail is insufficient to judge its validity.

Reference (27)

Repeated-Dose Toxicity

TEST SUBSTANCE

Category Chemical:	1338-24-5 Naphthenic acids
Test Substance:	1338-24-5 Naphthenic acids
Test Substance Purity/Composition and Other Test Substance Comments:	The 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 tablulated (Holowenko et al., 2002). Based on these data it was determined that there were no significant differences among these samples (Fedorak, 2009). The data indicated that the test material 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 (31%), 3 rings (5%) and 4 rings (1%).
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	N/A

METHOD

Route of Administration:	Oral
Other Route of Administration:	N/A
Type of Exposure:	Gavage
Species:	Rat
Other Species:	N/A
Mammalian Strain:	Sprague-Dawley

Other Strain:	N/A
Gender:	Male/female
Number of Animals per Dose:	12/sex/dose group
Concentration:	The naphthenic acids were suspended in corn oil to the appropriate concentrations and administered in 10 ml/kg doses.
Dose:	100, 300, 900 mg/kg/day
Year Study Performed:	2010
Method/Guideline Followed:	OPPTS 870.3650, 2000/OECD 422
GLP:	Yes. Code of Federal Regulations, Title 21, Volume 1, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies, revised April 1, 2007. OECD. Guideline for the Testing of Chemicals, Section: Health Effects, Subsection 474. Updated and adopted 21 July, 1997.
Exposure Period:	**Value or** **Lower Exposure Duration:** Male dosing was for 28-29 days **Upper Exposure Duration:** Depending on the time at which mating occurred, females were dosed for 39-53 days
Frequency of Treatment:	Daily
Post-Exposure Period:	None

Method/Guideline and Test Condition Remarks:

All rats were examined twice daily for mortality and general health. Detailed physical examinations of all animals were conducted weekly. Additionally, all animals were examined approximately 1 hour after each treatment, and all unusual observations were recorded.

Body weights of male rats were recorded one week prior to test substance administration, on the first day of dose administration, on a weekly basis during the study and at termination. Body weights of female rats were recorded once week prior to test substance administration, on the first day of dose administration and weekly until evidence of copulation was obtained. From that point body weights of female rats were recorded on gestation days (GD) 0, 4, 7, 11, 14, 17, and 20 and on lactation days (LD) 0, 1 and 4 (termination). For females for which there was no evidence of copulation, body weights were recorded weekly until termination. Body weights of offspring were recorded on post-natal day (PND) 1 and then on PND 4, prior to termination. Food consumption by adult animals was also recorded on the same schedule as the body weights.

The potential for nervous system effects was assessed using a functional observation battery (FOB). All rats in the vehicle (corn oil) and naphthenic acid-treated groups were examined prior to dosing, after approximately 28 days of dosing, and, for females, prior to termination. The FOB procedures were based on previously developed protocols (Gad, 1982; Haggerty, 1989; Irwin, 1968; Moser et al., 1988; 1991; O'Donoghue, 1989). The testing was conducted in a sound attenuated room with a white noise generator set to operate at 70 $\pm$ 10 dB. The investigators conducting the FOB were not aware of the treatment groups from which the respective animals were taken. The FOB consisted of the following: home cage observations; handling observations; open field observations; sensory observations and neuromuscular observations (Table 2). In addition there were physiological observations including body weight, body temperature and examination for catalepsy. There was also an assessment of locomotor activity which was measured electronically using a computer-controlled system with a series of infrared photobeams in a clear plastic rectangular cage. Animals were tested separately in 60 minute sessions divided into 5 minute intervals.

On the day of scheduled termination, blood samples were taken from all rats in the corn oil (vehicle) and naphthenic acid-treated rats for assessment of hematological and serum chemistry parameters. The hematological investigation included measurements of total leukocyte count, erythrocyte count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, prothrombin time, activated partial prothrombin time, reticulocyte count, mean platelet volume, red cell distribution width, hemoglobin distribution width, differential leukocyte count, and red cell morphology. The serum chemistry investigation included measurements of concentrations of albumin, total protein, globulin, albumin/globulin ratio, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminostransferase, gamma glutamyltransferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium, triglycerides, and bile acids.

At termination rats were euthanized by carbon dioxide inhalation. Necropsies were conducted on all animals sacrificed *in extremis* or at study termination. Organs were removed weighed if this was planned, and placed in 10% neutral buffered formalin for histologic examination. The disposition of organs and tissues was as listed in the table below. Note that the target organ investigation encompassed male and female reproductive organs to assist in the assessment of potential reproductive effects.

Tissues collected for weights and/or histological evaluation

Tissue Collected	Weight	Pathological Examination
Adrenals	Yes	Yes

Aorta	No	Yes
Bone with marrow (sternebrae)	No	Yes
Bone Marrow Smear	No	Yes
Brain (Cerebrum, Cerebellum)	Yes	Yes
Coagulating Gland	No	Yes
Eyes with Optic Nerve	No	Yes
Esophagus	No	Yes
Stomach	No	Yes
Duodenum	No	Yes
Jejunum	No	Yes
Ileum	No	Yes
Cecum	No	Yes
Colon	No	Yes
Rectum	No	Yes
Heart	Yes	Yes
Kidneys (2)	Yes	Yes
Left femur	No	Yes
Liver (2 lobes)	Yes	Yes
Lungs (fixed by inflation)	Yes	Yes
Lymph nodes (axillary, mesenteric, mandibular)		No Yes
Ovaries with oviducts	Yes	Yes
Pancreas	No	Yes
Peripheral nerve (sciatic)	No	Yes
Pituitary	No	Yes
Prostate	No	Yes
Salivary glands	No	Yes
Seminal Vesicles	No	Yes
Skeletal Muscle (rectus femoris)	No	Yes
Skin with mammary gland	No	Yes
Spinal cord (cervical)	No	Yes
Spleen	Yes	Yes
Testes with epididymides	Yes	Yes
Thymus	Yes	Yes
Thyroids (with parathyroids)	Yes	Yes
Trachea	No	Yes
Urinary Bladder	No	Yes
Uterus with Cervix and Vagina	Yes	Yes
Gross Lesions	No	Yes

Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, body weight changes, absolute and relative organ weights, clinical pathology values (except for gamma glutamyltransferase), and continuous FOB values were evaluated by one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences between the vehicle control and test substance-treated groups. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunnett test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. Histopathological findings in the test substance-treated groups and FOB parameters yielding scalar or descriptive data were compared to the vehicle control group

using Fisher's Exact Test (Steel and Torrie, 1980). Gamma glutamyltransferase data were evaluated using the Kruskal-Wallis nonparametric ANOVA (Kruskal and Wallis, 1952) to determine intergroup differences between the vehicle control and test substance-treated groups. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunn Test (Dunn, 1964) was used to compare the test substance-treated groups to the vehicle control group.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male Sprague-Dawley Rats	Systemic Toxicity	100		Mg/kg/day
NOAEL	Female Sprague-Dawley Rats	Systemic Toxicity	100		Mg/kg/day
NOAEL	Male Sprague-Dawley rats	Neurotoxicity	900		Mg/kg/day
NOAEL	Female Sprague-Dawley rats	Neurotoxicity	900		Mg/kg/day

Results Remarks:	Two high dose females were terminated on LD 2; one was sacrificed *in extremis* due to acute inflammation of the uterus; the other was sacrificed due to total litter loss. All other rats survived to scheduled termination. Clinical observations, which were noted only in high dose group females and approximately an hour of dosing included hunched posture; rocking, lurching, and/or swaying while ambulating; walking on tiptoes; hypoactivity; and shallow respiration. Some of the high dose group males also exhibited hunched posture. Body weight gain was reduced in high dose group males but the overall difference was less than 10% and the differences were not statistically significant. Among the females, the body weight gain in the high dose group was approximately 4% below control values but not significantly different at the end of the mating period. These differences in weight gain were associated with significantly reduced food consumption in the high dose group animals. There were no statistically significant differences in parameters assessed as part of the functional observation battery including home cage observations, handling parameters, open field observations, sensory observations or neuromuscular observations. There were some small differences in body weight gain as indicated previously but other physiological parameters (catalepsy, body temperature) were not affected by treatment. There were also no differences in locomotor activity patterns (data not shown). There were some hematology changes, primarily reductions in parameters related to hemoglobin content which were considered to have been treatment related. However, as is apparent from Table 1, the differences were small and there was no consistency between males and females. The clinical chemistry values showed a similar pattern. Among males the only statistically significant differences between control were for creatinine (control value = 0.3 $\pm$ 0.1 mg/dL versus a value of 0.4 $\pm$ 0.0 in the high dose group, $p < 0.01$), and chloride (control value = 104 $\pm$ 1.1 mEq/L versus a value of 102 $\pm$ 1.3 in the high dose group, $p < 0.01$). Among the female rats, statistically significant differences were found for albumin (control = 4.3 $\pm$ 0.2 g/dL versus 4.7 $\pm$ 0.3 in the high dose group, $p < 0.05$), total protein (control = 6.3 $\pm$ 0.3 g/dL versus 6.7 $\pm$ 0.4 in the high dose group, $p < 0.05$), glucose (control = 115 $\pm$ 11 mg/dL versus 130 $\pm$ 8.0 in the high dose group, $p < 0.05$), cholesterol (control = 69 $\pm$ 14 mg/dL in the control versus 89 $\pm$ 19 in the high dose group, $p < 0.05$), calcium (control = 10.6 $\pm$ 0.4 mg/dL in the control versus 11.5 $\pm$ 0.6 in the high dose group, $p < 0.01$), and phosphorus (control = 3.9 $\pm$ 0.6 mg/dL versus 5.5 $\pm$ 1.2 in the high dose group, $p < 0.05$). All of the differences were small and within the historical range of the laboratory. Additionally, most were significant at only the 0.05 level, and there was no consistency of response between the sexes. In the absence of any corresponding pathological findings, these differences were most likely incidental. The only notable gross observations were those of pale kidneys in the high dose males and a reduction in the number of *corpora lutea* in the high dose group females. Otherwise, the results of the gross examination were not remarkable. Organ weight determinations in males revealed significant increases in weights of liver, kidney, thyroid/parathyroid and epididymis although the differences in thyroid/parathyroid and epididymal weights were only statistically different when compared on a relative to body weight basis. In females, there was a significant increase in liver weights and significant reductions in lung weights, and absolute uterine weights (table 3). The lung weights were within the historical range for the laboratory, and were not associated with any pathological changes. The uterine weights were not significantly different when compared relative to body weights. All gravid females were in lactational anaestrus and undergoing involution. Uterine weight values all fell within the historical range for the laboratory and were not associated with any gross, histopathologic or clinical pathology changes. Other than the uterine weights there were no microscopic differences in the reproductive organs of the male and female rats.

The results of the pathological investigation are summarized in Table 3. 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. The microscopic examination also revealed minimal cardiomyopathy which occurred with increased incidence in the males in the 100, 300 and 900 mg/kg/day groups. The pathologist noted that cardiomyopathy is a common finding in rats (Greaves, 2007a), that the incidence of cardiomyopathy in the treated animals was within the historical range of the laboratory, and that the severity of cardiomyopathy in the treated male rats was similar to or less than the degree of severity found in the control animals. The pathologist also noted that the cardiomyopathy was not associated with any gross observations, organ weight changes or alterations in clinical pathology parameters.

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 were 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 these 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).

Minimal cardiomyopathy was reported to have increased in a dose-related fashion in male rats but was not considered to have been toxicologically important. In part because this is a common observation in control rats (Greaves et al., 2007b), and, additionally because the incidence was within the historical control range of the laboratory, the severity was not greater than that seen in the control groups, and because these microscopic observations were not associated with any other gross or clinical findings.
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, 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, 2007b) although cytoplasmic vacuolation of the adrenal cortex can also occur spontaneously (Frith et al., 2000) or as the result of pharmacological effects (Greaves, 2007c). The overall no effect level for all systemic effects was 100 mg/kg/day.

Table 1 Results o

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

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 corpusc ular 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)

Table 2. Statistically significant changes in terminal body weights and organ weights. The 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[b]
Kidney	3.51 ± 0.25	3.21 ± 0.20[a]	3.38 ± 0.39	3.53 ± 0.33	3.77 ± 0.46[b]
Heart	1.46 ± 0.09	1.46 ± 0.21	1.41 ± 0.14	1.43 ± 0.13	1.32 ± 0.13
Thyroid/parathyroid	0.019 ± 0.002	0.019 ± 0.001	0.020 ± 0.002	0.020 ± 0.002	0.020 ± 0.002
Epididymis (LT)	0.57 ± 0.14	0.60 ± 0.05	0.60 ± 0.04	0.66 ± 0.05[a]	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 [b]
Kidney	2.39 ± 0.17	2.07 ± 0.18[a]	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[a]	1.20 ± 0.12[b]	1.20 ± 0.07[b]
Uterus/Vagina	1.07 ± 0.19	1.00 ± 0.14	0.86 ± 0.08[a]	0.88 ± 0.11[a]	0.85 ± 0.12[a]

a = P < 0.05, b = P < 0.0

Table 3. Summary of microscopic findings from rats following repeated treatment with refined naphthenic acids.

									Males
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[b]	11[b]	0	0	0	0	
Minimal	0	3	9[b]	9[b]	0	0	0	0	
Mild	0	0	1	2					
Nephropathy	0	0	2	9[b]	0	0	0	0	
Minimal	0	0	2	5[a]	0	0	0	0	
Mild	0	0	0	4					
Liver									
Hypertrophy, hepatocellular, centrilobular			0	0	0	8[b]	0	0	0
	10[b]								
Minimal	0	0	0	8[b]	0	0	0	10[b]	
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[a]
Minimal	0	0	0	0	0	1	0	4	
Mild	0	0	0	0	0	0	0	1	
Thyroid									
Hypertrophy, epithelial		0	6[a]	9[b]	11[b]	0	3	4	8[b]
Minimal	0	6[a]	9[b]	11[b]	0	3	4	6[a]	
Mild	0	0	0	0	0	0	0	2	
Vacuolation, cytoplasmic		0	6[a]	9[b]	10[b]	0	3	4	8[b]
Minimal	0	6[a]	9[b]	10[b]	0	3	4	8[b]	

	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 8 5 4 4 3 3 2 4 Mild 4 7 8[a] 8[a] 0 0 0 0 a = p < 0.05, b = p < 0.01
Conclusion:	The overall no effect level for all systemic effects was 100 mg/kg/day.
RELIABILITY/DATA QUALITY	
Reliability:	1
Reliability Remarks:	Reliable without restrictions
Key Study Sponsor Indicator:	Key study for repeated dose toxicity and for reproductive toxicity
REFERENCE	
Reference:	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

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Bacterial reverse mutation assay
System of testing	:	S. typhimurium TA100, TA1535, TA97, TA98
Test concentration	:	Not indicated
Cycotoxic concentr.	:	With and without metabolic activation: >333 µg/plate
Metabolic activation	:	with and without
Result	:	Negative
Method	:	other: US National Toxicology Program protocols
Year	:	1993
GLP	:	Yes
Test substance	:	other TS: Sodium naphthenate [CAS number 61790-13-4] - Study indicates that it is a C7 naphthenic acid
Remark	:	Test material is a C7 naphthenic acid, whereas those produced commercially are mixtures of naphthenic acids predominantly in the C10-C30 range. Consequently, the results of this study are to be used as supplemental data only.
Reliability	:	(1) valid without restriction
Reference		(24)

Type	:	Cytogenetic assay
System of testing	:	Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary Cells (CHO)
Test concentration	:	Without activation: 54, 116 & 250 µg/ml. With activation: 25, 54, 116 & 250 µg/ml.
Cycotoxic concentr.	:	Not indicated
Metabolic activation	:	with and without
Result	:	Negative
Method	:	other:US National Toxicology program protocols
Year	:	1991
GLP	:	Yes
Test substance	:	other TS: Sodium naphthenate [CAS number 61790-13-4] - Study indicates that it is a C7 naphthenic acid
Remark	:	Solvent control: water Positive controls: Without metabolic activation Mitomycin C (0.4 ug/ml) With metabolic activation Cyclophosphamide (20 ug/ml) Metabolic activation Arochlor 1254 induced, Sprague-Dawley male rat liver S9 fraction Test material is a C7 naphthenic acid, whereas those produced commercially are mixtures of naphthenic acids predominantly in the C10-C30 range. Consequently, the results of this study are to be used as supplemental data only.
Reliability	:	(1) valid without restriction
Reference		(24)

Type	:	Cytogenetic assay

System of testing	:	Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary Cells (CHO)
Test concentration	:	
Cycotoxic concentr.	:	Not indicated
Metabolic activation	:	with and without
Result	:	
Method	:	other:US National Toxicology program protocols
Year	:	1991
GLP	:	Yes
Test substance	:	other TS: Sodium naphthenate [CAS number 61790-13-4] - Study indicates that it is a C7 naphthenic acid
Remark	:	Without activation: 17, 59, 167, 500 ug/ml (Trial 1) 100, 150, 200, 250 ug/ml (Trial 2) With activation:17, 59, 167, 500 ug/ml Solvent control: water Positive controls: Without metabolic activation - Mitomycin C (0.001 and 0.004 ug/ml) With metabolic activation - Cyclophosphamide (0.125 and 0.500 ug/ml) Metabolic activation: Arochlor 1254 induced, Sprague-Dawley male rat liver S9 fraction Test material is a C7 naphthenic acid, whereas those produced commercially are mixtures of naphthenic acids predominantly in the C10-C30 range. Consequently, the results of this study are to be used as supplemental data only.
Result	:	Weakly positive (trial 1- without metabolic activation) Positive (trial 2 - without metabolic activation) Negative (with metabolic activation)
Reliability	:	(1) valid without restriction
Reference		(24)

5.6 GENETIC TOXICITY 'IN VIVO

Genetic Toxicity *in vivo*	
TEST SUBSTANCE	
Category Chemical:	1338-24-5 Naphthenic acids
Test Substance:	1338-24-5 Naphthenic acids
Test Substance Purity/Composition and Other Test Substance Comments:	The 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 tablulated (Holowenko et al., 2002). Based on these data it was determined that there were no significant differences among these samples (Fedorak, 2009). The data indicated that the test material 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 (31%), 3 rings (5%) and 4 rings (1%).
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	In vivo mutagenesis (chromosomal aberrations)
Type of Test:	Micronucleus Test (OPPTS 870.5395)/OECD 474
Route of Administration:	Oral gavage
Species:	Rats
Strain:	Sprague-Dawley
Gender:	Male and Female
Dose:	100, 300, 900 mg/kg/day

Year Study Performed:	2010
Method/Guideline Followed:	OPPTS 870.5395/OECD 474
GLP:	Yes
Duration of Treatment/Exposure Period and Units:	Approximately 30 days
Frequency of Treatment:	Daily
Positive, Negative and Solvent Control Substance(s):	Sham control (no material administered) Negative control (corn oil) Positive control (cyclophosphamide, 60 mg/kg/day)
Post-Exposure Period:	None
Number of Animals per Sex per Dose:	6 males/6 females
Method/Guideline and Test Condition Remarks:	The micronucleus test was consistent with the US EPA guidelines for studies of this type (OPPTS 870.5395) and with OECD 474. The testing was in accordance with Good Laboratory Practice Guidelines of the OECD (OECD, 1997) and the U.S. EPA (CFR, 2007). Bone marrow was collected from all animals at terminal sacrifice and flushed into a centrifuge tube using a syringe containing heat inactivated fetal bovine serum (HI FBS). The bone marrow was centrifuged, the majority of the HI FBS was decanted, and the pellet was re-suspended. Bone marrow smears were prepared by placing single drops of suspension on microscope slides (minimum of two per preparation). The slides were coded, air dried, fixed in methanol and allowed to air dry a second time. Coded slides were stained with acridine orange (Hayashi et al., 1983). 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. The percentages of PCEs , micronucleated cells in NCEs, and the ratios of PCEs to total erythrocytes in the test substance- and vehicle-treated groups were compared using ANOV A (Snedecor and Cochran, 1980). If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunnett Test (Dunnett, 1964) was used to compare each test substance-treated group to the vehicle control group. In addition, the positive control and vehicle control groups were compared using a separate parametric one-way ANOVA (Snedecor and Cochran, 1980).
TEST RESULTS	
Systemic Toxicity:	No treatment-related bone marrow effects.

Genotoxic Effect:	Not mutagenic. 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 providing evidence that the test had worked as expected.
Results Remarks:	Summary of results of micronucleus data from rats following repeated treatment with refined naphthenic acids.

Treatment (N=5)	**Gender**	**Ratio of PCEs to Total Erythrocytes**	**Total MN PCEs/2000 PCEs (N=5)**	**% MN PCEs**	**Total MN NCEs/2000 NCEs**
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
Positive Control (Cyclophosphamide)					
60 mg/kg/day	Males	128	1.28 ± 0.14[a]	13	0.40 ± 0.21
	Females	97	0.97 ± 0.19a	16	0.51 ± 0.12[a]

a. $P < 0.05$

MN – Micronucleated erythrocytes. PCE – polychromatic erythrocytes. NCE – Normochromatic erythrocytes.

Conclusion:	Naphthenic acids did not induce chromosomal aberrations under the conditions of the test
RELIABILITY/DATA QUALITY	
Reliability:	
Reliability Remarks:	Reliable without restrictions
Key Study Sponsor Indicator:	Key study for in vivo mutagenic potential
REFERENCE	

Reference:	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

5.7 CARCINOGENICITY

Species	:	Mouse
Sex	:	Female
Strain	:	other: No information available
Route of admin.	:	Dermal
Exposure period	:	2 Years
Frequency of treatm.	:	2 times/day
Post exposure period	:	
Doses	:	0.05 ml undiluted
Result	:	
Control group	:	no data specified
Method	:	
Year	:	1987
GLP	:	no data
Test substance	:	other TS: Calcium naphthenate [CAS number 61789-36-4]
Method	:	Not described; listed in summary as "non-TSCA Protocol/Guideline (voluntary test)"
Result	:	The following statements appeared in the abstract: Clinical observations included mild irritation, hair loss, shiny patches on the skin, and flaking skin surfaces. These progressed to moderate irritation (observed with sores and scabs on the treated site), or severe irritation caused by large sores or visible ulcers. In the negative control group, no cutaneous tumors developed at or distant to treated sites. Twelve epidermal and one dermal tumor at the treated sites were observed in eight mice that were exposed to the test material. Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present.
Reliability	:	(4) not assignable This information is taken from an EPA site that summarizes results of testing submitted under TSCA. The protocol of the study does not appear to be comparable a guideline study as indicated in the summary. In addition, the material used (calcium naphthenate) was judged not to be similar to commercially available naphthenic acids. Consequently, the study is for supplemental use only.
Reference		(37)

5.8.1 TOXICITY TO FERTILITY

Type	:	One generation study
Species	:	Rabbit
Sex	:	male/female
Strain	:	New Zealand white
Route of admin.	:	Dermal
Exposure period	:	10 weeks
Frequency of treatm.	:	6 hr/day, 5 days/week
Premating exposure period		
Male	:	10 weeks
Female	:	Not exposed
Duration of test	:	10 week exposure period prior to mating, gestation and delivery. Total duartion of study was approximately 22 weeks

No. of generation studies	:	1
Doses	:	Undiluted
Control group	:	other: carrier oil as present in the test substance
Method	:	
Year	:	1984
GLP	:	no data
Test substance	:	other TS: Calcium naphthenate, Shell SAP Oil [CAS number 61789-36-4]
Method	:	A group of 12 male rabbits was dermally exposed to 2 ml undiluted test substance or control vehicle for six hours daily for 5 days per week for 10 weeks. Body weights were recorded weekly and at the end of 10 weeks, each male was mated with 2 untreated female rabbits. Half of the males of each group were killed and necropsied after mating. The remaining males were weighed weekly and necropsied approximately 12 weeks later. Macroscopic and microsopic examinations of the male reproductive tracts were carried out on all rabbits. The females were necropsied on day 29 of gestation. Numbers of corpora lutea, total implantations, pre-and post-implantation losses and numbers of viable fetuses were recorded.
Result	:	All male rabbits survived with the exception of one control that died after 9 weeks of exposure, having shown no unusual clinical signs. There were no systemic toxicity, application site toxicity, or statistically significant changes in body weights observed in the test animals during the 10 week exposure period or the 12 week post-exposure period. In the male animals, there were no significant changes in the testes weights. In the females, there were no significant differences in the number of implantations, or in pre-and post-implantation losses. In addition, there were no differences in viable fetuses to those females that were mated with exposed males compared to those mated with unexposed males. The study also reported that there were no macroscopic or microscopic pathological findings in the male reproductive tract.
Reliability	:	(2) valid with restrictions The study has sufficient detail, however, the protocol does not appear to be comparable to a guideline study. In addition, the material used (calcium naphthenate) was judged not to be similar to commercially available naphthenic acids. Consequently, the study is for supplemental use only.
Reference		(32)

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	1338-24-5 Naphthenic acids
Test Substance:	1338-24-5 Naphthenic acids
Test Substance Purity/Composition and Other Test Substance Comments:	The 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 tablulated (Holowenko et al., 2002). Based on these data it was determined that there were no significant differences among these samples (Fedorak, 2009). The data indicated that the test material 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 (31%), 3 rings (5%) and 4 rings (1%).
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	N/A
METHOD	
Route of Administration:	Oral
Other Route of Administration:	N/A
Type of Exposure:	Gavage
Species:	Rat
Other Species:	N/A
Mammalian Strain:	Sprague-Dawley
Other Strain:	N/A

Gender:	Male/Female
Number of Animals per Dose:	12/sex/dose group
Concentration:	The test materials were suspended in corn oil to the appropriate concentrations and administered daily in bolus doses of 10 ml/kg
Dose:	100, 300, 900 mg/kg/day
Year Study Performed :	2010
Method/Guideline Followed:	OPPTS 870.3650, 2000/OECD 422
GLP:	Yes. The testing was in accordance with Good Laboratory Practice Guidelines of the OECD (OECD, 1997) and the U.S. EPA (CFR, 2007).
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** Males were exposed 28-29 days Females were exposed for 39-53 days depending on the day on which mating occurred.
Frequency of Treatment:	Daily
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	Dosing of males was initiated 14 days prior to pairing and throughout a 14 day mating period for a total of 28-29 doses. Dosing of females was also initiated 14 days prior to pairing and continued throughout the mating and gestational periods until study termination on post-natal day 3. The total number of doses ranged from 39-53 depending on the time at which mating occurred. Body weights of female rats were recorded once week prior to test substance administration, on the first day of dose administration and weekly until evidence of copulation was obtained. From that point body weights of female rats were recorded on gestation days (GD) 0, 4, 7, 11, 14, 17, and 20 and on lactation days (LD) 0, 1 and 4 (termination). For females for which there was no evidence of copulation, body weights were recorded weekly until termination. Body weights of offspring were recorded on post-natal day (PND) 1 and then on PND 4, prior to termination. Food consumption by adult animals was also recorded on the same schedule as the body weights.

	Mating was initiated after 14 days of dosing. Rats were mated on a 1:1 basis within each treatment group, females were co-housed with the males. Each mating pair was evaluated on a daily basis during the mating period. Successful mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. The day on which mating was confirmed was designated as gestational day 0. All females confirmed to have mated were placed in plastic maternity cages once mating was confirmed. Females for which copulation was not detected were placed in maternity cages at the end of the 14 day mating period. All females were allowed to deliver and to rear their young to post-natal day 4. On the day of parturition, all pups were examined for viability, for the presence of gross malformations and to assess gender. The numbers of live and stillborn pups were recorded. Length of gestation was calculated as the time from confirmation of mating to the onset of delivery. Females for which there was no evidence of mating were sacrificed on post-cohabitation day 25, those that showed evidence of mating but failed to deliver were euthanized on post-mating day 25, and all others were euthanized on post-natal day 4. Uteri with no microscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss (Salewski, 1964). All offspring were uniquely identified and examined daily for signs of mortality and ill health. All offspring were individually weighed on PND 1 and 4. Gender was assessed on PND 0 and 4. At scheduled termination, PND 4, all surviving offspring were euthanized and discarded without further examination. Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction (Hollander and Wolfe, 1999). Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, offspring body weights and body weight changes, gestation length, numbers of former implantation sites, numbers of corpora lutea, number of pups born, live litter size on PND 0, unaccounted for sites, absolute and relative organ weights, and pre-coital intervals were evaluated by one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences between the vehicle control and test substance-treated groups. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunnett test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. Note that an examination of target organs including male and female reproductive organs was also carried out as part of this test. Organs examined included: ovaries with oviduct, uterus with cervix and vagina, testes with epididymides, prostate and seminal vesicles. The ovaries, testes and uteri were weighed and all were examined histologically. The absolute epididymal weights were increased in the 900 mg/kg/day group but were not significantly different when expressed on a per body weight basis. The uterine weights were also significantly elevated but this was considered to have been a consequence of the fact that the females were all in lactational anaestrous. The uterine weights were within the historical range of the laboratory and were not considered to have been toxicologically important. There were no weight differences in any of the other organs and no pathological changes in any of the reproductive organs at the highest dose tested (900 mg/kg/day).
Pre-Mating Exposure / Males :	14 days
Pre-Mating Exposure / Females:	14 days

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male Rats	Mating	900		Mg/kg/day
NOAEL	Female Rats	Mating	900		Mg/kg/day
NOAEL	Male Rats	Reproductive Organ Effects	900	Mg/kg/day	
NOAEL	Female Rats	Reproductive Organ Effects	900	Mg/kg/day	

Results:

There was no evidence of treatment-related effects on mating. More specifically, there were no apparent treatment-related differences in frequency of mating, time to mate, mating success or length of the gestational period (table 1).

Note also as indicated above that there were no toxicologically important changes in the reproductive organs.

Table 1. Summary of reproductive parameters assessed in the repeated dose/reproductive toxicity 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**[c]
Post-Implantation loss (%) [d]	6.0	5.1	7.7	11.5

a. Pregnant = uterine implantation sites.
b. Data summarized as mean ± standard deviation.
c. $p < 0.05$

Results Remarks: No reproductive effects were identified.

Conclusion: The NOAEL for reproductive effects of refined naphthenic acids is 900 mg/kg/day

RELIABILITY/DATA QUALITY

Reliability:	**1**
Reliability Remarks:	Reliable without restrictions.
Key Study Sponsor Indicator:	Key study for the assessment of reproductive toxicity
REFERENCE	
Reference:	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

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	Rat
Sex	:	Female
Strain	:	Wistar
Route of admin.	:	Gavage
Exposure period	:	
Frequency of treatm.	:	Daily
Duration of test	:	
Doses	:	0.6, 6 & 60 mg/kg/day
Control group	:	
Method	:	
Year	:	2002
GLP	:	no data
Test substance	:	other TS: Naphthenic acid isolated from Athabasca oil sands tailings. [Associated with CAS nymber 1338-24-5 in Toxline search]
Method	:	Oral doses of 60 mg/kg/day were given to female rats during pre-breeding, breeding and gestation.
Result	:	The following description was given: Reproductive toxicity testing demonstrated dramatic effects on female fertility at an oral dosage of 60 mg/kg/day during pre-breeding, breeding and gestation. While control and low dose (6 mg/kg/day) animals achieved 93 and 100% reproductive success, respectively, only 7% of females dosed at 60 mg/kg/d successfully bore a litter. Total cholesterol of the latter group was 30% lower than controls. Mating and ovulation were comparable amongst control and dose groups, while fetal malformations were not apparent in any offspring. Results suggest that the dose-related infertility may be associated with poor embryonic implantation, an effect that might be secondary to depressed sex hormone production requiring cholesterol as a precursor.
Reliability	:	(4) not assignable This information is taken from an abstract. No other details of the study could be obtained. The protocol of the study does not appear to be comparable to a guideline study, and the level of detail is insufficient to judge. However, it may be useful in establishing dose levels for a more in-depth study.
Reference		(31)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	1338-24-5 Naphthenic acids
Test Substance:	1338-24-5 Naphthenic acids
Test Substance Purity/Composition and Other Test Substance Comments:	The 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 tablulated (Holowenko et al., 2002). Based on these data it was determined that there were no significant differences among these samples (Fedorak, 2009). The data indicated that the test material 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 (31%), 3 rings (5%) and 4 rings (1%).
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	N/A

METHOD

Route of Administration:	Oral
Other Route of Administration:	N/A
Type of Exposure:	Oral gavage
Species:	Rat
Other Species:	N/A
Mammalian Strain:	Sprague-Dawley
Other Strain:	N/A
Gender:	Female
Number of Animals per Dose:	12
Concentration:	The naphthenic acids were suspended in corn oil to the appropriate concentrations and administered in 10 ml/kg doses.
Dose:	100, 300, 900 mg/kg/day

Year Study Performed :	2010
Method/Guideline Followed:	OPPTS 870.3650, 2000/OECD 422
GLP:	Yes. The testing was in accordance with Good Laboratory Practice Guidelines of the OECD (OECD, 1997) and the U.S. EPA (CFR, 2007).
Exposure Period:	**Value or** **Lower Exposure Duration : 39 days** **Upper Exposure Duration : 53 days** **Dosing was initiated 14 days prior to mating and continued until post-natal day 3.**
Frequency of Treatment:	Daily
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	The rats were obtained from Charles River Laboratories, Raleigh, North Carolina. They were held in the laboratory for a 16 day acclimation period and then randomly divided into treatment groups by weight. Mating was initiated after 14 days of dosing. Rats were mated on a 1:1 basis within each treatment group, females were co-housed with the males. Each mating pair was evaluated on a daily basis during the mating period. Successful mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. The day on which mating was confirmed was designated as gestational day 0. All females confirmed to have mated were placed in plastic maternity cages once mating was confirmed. Females for which copulation was not detected were placed in maternity cages at the end of the 14 day mating period. All females were allowed to deliver and to rear their young to post-natal day 4. On the day of parturition, all pups were examined for viability, for the presence of gross malformations and to assess gender. The numbers of live and stillborn pups were recorded. Length of gestation was calculated as the time from confirmation of mating to the onset of delivery. Females for which there was no evidence of mating were sacrificed on post-cohabitation day 25, those that showed evidence of mating but failed to deliver were euthanized on post-mating day 25, and all others were euthanized on post-natal day 4. Uteri with no microscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss (Salewski, 1964). All offspring were uniquely identified and examined daily for signs of mortality and ill health. All offspring were individually weighed on post-natal days 1 and 4. Gender was assessed on post-natal days 0 and 4. At scheduled termination, post-natal day 4, all surviving offspring were euthanized and discarded without further examination. Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction (Hollander and Wolfe, 1999). Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, offspring body weights and body weight changes, gestation length, numbers of former implantation sites, numbers of corpora lutea, number of pups born, live litter size on PND 0, unaccounted for sites, and pre-coital intervals were evaluated by one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences between the vehicle control and test substance-treated groups. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunnett test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. Mean litter proportions (percent of litter) of males at birth and post-natal survival were evaluated using the Kruskal-Wallis

nonparametric ANOVA (Kruskal and Wallis, 1952) to determine intergroup differences between the vehicle control and test substance-treated groups. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunn Test (Dunn, 1964) was used to compare the test substance-treated groups to the vehicle control group.

TEST RESULTS

Concentration (LOAEL/LOAEC/NOAEL/NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration: Units:
NOAEL	Female Sprague-Dawley Rats		Maternal Effects 900	Mg/kg/day
NOAEL	F1 offspring	Offspring delivered	300	Mg/kg/day
NOAEL	F1 offspring	Offspring live born	100	Mg/kg/day
NOAEL	F1 offspring	Offspring body weights	300	Mg/kg/day

Results Remarks:

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. Note that there was a significant increase in pre-coital interval in the 300 mg/kg group, but this was due to a single female, and, as noted was within the historical range of the laboratory. Accordingly, it was not considered to have been a treatment-related effect.

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 (see Table 1 below). However, there was a significant reduction in the number of offspring born/litter in the high dose group (Table 2). There was also a significant reduction in survival in offspring in the high dose group, and those 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).

Table 1. Summary of reproductive parameters assessed in the repeated dose/reproductive toxicity 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**[c]	
Post-Implantation loss (%) [d]		6.0	5.1	7.7	11.5

a. Pregnant = uterine implantation sites.
b. Data summarized as mean ± standard deviation.
c. $p < 0.05$

Table 2. Survival, viability and growth of offspring following *in utero* exposure to refined naphthenic acids. The data are given as mean ± SD.

Dose (mg/kg/day)	Corn Oil	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[a]**	**9.6 ± 4.0[b]**
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[a]**
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[b]**
Pup weight PND 4 – females		9.1 ± 1.0	9.0 ± 1.0	8.8 ± 0.7	**7.3 ± 1.5[b]**

a. $P < 0.05$, b. $p < 0.01$

Conclusion:	Treatment of Sprague-Dawley rats with refined naphthenic acids had no apparent effects on mating and did not produce malformations at the highest dose tested (900 mg/kg/day). However, there were significant reductions in number of offspring, number live born and offspring body weights. The overall no observed adverse effect level was 100 mg/kg/day.
RELIABILITY/DATA QUALITY	
Reliability:	
Reliability Remarks:	Reliable without restrictions.
Key Study Sponsor Indicator:	Key study for the assessment of developmental toxicity
REFERENCE	
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5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

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