201-14906B

ROBUST SUMMARY
OF INFORMATION ON

Substance Group: **RECLAIMED SUBTANCES: NAPHTHENIC ACID**

Summary prepared by: **American Petroleum Institute**

Date of last Update: **DECEMBER 15, 2003**

Number of pages: **40**

Reliability of data included in this summary has been assessed using the approache described by Klimisch, et al.

Klimisch, H.J., Andreae, M. and Tillman, U (1997)
A systemic approach for evaluating the quality of experimental toxicological and exotoxicological data.
Regulatory Toxicology and Pharmacology 25: 1-5

1. General Information

1.1 GENERAL SUBSTANCE INFORMATION

Substance Type: Naphthenic Acids

Physical status: Naphthenic acid fractions are oily liquids. The salts may be liquid or solid. Naphthenic acids (CASRN 1338-24-5) 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.

2. Physical and Chemical Data

2.1 MELTING POINT

Test Substance: Naphthenic Acids, commercial mixtures

Method: Not stated

Year (Guideline): Not stated

Type (test type): Not stated

GLP: Unknown

Test Conditions: Unknown

Results:

-35 °C to +0 °C	Ref (1)
-35 °C to +2 °C	Ref (2)
+30 °C	Ref (3)

Remark: Values cited represent ranges of melting points cited in product literature data and Material Safety Data Sheet for commercial naphthenic acid products.

Source:

(1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

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

(3) Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.

Reliability: (4) Not assignable. Original source data were not available for review.

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method/Guideline: Calculated values using MPBPWIN Version 1.40, a subroutine of the computer program EPIWIN Version 3.10

Year (guideline): 2000

Type (test type): Not applicable

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10

Results:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Melting Point, °C
1-ring cyclopentane	16	254	117
1-ring cyclohexane	21	325	155
2-ring cyclopentane	17	266	127
2-ring cyclohexane	21	323	157
3-ring cyclohexane	17	264	128
3-ring cyclohexane	21	321	160
4-ring cyclohexane	17	262	131
4-ring cyclohexane	21	319	156

Remark: Substances in this category do not have a specific melting point but a range of melting points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual melting point ranges will vary dependent upon their constituent composition.

Melting point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated melting points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products, as evidenced by comparing the estimated melting point values to those cited in product literature and MSDS data (SocTech, S.A. 2003; AGS Chemicals Limited. 2003; Mallinckrodt Baker, Inc. 1997).

Source: U.S. EPA. 2000. API (Estimation programs interface) suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

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

Reliability: (2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of melting point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

2.2 BOILING POINT

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Not stated

Year: Not stated

Type: Not stated

GLP: Not stated

Year (study performed): Not stated

Test Conditions: Not stated

Results:

250 °C to 350 °C	Ref (1)
140 °C to 200 °C	Ref (2)
200 °C to 370 °C	Ref (3)

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.

Source:

(1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

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

(3) Brient, J.A., P.J. Wessner, and M.N. Doyle. 1995. Naphthenic Acids. In: Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, Inc.

Reliability: (4) Not assignable

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, MPBPWIN V1.40 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10

Results: Boiling point values for various cycloaliphatic carboxylic acids in naphthenic acid mixtures are:

Compound	Estimated Boiling Point, °C
C7 cyclohexane	233
C9 dicyclopentane	266
C10 cyclopentane	284
C11 cyclohexane	301
C13 dicyclopentane	326
C14 cyclopentane	340
C15 cyclohexane	352
C17 dicyclopentane	373
C17 tricyclohexane	375

Remark: Substances in this category do not have a specific boiling point but a range of boiling points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual boiling point ranges will vary dependent upon their constituent composition.

Boiling point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated boiling points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Reliability: (2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of boiling point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

2.4 VAPOR PRESSURE

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, MPBPWIN V1.40 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

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

Results: Estimated vapor pressures for various naphthenic acid compounds:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Vapor Pressure, Pa
1-ring cyclopentane	16	254	$1.8x10^{-3}$
1-ring cyclohexane	21	325	$1.5x10^{-5}$
2-ring cyclopentane	17	266	$4.8x10^{-4}$
2-ring cyclohexane	21	323	$1.5x10^{-5}$
3-ring cyclohexane	17	264	$4.2x10^{-4}$
3-ring cyclohexane	21	321	$1.4x10^{-5}$
4-ring cyclohexane	17	262	$1.6x10^{-5}$
4-ring cyclohexane	21	319	$4.4x10^{-4}$

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 hydrocarbon acidic structures reported by Rogers et al. (2002) to predominate in naphthenic acids 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 representative individual naphthenic acid molecules 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.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

OECD (Organization for Economic Cooperation and Development). 1995. OECD Guideline 104, Vapor Pressure. OECD, Paris, France.

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

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

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

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

2.5 PARTITION COEFFICIENT

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, KOWWIN V1.66 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, vapor pressures were calculated by KOWWIN, V1.66, EPIWIN V3.10

Results: Tabulated values for various naphthenic acid molecules are:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Log Kow
1-ring cyclopentane	16	254	6.7
1-ring cyclohexane	21	325	9.2
2-ring cyclopentane	17	266	6.3
2-ring cyclohexane	21	323	8.3
3-ring cyclohexane	17	264	5.4
3-ring cyclohexane	21	321	7.3
4-ring cyclohexane	17	262	6.5
4-ring cyclohexane	21	319	5.1

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 predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected, however, that the lowest molecular weight structures will have the lowest partition coefficients of the compounds in the complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures of molecular weights below 250 will likely show log Kow values lower than those estimated here.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

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

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

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in: Water

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculation, EPIWIN©, WSKOWWIN V1.40 (U.S. EPA 2000)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, water solubility values were calculated by WSKOWWIN, V1.40, EPIWIN V3.10

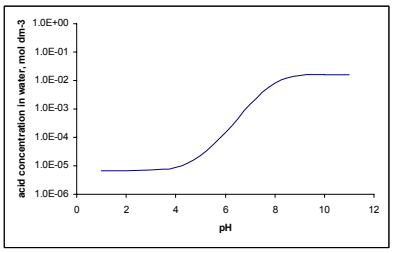
Results: Tabulated estimates at 25°C for various naphthenic acid molecular structures are:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Water Solubility, mg/l
1-ring cyclopentane	16	254	0.11
1-ring cyclohexane	21	325	0.0003
2-ring cyclopentane	17	266	0.19
2-ring cyclohexane	21	323	0.002

3-ring cyclohexane	17	264	1.2
3-ring cyclohexane	21	321	0.01
4-ring cyclohexane	17	262	0.08
4-ring cyclohexane	21	319	2.1

Remark:

No water solubility measurements were found for naphthenic acids, but their dissociation equilibrium in aqueous systems provides a general understanding of their behavior. These compounds exist as weak acids, with most pKa values being reported at about 5 (Havre, 2002). At low pHs, these compounds exist in their undissociated form and tend to partition onto solids (Rogers et al., 2002). At high pHs, they exist in their dissociated form and become more mobile (Havre, 2002). The following plot shows a theoretical model of the concentration of the acid in the water phase with water pH. This relationship is used as the basis for extraction of naphthenic acids from crude oil, where an alkaline hot water extraction process is used (CEATAG 1998; Brient et al., 1995). However, solubility does not follow an exact acid/base equilibrium, and the equilibrium between oil and water becomes increasingly complex as pH rises. This is due to the tendency of these substances to form micelles and reversed micelles at alkaline pHs. In this system, the existence of 4 or 5 isotropic phases can be observed, making exact solubility measurements difficult (Havre, 2002).



from Havre, 2002

To gain an overview of the water solubility of a range of molecular weight naphthenic acids, the EPIWIN© computer model (U.S. EPA 2000) was used to generate solubility estimates for different molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected that the lowest molecular weight structures will have the greatest water solubility of the compounds in complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures having molecular weights below 250 will likely show water solubilities greater than those estimated here.

Source:

U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Havre, T.E. 2002. Formation of calcium naphthenate in water/oil systems, naphthenic acid chemistry and emulsion stability. Ph.D. Thesis, Department of Chemical Engineering, Norwegian University of Science and Technology, Trondheim, Norway. October 2002.

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

CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

Brient, J.A., P.J. Wessner, and M.N. Doyle. 1995. Naphthenic acids. In: Kroschwitz, J.I. (ed.). Encyclopedia of Chemical Technology, Vol. 16, 4th ed. John Wiley & Sons, Inc., New York. pp 1017 – 1029.

Reliability: (2) Reliable with restrictions
Estimated water solubility values were obtained from a validated computer program.

2.14 ADDITIONAL REMARKS

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).

Source: CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: http://www.soctech.ro/English/Produse/1acizinaft.htm

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

Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.

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

3. Environmental Fate Data

3.1.1 PHOTODEGRADATION

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Calculations by EPIWIN© V3.10; Subroutine AOPWIN V1.90.

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: Not applicable, photodegradation potential was calculated by AOPWIN, V1.90, EPIWIN V3.10

Results:

Type	Carbon Number	Molecular Weight	Half Life (days)
1-ring cyclopentane	16	254	0.6
1-ring cyclohexane	21	325	0.4
2-ring cyclopentane	17	266	0.5
2-ring cyclohexane	21	323	0.3
3-ring cyclohexane	17	264	0.3
3-ring cyclohexane	21	321	0.3
4-ring cyclohexane	17	262	0.3
4-ring cyclohexane	21	319	0.3

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 predominate in Athabasca oil sands extracts (Rogers et al., 2002).

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.

Source: U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

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

Reliability: (2) Reliable with restrictions
Estimated water solubility values were obtained from a validated computer program.

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.

Source: Harris, J.C. 1982. Rate of hydrolysis. In; Handbook of Chemical Property Estimation Methods. W.L. Lyman, W.F. Reehl, and D.H. Rosenblastt, eds. Mcgraw-Hill Book Co., New York, NY.

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Test Substance: Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

Method: Level 1 Fugacity-Based Environmental Equilibrium Partitioning Model (Version 2.11)

Year: 2000

Type: Estimation, computer model

GLP: Not applicable

Year (study performed): Not applicable

Test Conditions: 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.

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

Type (C-number)(Molecular Weight)

Distribution In:

Air	Water	Soil	Sediment	Suspended Sediment	Biota
1-ring cyclopentane (C16)(254)					
<0.1	<0.1	98	2	<0.1	<0.1
1-ring cyclohexane (C21)(325)					
<0.1	<0.1	98	2	<0.1	<0.1
2-ring cyclopentane (C17)(266)					
<0.1	<0.1	98	2	<0.1	<0.1
2-ring cyclohexane (C21)(323)					
<0.1	<0.1	98	2	<0.1	<0.1
3-ring cyclohexane (C17)(264)					
<0.1	0.4	97	2	<0.1	<0.1
3-ring cyclohexane (C21)(321)					
<0.1	<0.1	98	2	<0.1	<0.1
4-ring cyclohexane (C17)(262)					
<0.1	<0.1	98	2	<0.1	<0.1
4-ring cyclohexane (C21)(319)					

Remark: Multimedia distribution was calculated for a range of naphthenic acids covering predominant molecular weight and ring structures of such constituents found in Athabasca oil sands extracts (Rogers et al., 2002). The principle distribution of these constituents following an environmental release would be to soil and/or sediment, with overwhelming partitioning to soil.

Source: Mackay, D. 1991. Multimedia environmental models; The fugacity approach Lewis Publ. CRC Press, Boca Raton, Florida.

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

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

3.8 ADDITIONAL REMARKS

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 NH_4NO_3, K_2HPO_4, and KH_2PO_4 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		1MCCH		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.

Source: Herman, D.C., P.M. Fedorak, and J.W. Costerton. 1993. Biodegradation of cycloalkane carboxylic acids in oil sand tailings. Can. J. Microbiol. 39:576-580.

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

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 CO_2 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 H_2SO_4 to convert all forms of inorganic carbon into CO_2. A 0.5 ml headspace sample from each bottle was analyzed for CO_2 content by gas chromatography. Mineralization of the organic substrate was first corrected for the amount of CO_2 in the control bottles, then expressed either as the total amount of CO_2 produced within the bottle or as the percentage of organic carbon converted to CO_2.

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 CO_2 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 CO_2 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 CO_2 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 CO_2 determined by GC. The level of CO_2 production was corrected for the amount of CO_2 within the control bottles and expressed as the percentage of organic substrate converted to CO_2.

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

Mineralization by day 24, % organic C converted to CO_2:

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 CO_2 production and O_2 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 CO_2 and O_2 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 CO_2 production and O_2 utilization when compared with TPW alone. Sterilized TPW demonstrated no CO_2 production or O_2 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 CO_2 evolution and O_2 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.

Source: Herman, D.C., P.M. Fedorak, M.D. MacKinnon, and J.W. Costerton. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oils sands tailings.

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

4. Ecotoxicity

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Test Substance: Naphthenic acids

Method/Guideline: Hart, et al. 1945; Doudoroff et al. 1951

Year (guideline): N/A

Type (test type): Static

GLP: No

Year (study performed): 1965

Species: zebra fish (*Brachydanio rerio*)

Analytical Monitoring: No

Exposure Period: 96 hours

Statistical Method: (FT - ME) Graphical interpolation for determining the LC50.

Test Conditions: (FT - TC)

- **Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.**

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.

Results: (FT - RS) 96-hour TLm = 16.3 ppm

Units/Value:

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

- **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.** The article reported that pH and dissolved oxygen concentrations were taken during the test, but these data were not reported.

Conclusion: (FT - CL)

Reliability: (FT - RL) (2) Reliable 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.

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

Hart, W.B., P. Doudoroff, and J. Greenbank. 1945. The evaluation of the toxicity of the industrial wastes, chemicals and other substances to freshwater fishes – The Atlantic Refining Company, Philadelphia, PA. 315 pp.

Dourdoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galstoff, W.B. Hart, T. Patrick, E.R. Strong, E.W. Surber, and W.M. VanHorn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sew. and Ind. Wastes. 23(11):1380-1397.

Other (source): (FT - SO)

FT - Freetext
ME - Method
TC - Test Conditions
RS - Results
CL - Conclusion
RL - Reliability
RE - Reference
SO - Source

Test Substance: Naphthenic acid mixture (commercially available from Eastman Chemicals)

Method/Guideline: Peltier and Weber 1985

Year (guideline): 1985

Type (test type): static acute

GLP: not stated

Year (study performed):

Species: three-spine stickleback (*Gasterosteus aculeatus*)

Analytical Monitoring: no

Exposure Period: 96 hours

Statistical Method: (FT - ME)

Test Conditions: (FT - TC)

- **Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.**

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.

Results: (FT - RS)

Units/Value: 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

- **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.**

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.

Conclusion: (FT - CL)

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

Source: (FT - RE) Dorn, P.B. 1992. Case Histories – The petroleum refining industry. In: Ford, D.L. (ed.). Water Quality Management Library, Volume 3, Toxicity Reduction Evaluation

and Control. Technomic Publishing Co., Lancaster, PA. pp 183 – 223.

Peltier, W.H., and C.I. Weber, eds. 1985. Method for measuring acute toxicity of effluents to freshwater and marine organisms, 3rd edition. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/4-85-014. 230 pp.

Stephan, C.E. 1977. Method for calculating an LC50. In: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA. pp 65-84.

Other (source): (FT - SO)

FT - Freetext
ME - Method
TC - Test Conditions
RS - Results
CL - Conclusion
RL - Reliability
RE - Reference
SO - Source

4.9 ADDITIONAL REMARKS

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

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

Hisaoka, K.K., and H.I. Battle. 1958. The normal development stages of the zebra-fish, *Brachydanio rerio* (Hamilton-Buchanan). J. Morph. 102(2):311-327.

Reliability: (2) Reliable 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.

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

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

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

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

Cairns, J. Jr., and A. Scheier. 1962. The effect of temperature and hardness of water upon the toxicity of naphthenic acids to the common bluegill (Lepomis macrochirus Raf.) and the pond snail (Physa heterostropha Say). Notulae Naturae. No. 353: 111 pp. Acad. Nat. Sci. Philadelphia.

Reliability: (3) Not reliable. The endpoint was cited in the text of a journal article without details of the test.

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

Value: 96-hour LC50 = 0.0026 mg/l

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.

Source: Exxon Corporation. 1980. Aquatic bioassay testing of Exxon Corporation's experimental compounds (MRD 78-100). Report by Battelle Columbus Laboratories, Columbus, Ohio.

5. Acute Toxicity

5.1.1 ACUTE ORAL TOXICITY

Type:	LD_{50}
Value:	5.88 (4.31-8.02) g/kg bw
Species:	Rat
Strain:	Wistar
Sex:	Male
Number of Animals:	5 per dose level (7 dose levels)
Vehicle:	None – administered undiluted
Year:	1979
GLP:	Unable to determine
Test Substance:	MRD-79-10 (Raw naphthenic acid derived from kerosene)
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 LD_{50} was determined to be 5.88 (4.31-8.02) g/kg bw
Reliability:	(1) Reliable without 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.

Source	Exxon, Acute Oral Toxicity of MRD-79-10 in Rats, MB 79-3702, 1979.
Type:	LD_{50}
Value:	3.0 g/kg bw (fraction from crude kerosene acids) 5.2 g/kg bw (fraction from mixed crude oils)
Species:	Rat
Strain:	No information
Sex:	No information available
Number of Animals:	"Sufficient animals …so the the LD50 dose could be computed by either the Weil or the Litchfield and Wilcoxon method"
Vehicle:	None – administered undiluted
Year:	1955
GLP:	Unable to determine
Test Substance:	1) 7-93% Naphthenic acid fraction from crude kerosene acids 2) 65-69% Naphthenic acid fraction from mixed crude oils
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.
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 LD_{50}s 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)
Reliability:	(2) Reliable 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.
Source	Rockhold, W.T. 1955. The toxicity of naphthenic acids and their metal salts. Archs Ind Hlth 12, 477-482.

Type:	LD_{50}
Value:	3550 mg/kg bw
Species:	Mice
Strain:	White – no other information
Sex:	Male
Number of Animals:	No information available
Vehicle:	No information available
Year:	1977
GLP:	Unlikely
Test Substance:	Naphthenic Acid – no further description
Method	Not described
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.
Source	Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181.

Type:	Acute Oral Toxicity Study (Not LD50)
Value:	Not applicable
Species:	Rat
Strain:	Wistar
Sex:	Male/Females
Number of Animals:	10 Females/dose (3 doses, plus control) 10 Males/dose (1 dose, plus control)
Vehicle:	Aqueous solutions of naphthenic acids/Water
Year:	2002

GLP:	Unable to determine
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.
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 $p \leq 0.05$ 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.
Reliability:	(2) Reliable with restriction. 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.
Source	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.

5.1.2 ACUTE DERMAL TOXICITY (WITH IRRITATION)

Type:	LD_{50}
Value:	> 3.16 g/kg bw
Species:	Rabbit

Strain:	NZ White
Sex:	Male/Female
Number of Animals:	2 per sex
Vehicle:	None – administered undiluted
Year:	1979
GLP:	Unable to determine
Test Substance:	MRD-79-10 (Raw naphthenic acid derived from kerosene)
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 LD_{50} 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) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.

Source Exxon, Acute Dermal Toxicity of MRD-79-10 in Rabbits, MB 79-3702, 1979.

5.2.1 EYE IRRITATION

Type: EYE IRRITATION

Species: Rabbit

Strain: NZ White

Sex: Male/Female

Number of Animals: 3 per sex

Concentration :	None – administered undiluted
Year:	1979
GLP:	Unable to determine
Test Substance:	MRD-79-10 (Raw naphthenic acid derived from kerosene)
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. abraded sites. 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) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.
Source	Exxon, Eye Irritation Study of MRD-79-10 in Rats, MB 79-3702, 1979.

5.4 REPEATED DOSE TOXICITY

Type:	Subchronic (90 Day)
Species:	Rat
Sex:	Females
Strain:	Wistar
Route of administration:	Oral

Exposure period: 90 days

Frequency of treatment: 1 dose/day (Mon. – Fri, 5 days/week)

Doses/No. of animals: 0.6, 6 or 60 mg/kg bw (aqueous solutions of naphthenic acids); 12 animals per dose level

Control group: Water – 7.0 ml tap water

Year: 2002

GLP: Unable to determine

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 sands tailings.

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 $p \leq 0.05$ 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.
Reliability	(2) Reliable with restriction. 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.
Source:	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.
Type:	Subchronic (30 Day)
Species:	Mice
Sex:	Male
Strain:	Wistar
Route of administration:	Oral
Exposure period:	30days
Frequency of treatment:	Daily
Doses/No. of animals:	1000 mg/kg bw (no information on number of animals per dose)
Control group:	No information available
Year:	1977
GLP:	Unlikely
Test Substance:	Naphthenic acid – no further information.
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.

Source:

Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181. [meeting abstract]

5.5 GENETIC TOXICITY IN VITRO

The following salts of naphthenic acid were tested using National Toxicology Program protocols and conducted in accordance with GLP's. Consequently they have ratings of (1), reliable without restriction:

	Calcium Naphthenate	Sodium Naphthenate
Salmonella Mutagenicity Test	Negative	Negative
Chromosome Aberration Test	---	Negative
Sister Chromatid Exchange Test	---	Positive

Source: NTP. 2003. http://ntp-server.niehs.nih.gov/htdocs/Overviews/GenProtocolsPg.html.

5.6 GENETIC TOXICITY IN VIVO

No data available.

5.7 CARCINOGENICITY

Species:	Mice
Sex:	Female
Strain:	No information available
Route of administration:	Dermal
Exposure period:	2 yr
Frequency of treatment:	2 times/day
Doses/No. of animals:	0.05 ml neat - 50 animals
Control group:	No information available
Year:	1987
GLP:	Unknown
Test Substance:	Calcium naphthenate
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.
Source:	U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). http://www.epa.gov/opptintr/chemtest/naphthst.htm.

5.8 EFFECTS ON REPRODUCTION

Type:	One Generation Reproduction
Species:	Rabbit
Sex:	Male (10)/Female (2)
Strain:	No information available
Route of administration:	Dermal
Frequency of treatment:	6 hr/day, 5 d/wk, 10 weeks
Doses/No. of animals:	2 ml (neat) – 10 male (2 female animals not treated)
Control group:	No information available
Method:	10 week exposure of males prior to mating
Year:	1984
GLP:	Unknown
Test substance:	Calcium naphthenate
Method:	Not described; listed in summary as "non-TSCA Protocol/Guideline (voluntary test)"
Result:	The following statements appeared in the available summary: 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:	(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.
Source:	U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). http://www.epa.gov/opptintr/chemtest/naphthst.htm.

5.9 DEVELOPMENTAL TOXICITY

Species:	Rat
Sex:	Female
Strain:	Wistar
Route of administration:	Oral
Dose:	0.6, 6 or 60 mg/kg bw
Exposure period:	"Pre-breeding, breeding and gestation" - no other details provided
Frequency of treatment:	Daily
Year:	2002
GLP:	Unknown
Test Substance:	Naphthenic acid isolated from Athabasca oil sands tailings.
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. 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.
Source:	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002b. Mammalian toxicity of naphthenic acids derived from the Athabasca Oil Sands (AOS). Toxicologist 66(1-S): 64-5. [meeting abstract]