Review of Health Effects of Naphthenic Acids: Data Gaps and Implications for Understanding Human Health Risk

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Oil Sands Research and Information Network

OSRIN is a university-based, independent organization that compiles, interprets and analyses available knowledge about returning landscapes and water impacted by oil sands mining to a natural state and gets that knowledge into the hands of those who can use it to drive breakthrough improvements in reclamation regulations and practices. OSRIN is a project of the University of Alberta's School of Energy and the Environment (SEE). OSRIN was launched with a start-up grant of $4.5 million from Alberta Environment and a $250,000 grant from the Canada School of Energy and Environment Ltd.

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REPORT SUMMARY

Oil sands mining involves removal of water from the Athabasca River basin in northeastern Alberta. Water produced during the extraction of bitumen from oil sands is referred to as oil sands process water (OSPW). Information on the likelihood of human exposure to OSPW-derived naphthenic acids and toxicological (dose-response) data are needed to have a complete understanding of the human health risk of these compounds. A review of literature was undertaken as a first step in framing potential human health risk associated with exposure to OSPW-derived naphthenic acids in surface water. Specifically, this review focused on chemical characteristics of, and potential toxicological effects related to, OSPW-derived naphthenic acids.

General Chemical Characteristics of Naphthenic Acid Mixtures in OSPW

There are several important findings of the review with regard to chemical characteristics of naphthenic acid mixtures in oil sand process waters:

- OSPW represents a complex mixture of naphthenic acids along with other organic chemicals that can also contribute to potential toxicity of the mixture.
- There is a difference in the distribution of organic compounds and their contribution to potential toxicity of OSPW that is fresh (i.e., OSPW recently produced from the oil sands extraction process) versus OSPW that is allowed to age (i.e., OSPW that has been aged for a number of years in inactive storage ponds or pit lakes). Aged OSPW contains higher molecular weight, multi-ring naphthenic acids that have been shown to be more resistant to microbial degradation and less potent in toxicity to biological organisms.
- An understanding of the forms and composition of OSPW-derived naphthenic acids and other organic compounds present in fresh and aged OSPW, and the effect of aging and aging environment on this composition, and variation in OSPW composition across oil sands processes is incomplete.

Human Exposure Evidence

OSPW-derived naphthenic acids are not used by the human population and the potential for human exposure in the oil sands region will arise from their presence in surface water or from potential future release of reclaimed OSPW to surface water. Based on the information reviewed, it was found that:

- Direct contact activities with surface water (e.g., ingestion and skin contact) represent a plausible way in which human exposure may occur to OSPW-derived naphthenic acids.
- Low water-to-air transfer properties and dilute concentrations of aged and reclaimed OSPW-derived naphthenic acids provide no meaningful scientific evidence to support the inhalation pathway as being important for potential human exposure.

- Low octanol water partition values and apparent rapid depuration of aged OSPW-derived naphthenic acids offer no meaningful scientific evidence to support the fish ingestion pathway as being important for potential human exposure to these compounds.

Toxicological Evidence

Toxicity information of interest for understanding human health risk from chemicals in the environment includes: acute toxicity, subchronic/chronic adverse responses (e.g., weight loss, immunosuppression, etc.), neurotoxicity, developmental and reproductive toxicity, and genetic toxicity (mutagenicity and carcinogenicity).

A general finding of this review is:

- Toxicological evidence observed for commercial naphthenic acids derived from crude oils and/or commercial naphthenic acid salts will not be representative of naphthenic acids in aged and reclaimed OSPW. Higher molecular weight, multi-ring naphthenic acids, which are more resistant to microbial degradation and less potent in toxicity to biological organisms, are the forms reported to be present in aged and reclaimed OSPW.
- OSPW-derived naphthenic acids come from bitumen which is considered to be extensively biodegraded petroleum, whereas commercial naphthenic acids are typically prepared from petroleum sources that have not undergone extensive biodegradation. Therefore, potential human toxicity and corresponding human exposure limits for OSPW-derived naphthenic acids should not be inferred from studies of commercial naphthenic acids.

Acute Toxicity

Naphthenic acids found within crude oils exhibit similar oral toxicity to table salt. Acute toxicity testing in rats revealed behavioral and histopathological effects from a single administration of OSPW-derived naphthenic acids, but at a dosage 50 times a worst case environmental exposure for small mammalian wildlife. This dosage is a not realistic exposure condition that would apply to humans in the oil sands region.

Subchronic/Chronic Noncarcinogenic Toxicity

A finding of this review is:

- Based upon limited information reviewed, uncertainty remains in the understanding of toxicokinetic (fate in the body) and toxicodynamic (mode of action and dose-response) information needed to infer noncarcinogenic human exposure-related responses to naphthenic acids and other acid-extractable organics present in aged and reclaimed OSPW.

A recommendation of this review is:

- There is a need to further examine potential subchronic/chronic toxicity of naphthenic acids and other acid-extractable organics present in aged and reclaimed OSPW.

Developmental and Reproductive Toxicity

A finding of this review is:

- Based upon limited information reviewed, uncertainty remains about knowledge of developmental and reproductive toxicity of naphthenic acids and other acid-extractable organics present in aged and reclaimed OSPW.

A recommendation of this review is:

- There is a need to further examine developmental and reproductive toxicity endpoints of naphthenic acids and other acid-extractable organics present in aged and reclaimed OSPW using *in vitro*/*in vivo* bioassay testing focusing on cellular response pathways.

Genetic Toxicity

A finding of this review is:

- Based upon limited information reviewed, uncertainty remains about knowledge of genetic toxicity of naphthenic acids and other acid-extractable organics present in aged and reclaimed OSPW.

A recommendation of this review is:

- There is a need to further examine genetic toxicity endpoints (including carcinogenic endpoints) of naphthenic acids and other acid-extractable organics present in aged and reclaimed OSPW using *in vitro* genetic (micronucleus) testing and/or other suitable tests focusing on cellular response pathways.

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OSRIN is grateful to Alberta Environment and Water and Environment Canada for their review of the report drafts. *The authors carefully reviewed and considered the comments and incorporated them wherever possible into the final report. There were instances, however, where the authors disagreed with the comments and therefore did not incorporate them.*

DISCLAIMER

This report focuses on chemical characteristics of, and potential toxicological effects related to, OSPW-derived naphthenic acids. The emphasis is on presentation of current toxicological evidence and human health risk that may be posed by exposure to OSPW-derived naphthenic acids, primarily in the reclaimed landscape setting.

- This report does not focus on the physical characteristics of naphthenic acids.
- This report does not address other organic chemicals (e.g., PAH's, phenols, sulphur heterocylic compounds, etc.) that may be present in oil sands process-affected water.
- This report does not address inorganic chemicals (e.g., metals, salts, etc.) that may be present in oil sands process-affected water.
- This report does not address environmental or ecological impacts of naphthenic acids or other organic chemicals that may be present in oil sands process-affected water.

Readers interested in these broader subjects are encouraged to read the extensive literature available.

1 INTRODUCTION

Toxicity is a property of the dose of a chemical. All chemicals are potentially toxic to biological organisms depending upon their physical/chemical properties, and the amount and frequency of exposure (i.e., the dose). Small doses, versus large doses of a chemical, have two very different meanings in terms of whether or not it is actually toxic to biological organisms. A recent review of oil sands water toxicity by Natural Resources Canada (2010) concluded that there is currently no clearly demonstrated connection with oil sands development projects and toxic effects in off-site downstream surface waters.

Oil sands mining involves removal of water from the Athabasca River basin in northeastern Alberta. Water produced from the extraction of bitumen from oil sands is referred to as oil sands process water (OSPW)[1]. Naphthenic acids and other organic compounds are dissolved and concentrated in OSPW (Grewer et al. 2010). Aged OPSW refers to process water that has been stored for more than 3 to 5 years after oil sands tailings deposition stops. Reclamation of OSPW could be accomplished through multi-barrier treatment approaches that can comprise both natural and engineered treatment systems.

Naphthenic acids (NAs) are also known to be produced during in-reservoir (i.e., in situ) biodegradation of petroleum hydrocarbons (Meredith et al. 2000) and they are found everywhere in petroleum deposits (Brient et al. 1995, Clemente and Fedorak 2005). Tissot and Welte (1984) stated that NAs in the Athabasca oil sands region were produced by natural biodegradation of mature petroleum. Thus, these compounds can enter surface waters through natural discharge of groundwater and erosion of riverbank oil deposits (Headley and McMartin 2004). NAs and other organic chemicals concentrated in OSPW can be introduced to surface water in northeastern Alberta through seepage of oil sands tailings ponds into groundwater. These compounds can also make their way into surface waters through potential future release of reclaimed oil sands pit lake water.

MacKinnon and Boerger (1986) identified NAs as components of OSPW that can pose potential toxicity to aquatic organisms. Many of the possible surface water exposure pathways to OSPW-derived NA that have the potential to compromise human health are the same as, or shared with, those that can compromise aquatic ecosystem health[2].

Two approaches are generally used to assess the potential of chemicals of concern in surface water to compromise human health:

- Undertake human health risk assessment of chemicals of concern that do not have established water quality guidelines or limits.

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[1] Also commonly referred to as process-affected water (PAW).

[2] This report focuses on studies of animal toxicity that are used to derive human health endpoints (e.g., rats). There are numerous studies of toxicity to fish and other environmental bioindicators that are not included here as they are not relevant to human health. See, for example, Cruz-Martinez, L. and J.E.G. Smits, 2012. *Potential to Use Animals as Monitors of Ecosystem Health in the Oil Sands Region*. OSRIN Report No. TR-18. 52 pp.

- Direct comparison of chemicals of concern to established water quality guidelines or limits (or existing and/or background water quality concentrations).

Human health risk assessment is done by making a comparison of measured or estimated (model predictions) environmental contaminant exposure concentrations to toxicological response values identified from animal bioassay testing. These comparisons involve use of toxicological (dose-response) data from surrogate species – such as laboratory mice or rats – for assessing risk in humans.

However, traditional animal bioassay tests have inherent uncertainties associated with (U.S. EPA 2009a): (1) human relevance of laboratory animal studies (species extrapolation); (2) use of high doses in animals to estimate human responses associated with much lower environmental exposures (dose extrapolation); and (3) predicting effects in susceptible populations. Thus, implicit in these approaches are a number of conservative steps that are taken to ensure that the human population is protected from almost all eventualities (Ritter et al. 2002).

Water quality guidelines include, for example, Alberta Environment and Water surface water quality guidelines (Alberta Environment 1999) or guidelines for Canadian drinking water quality (Health Canada 2010). Where these types of guidelines apply to human health, they are usually developed using human health risk assessment approaches. For chemicals of concern, direct comparisons are normally made of a measured value or model prediction of a surface water concentration to a water quality guideline that is protective of human health.

However, a water quality guideline does not currently exist for NA. Another approach that is used is to compare an estimate or model prediction of NA concentration in surface water to existing and/or modeled future background concentrations in the surface water. The notion is that, if environmental contaminant releases from a development project do not contribute to an increase in the existing and/or modeled future background concentrations in surface water, the incremental risk to human health from these releases will be small and unimportant. As will be shown, this is the health risk assessment approach used for previously approved oil sands mine development projects in the Athabasca oil sands region.

Adequate information on likelihood of human exposure to OSPW-derived NAs and toxicological (dose-response) data are needed to have a complete understanding of human health risk of these compounds. A review of literature was undertaken as a first step in framing potential human health risk associated with exposure to OSPW-derived NAs in surface water. Specifically, this review focused on characteristics of and potential toxicological effects related to OSPW-derived NAs.

The remainder of this report includes the following sections:

- Methods used for the literature review (described here).
- A description of naphthenic acids (Section 2).
- A recent example of how OSPW-derived NAs were evaluated with respect to human health risk in environmental impact assessment of oil sands projects (Section 3).

- A discussion of the potential for human exposure to OSPW-derived NAs (Section 4).
- Current toxicological evidence for OSPW-derived NAs that is relevant for understanding human health risk (Section 5).
- Summary and recommendations (Section 6).

1.1 Methods

The literature review was performed using the following approach:

- Electronic on-line databases at the University of Alberta Science and Technology Library (1960 to 2010) were searched. These included – Toxicology Abstracts, MEDLINE and TOXNET.
- The U.S. Environmental Protection Agency Office of Research and Development, National Center for Environmental Assessment (NCEA, Washington, DC)[3] was contacted and interviewed to inquire about toxicity investigations undertaken and documented toxicological information on naphthenic acids or naphthenic acid mixtures. NCEA is responsible for provision of guidance and risk assessments to the U.S. Environmental Protection Agency aimed at protecting human health and the environment.
- The National Toxicology Program[4], National Institute of Environmental Health Sciences, U.S. Department of Health and Human Services was contacted and interviewed to inquire about toxicological investigations undertaken with naphthenic acids or naphthenic acid mixtures. [Note: Study investigators were referred to the U.S. Environmental Protection Agency National Center for Environmental Assessment.]

2 DESCRIPTION OF NAPHTHENIC ACIDS

Oil sands mining is carried out in northeastern Alberta either by removing sand which contains bitumen using open pit mining methods, or by processing the sand while still in place using *in situ* methods. In general, separating the mined bitumen from the sand requires a number of steps (InfoMine Inc. 2011):

- The sand is mixed with water, large clumps are broken down and coarse material is removed.
- The resultant slurry is fed into a separation vessel where the sand settles to the bottom. A mixture of sand, water and bitumen remains suspended in the middle and impure bitumen froth floats to the top and is removed for further processing.

[3] See http://www.epa.gov/aboutepa/ncea.html

[4] See http://ntp.niehs.nih.gov/

- The sand at the bottom of the tank is pumped into tailings ponds and the mixture suspended in the middle goes through a secondary separation process where an additional 2% to 4% of the bitumen is removed as froth.
- The froth is mixed with a diluent such as naphtha to decrease its viscosity. Water and solids remaining in the bitumen froth are removed using centrifuges and settling units and the bitumen is sent for further processing to be converted to synthetic crude oil.

NAs are concentrated in the process waters (i.e., OSPW) during these steps. OSPW-derived NAs are non-volatile, chemically stable, and act as surfactants. A surfactant is a chemical that is capable of stabilizing mixtures of oil and water by reducing the surface tension at the interface between the oil and water molecules.

OSPW-derived NAs comprise a complex blend of saturated cyclic and noncyclic carboxylic acids having the general condensed chemical formula $C_nH_{2n+Z}O_2$ (Han et al. 2008). In the formula "n" indicates the carbon number and "Z" is a negative even integer related to the number of rings in the molecule (e.g.: Z = 0, no rings; Z = -2, 1 ring; Z = -4, 2 rings; etc.). A generic structure for OSPW-derived NA is shown in Figure 1. Molecular weights of different Z series and n families of OSPW-derived NA are shown in Table 1 (after McMartin 2003).

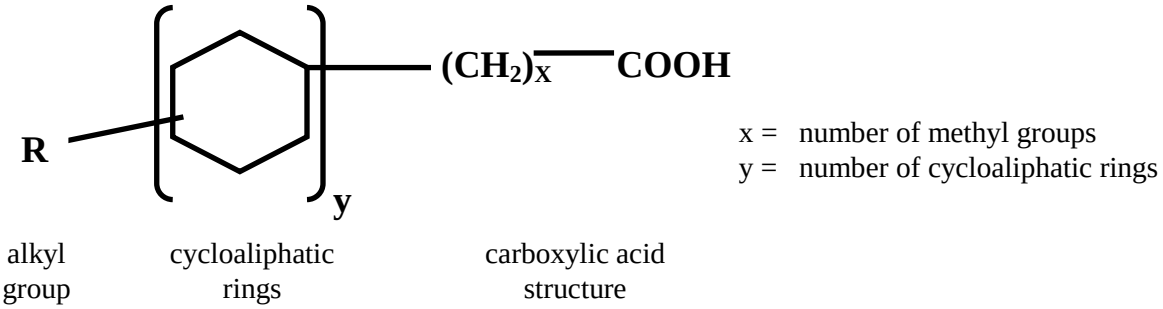

Figure 1. Generic structure for OSPW-derived naphthenic acids.

Table 1. Molecular weights (M.W.) of different Z series and n families of OSPW-derived naphthenic acids (after McMartin 2003).

Number of carbon atoms	M.W. z = 0 (open chain)	M.W. z = -2 (1 ring)	M.W. z = -4 (2 rings)	M.W. z = -6 (3 rings)
10	172	170	168	166
11	186	184	182	180
12	200	198	196	194
13	214	212	210	208
14	228	226	224	222
15	242	240	238	236

Number of carbon atoms	M.W. z = 0 (open chain)	M.W. z = -2 (1 ring)	M.W. z = -4 (2 rings)	M.W. z = -6 (3 rings)
16	256	254	252	250
17	270	268	266	264
18	284	282	280	278
19	298	296	294	292
20	312	310	308	306

OSPW-derived NAs have molecular weights that range from 140 to 450 and acid dissociation constants that range between 10^{-6} and 10^{-5} (Brient et al. 1995), and they are natural components of petroleum (Clemente and Fedorak 2005). As a group, OSPW-derived NAs have physical and chemical characteristics that can be used to describe the overall mixture as shown in Table 2.

Table 2. Physical and chemical properties of naphthenic acids.
After Brient et al. 1995, CEATAG 1998, Headley et al. 2002, Herman et al 1993, McMartin 2003, Schramm et al. 2000**.**

Parameter	General characteristic
molecular weight	140 to 450
solubility	>50 mg/L in water
acid dissociation constant	10^{-5} to 10^{-6}
boiling point	250 to 350ºC
octanol-water partition coefficient	250 (pH 7) to 130 (pH 10), commercial (non-metallic), derived from crude oils
	250 (pH 7) to 3 (pH 8.5), oil sands derived
odour	primarily impacted by presence of phenol and sulphur compounds, musty hydrocarbon

Figure 2 is a mass spectra showing the distribution of organic compounds in extracts from commercial NAs and Athabasca oil sands NAs (after Headley et al. 2010). Mass spectrometry is a technique for separating and identifying molecules based on mass. Mass spectra shown in Figure 2 display intensity versus mass-to-charge ratio (m/z) plots of mass spectrometry analysis. The spectrum of a sample is a pattern representing the distribution of ions by mass (i.e., mass-to-charge ratio) in the sample. The mass-to-charge ratio (m/z) on the x-axis is the relationship between the mass of a given ion and the number of elementary charges that it carries. The intensity on the y-axis is the intensity of the ion current measured for distribution of ions by the spectrometer. Figure 2 clearly shows that a sample of Athabasca oil sands (bottom graphic)

contains a much wider distribution of extractable organic molecules compared to commercial NA (top graphic).

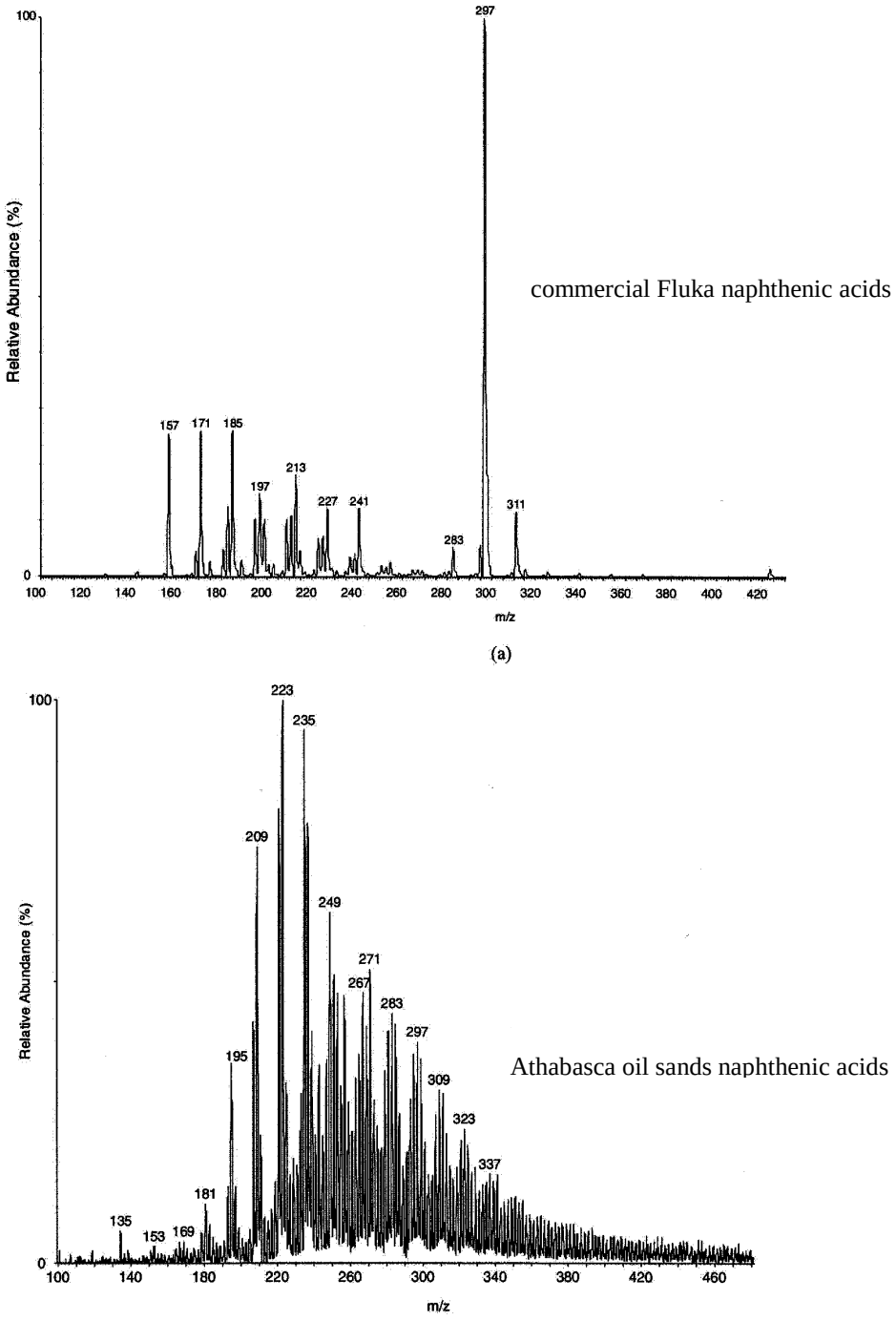

Figure 2. ESI low resolution mass spectra of commercial Fluka naphthenic acids and Athabasca oil sands naphthenic acids (after Headley et al. 2010).

Environmental and regulatory attention has focused on the NA fraction of OSPW due to its reported persistence in the environment and aquatic toxicity at levels found in certain oil sands extraction tailings pond waters (Grewer et al. 2010). As shown in Figure 2, there are literally hundreds of these and other extractable organic chemicals found as mixtures in oil sands materials ranging from trace to measureable amounts. It is unknown which specific components, or combinations thereof, are the most potent in terms of their contribution to reported OSPW toxicity (Natural Resources Canada 2010). Brient et al. (1995) stated that the potency of these mixtures does not necessarily correlate directly to NA concentration, but is reported to be more a function of content and complexity of numerous extractable organic chemicals present in the mixture.

Previous published studies – for example, Clemente et al. 2004, Han et al. 2008 and Scott et al. 2008 – have focused on characterization of OSPW-derived NA content because of its reported toxicity. However, more recently Grewer et al. (2010) and Headley et al. (2009) have shown that OSPW-derived NAs may account for less than 50% of all organic chemicals in OSPW.

Grewer et al. (2010) stated that a wide array of chemicals present in the acid-extractable organic fraction of OSPW have not been studied in detail (e.g., sulphur containing compounds). Based on their findings, Grewer et al. (2010) and Natural Resources Canada (2010) stated that it appears that the term "naphthenic acids" – which has traditionally been used to describe potentially toxic extractable compounds in OSPW – should be replaced by a term such as "oil sands tailings water acid extractable organics." Traditional "naphthenic acids compounds" (e.g., Figure 1) may only represent some of the components of potentially toxic acid-extractable compounds in OSPW.

OSPW from different oil sands sources have different NA composition (i.e., NA with different carbon numbers and Z values) (Armstrong et al. 2008, Clemente et al. 2003, Grewer et al. 2010), and variable biodegradation properties (Armstrong et al. 2008, Han et al. 2008, Scott et al. 2005). Grewer et al. (2010) and Natural Resources Canada (2010) state that identification of other OSPW organic chemicals and their potential effects, and evaluation of effects of NA preparations with different compositions, constitute an analytical and experimental challenge. Natural Resources Canada (2010) concludes that it is fairly certain that all of the potential biological toxic components of OSPW have not yet been identified.

There are several important points to conclude this discussion of NA characteristics in OSPW:

- In addition to NA, OSPW also contains a variety of organic chemicals that may contribute to its potential biological toxicity.
- Uncertainty exists in the understanding of potential biological toxicity of OSPW due to the lack of characterization of other chemicals in the OSPW organic fraction.
- "OSPW-derived NA" may only represent some of the components of potentially toxic extractable compounds in OSPW.
- OSPW from different oil sands sources have different NA compositions.

For the remainder of this report the reader needs to keep in mind that when reference is made to "OSPW-derived NA", ***the acid-extractable organic fraction of OSPW is what is meant*** *– i.e., an organic fraction of OSPW that contains unidentified compounds in addition to NA.*

3 NAPHTHENIC ACIDS IN HUMAN HEALTH RISK ASSESSMENT

An example of an approach for how OSPW-derived NAs are characterized in human health risk assessment is discussed based on a recently approved (January 2011) mineable oil sands development project in Alberta. On January 27, 2011, the Environment Canada and Energy Resources Conservation Board Joint Review Panel approved the TOTAL E&P Joslyn North Mine Project in northern Alberta (Joint Review Panel 2011). The project will be located about 70 kilometres north of Fort McMurray. It will consist of an oil sands surface mine and ore preparation and bitumen extraction facility. It is designed to produce about 16,000 m^3 per day (100,000 barrels per day) of liquid hydrocarbon (Joint Review Panel 2011). The project also includes tailings management facilities (i.e., tailings ponds) and other infrastructure.

As stated previously, a water quality guideline that is protective of human health does not exist for naphthenic acids. The health risk assessment approach used for OSPW-derived NAs for the Joslyn North Mine Project involved comparing model predictions of OSPW-derived NA concentrations in surface water to existing and modeled future surface water background concentrations. The hypothesis tested in the assessment was that, if environmental releases of OSPW-derived NA from the Joslyn North Mine Project only contribute to small increases in existing or future cumulative NA concentrations in surface water, the incremental risk to human health from Joslyn North Mine Project releases will be *de minimus* (i.e., of no significance or not worthy of consideration).

An original assessment (DCEL 2006) and revised assessment (Total 2010) were prepared for the Joslyn North Mine Project. These assessments considered potential OSPW-derived NA releases from the Joslyn North Mine Project to surface waters for three environmental impact assessment cases: (1) a baseline case; (2) an application case; and (3) a planned development case:

- The *baseline case* modeled conditions for potential OSPW-derived NA releases to surface waters from existing and approved development activities, both oil sands developments and other resource development activities. This represented a cumulative effects assessment of baseline conditions.
- The *application case* modeled conditions for potential OSPW-derived NA releases to surface waters from the Joslyn North Mine Project in addition to the baseline case. This represented an assessment of the cumulative baseline conditions along with the Joslyn North Mine Project.
- The *planned development case* allowed for a cumulative effects assessment whereby residual impacts of reasonably foreseeable proposed projects (i.e., other proposed oil sands developments and other resource developments) were added to those considered in the application case. Modeled conditions for potential OSPW-derived

NA releases to surface waters were assessed for reasonably foreseeable proposed projects in addition to the application case.

Several water quality models were used to predict OSPW-derived NA concentrations in surface waters (i.e., watercourses, water bodies, and pit lakes). These models included:

- Small Streams Model –A surface water quality model referred to as the Hydrological Simulation Program Fortran model was used for continuous simulation of OSPW-derived plus natural NA water quality in Joslyn Creek and the Ells River within the local study area. The Hydrological Simulation Program Fortran model is a dynamic modeling system developed by U.S. Environmental Protection Agency for simulation of watershed hydrology, point and nonpoint constituent loading, receiving water quality, and temperature. This model and modeling approach was consistent with approaches used in previously approved oil sands mine projects in the Athabasca oil sands region.
- Pit Lake Model – OSPW-derived plus natural NA water quality in a pit lake was modeled using a flow and mass-balance Pit Lake Model. Again, the model and modeling approach were consistent with previously approved oil sands mine projects in the Athabasca oil sands region.
- Athabasca River Model – A description of the Athabasca River Model – including derivation, formulation, and setup – is provided in Shell (2007). Two locations were modeled in the Athabasca River: downstream of Ells River and upstream of Embarras River. Again, the model and modeling approach were consistent with previously approved oil sands mine projects in the Athabasca oil sands region.

OSPW-derived plus natural NAs in surface waters were modeled in the following ways:

- As two distinct mixtures – a labile mixture (i.e., organic compounds susceptible to physical, chemical, and biological decomposition) and a refractory mixture (i.e., organic compounds resistant to physical, chemical, biological decomposition).
- As a total (labile + refractory) mixture.

The labile mixture is thought to be more potentially toxic to biological organisms; however it often only represents a small fraction of total NA forms. Changes to NA surface water concentrations were quantified for a pre-industrial reference condition and several different times (in 2013, 2036, 2044 and in the far-future) associated with each major phase of the Joslyn North Mine Project.

Small streams modeling results – Joslyn Creek:

- Baseline case – There were no existing or approved developments affecting water quality in the Joslyn Creek watershed and therefore, the baseline case was represented by pre-industrial reference conditions.
- Application case –Median and peak concentrations of refractory NAs in Joslyn Creek in 2013 were predicted to increase relative to baseline case concentrations.

Refractory NAs were predicted to increase in 2013 beyond 10% of baseline case peak concentrations. In 2036 and into the far-future, NA concentrations were predicted to be within baseline case levels.

- Planned development case – A planned development case was not assessed because there were no other planned developments in the watershed.

Small streams modeling results – Lower Ells River:

- Baseline case – The baseline case conditions are the same as pre-industrial conditions because there were no existing or approved developments affecting water quality in the Ells River watershed.
- Application case – In 2013, median values of refractory NA concentrations were predicted to increase due to muskeg drainage and overburden dewatering. Total NAs were predicted to increase only slightly compared to the baseline case concentrations.
- Changes to the watershed in 2036 were not predicted to result in appreciable changes to NA concentrations in the Ells River.
- In 2044 and in the far-future, release from the pit lake was predicted to increase concentrations of labile and refractory NAs relative to baseline case concentrations. Median concentrations of total NAs were predicted to be slightly higher in 2044 and in the far-future compared to baseline case concentrations. The highest concentration of total NAs was for the 2044 snapshot, but both the median and peak concentrations were predicted to decrease in the far-future. Labile NAs were predicted to be present but only as a small fraction of the total NAs.
- Planned development case – A planned development case was not assessed because there are no other planned developments in the watershed.

Pit lake modeling results:

- Maximum concentrations of labile and refractory NAs in the pit lake were predicted to be 0.71 and 9.7 mg/L, respectively during the 2044 snapshot, and to decline over time. Labile NAs represented a small fraction of the total NA concentrations (i.e., <7%).

Athabasca River Modeling Results:

- For the application case, worst-case (or maximum) modeled NA concentration for each development case and time period (i.e., in 2013, 2036, 2044 and in the far-future) at the two locations are shown in Table 3 (after Total 2010). Downstream of Ells River, median concentrations of refractory and total NAs were predicted to exceed baseline case concentrations in 2044 and the far-future from the pit lake release.

- Median concentrations of NAs were predicted to remain low during all snapshots at Ells River, whereas peak concentrations of NAs were predicted to increase in the 2044 snapshot and decrease in the far-future. These increases were indicated to be due to the less potentially toxic, refractory form of NA. Finally, planned development case concentrations of NA downstream of Ells River and at Embarras were predicted to be generally the same as those reported for the application case.

Table 3. Modeled naphthenic acid concentrations in Athabasca River for the Joslyn North Mine Project (after Total 2010).

Naphthenic acid concentrations in Athabasca River downstream of Ells River:

		Pre-industrial condition		Baseline case		Application case		Planned development case	
Naphthenic acids	Unit	median	peak	median	peak	median	peak	median	peak
Labile[1]	mg/L	0	0	<0.01	0.04	0.01	0.13	0.01	0.13
Refractory	mg/L	0.11	0.62	0.26	0.81	0.32	2.5	0.32	2.5
Total	mg/L	0.11	0.62	0.26	0.85	0.33	2.6	0.33	2.6

Naphthenic acid concentrations in Athabasca River at Embarras:

		Pre-industrial condition		Baseline case		Application case		Planned development case	
Naphthenic acids	Unit	median	peak	median	peak	median	peak	median	peak
Labile[1]	mg/L	0	0	<0.01	0.02	<0.01	0.03	<0.01	0.03
Refractory	mg/L	0.13	0.62	0.24	0.79	0.24	0.8	0.25	0.81
Total	mg/L	0.13	0.62	0.24	0.81	0.25	0.83	0.25	0.84

[1] Naphthenic acid form treated as being more potentially toxic to biological organisms.

4 POTENTIAL FOR HUMAN EXPOSURE TO NAPHTHENIC ACIDS

The National Research Council Committee on Toxicity Testing and Assessment of Environmental Agents (NRC 2006, 2007) reviewed established and emerging toxicity testing methods and strategies for chemicals. In developing strategies for toxicity testing of chemicals, one of the outcomes of their review was a recommendation for having a thorough understanding of the likelihood of human exposure to the chemicals.

OSPW-derived NAs are not used by the human population and the potential for human exposure in the oil sands region will arise from their presence in surface waters. Because of the

importance of understanding likelihood of human exposure to better characterize human health risk to OSPW-derived NAs, this issue was examined further.

Gaining an adequate understanding of human exposure to chemicals of concern in surface water requires consideration of release, behavior and fate characteristics. Following release to the environment, a chemical may move or partition into several different environmental media (e.g., the atmosphere, bottom sediment, suspended sediment, fish muscle tissue), where it may be subject to a numerous processes that act to modify its concentration and chemical characteristics. Mobility and extent to which a chemical undergoes transformation in the environment, and hence the pathways and degree to which humans may be exposed to it, depends in part on physical, chemical, and biological properties of the chemical.

4.1 Surface Water to Air Partitioning

For any organic chemical originally present in surface water to be of importance via the inhalation pathway, it must be present in substantial concentrations in the water (e.g., >0.1% by weight) and it must possess properties that enable it to readily partition (i.e., volatilize) from water to air. For illustration purposes, NA concentrations ranging from 1 to 10 mg/L only represent 0.0001% to 0.001 % by weight water concentrations – which are considered *very dilute* concentrations. Historical NA concentrations reported for surface waters of the Lower Athabasca River have been less than 3 mg/L (RAMP 1998, 2008) or <0.0003% by weight.

With respect to volatilization, Henry's Law Constant is an air-water partitioning property of an organic chemical indicating its potential to transfer between these media. It is strongly temperature-dependent for most environmental situations (Mackay et al. 2000). Han et al. (2009) estimated Henry's Law Constant for three model NA compounds similar to NA forms present in oil sands pit lakes in an effort to understand their water-to-air partitioning behavior. Estimated Henry's Law Constants for the three model NA compounds (condensed molecular formulas $C_{12}H_{24}O_2$, $C_{12}H_{22}O_2$, and $C_{12}H_{18}O_2$) were: 9.3×10^{-6}, 2.0×10^{-6} and 2.9×10^{-8} atm-m^3/mol, respectively.

These model NA compounds are compared against examples of environmentally significant volatile and semi-volatile organic compounds – benzene and anthracene, respectively. Benzene with a boiling point ~80°C is a volatile organic compound (i.e., boiling point less than 250 to 260°C). Anthracene is a polycyclic aromatic hydrocarbon (PAH) with a boiling point ~340°C and falls within a class of semi-volatile organic compounds (i.e., boiling point range ~250 up to 500°C).

The estimated Henry's Law Constant at for benzene is 2.7×10^{-3} to 3.0×10^{-3} atm-m^3/mol and for anthracene is 1.3×10^{-5} atm-m^3/mol at 10°C (U.S. EPA 2009b). The model NA compounds reported by Han et al. (2009) are 290 to 100,000 times less volatile than benzene. However, Henry's Law Constant for anthracene indicates that potential transfer of this compound from surface water to air occurs *only slowly* at a rate controlled by slow diffusion through air (Thomas 1982). Furthermore, model NA compounds reported by Han et al. (2009) are 1.4 to 450 times less volatile than anthracene.

Thus the expected conditions of OSPW-derived NAs (i.e., dilute concentrations and low surface water-to-air transfer properties) offer very little in the way of defensible scientific evidence to support the inhalation pathway as being important for potential human exposure.

4.2 Surface Water to Biological Tissue Partitioning (Food Chain Accumulation Potential)

For any organic chemical originally present in surface water to be of importance via a secondary non-inhalation pathway (i.e., consumption of fish), it must possess properties that enable it to readily transfer directly from water to fish tissue (i.e., bioconcentrate) or be readily taken up along the food chain of fish (i.e., bioaccumulate). A parameter referred to as the octanol-water partition coefficient, K_{ow}, is a key property in the study of environmental fate of organic chemicals as it is related to bioconcentration and bioaccumulation in fish and other aquatic organisms (Lyman 1982).

K_{ow} is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature. Octanol is an organic solvent that is used as a surrogate for aquatic biological tissue. Chemicals with low K_{ow} (i.e., <10) are considered to be hydrophilic (water-loving), have high solubility in water, and have a small ability to concentrate or accumulate in fish tissue (Lyman 1982). Environment Canada (1995) considers substances with K_{ow}'s ≥100,000 (log K_{ow} ≥5) to be bioaccumulative.

Schramm et al. (2000) reported K_{ow}'s for NA in oil sands process tailings to range from 30 down to 3 within the pH range of 7.5 to 8.4, and <10 above pH 8. This pH range (i.e., 7.5 to 8.4) is notable in that it is consistent with observations in various reaches of the Lower Athabasca River from 1976 to 1997 (RAMP 1998) and that observed more recently (RAMP 2008).

K_{ow}'s of two environmentally significant volatile and semi-volatile organic compounds – benzene and anthracene – are discussed for comparison purposes. U.S. EPA (1986) reports the K_{ow}'s for benzene and anthracene as 132 and 28,000, respectively. K_{ow}'s for both of these compounds are much greater than 10. In the case of anthracene it indicates an obvious potential for bioconcentration and bioaccumulation in fish tissue. In the case of benzene it indicates minor to insignificant potential for bioconcentration and bioaccumulation in fish tissue.

For NA in oil sands process tailings (K_{ow} <10 at pH's above 8), insignificant potential exists for bioconcentration and bioaccumulation in fish tissue. Young et al. (2008) measured uptake and depuration (purification) of commercial NA in laboratory experiments. Exposure of rainbow trout (*Oncorhynchus mykiss*) to 3 mg/L naphthenic acids for 9 days gave a bioconcentration factor (BCF) of ~2 at pH 8.2. About 95% of the NAs were depurated (i.e., cleared) within 24 hours after the fish were transferred to NA-free water.

Finally, Natural Resources Canada (2010) reviewed current evidence of fish tainting in the oil sands region and concluded that it is unlikely that NAs are the major fish tainting components in oil sands tailings waters. They stated that it is not known whether a single compound, a similar group of compounds, or a mixture of natural and introduced compounds might be causing fish tainting. Thus the expected conditions of OSPW-derived NA (i.e., very low K_{ow}) and apparent

rapid depuration offers little in the way of defensible scientific evidence to support the fish ingestion pathway as being important for potential human exposure for these compounds.

4.3 Biodegradation in Surface Water

The potential for biodegradation of an organic chemical in a microbiologically active environment – such as a river – is an important indicator of persistence. Persistence is one of three principal criteria used by governments to set regulatory priorities among constituents of concern in the environment; the other two being potential to bioaccumulate and toxicity (Webster et al. 1998). Environment Canada (1995) considers an environmental chemical to be persistent if it has a half-life ≥182 days in surface water. Half-life ($t_{½}$) is the time required for the concentration of an environmental chemical to decrease to half its original value.

Using established procedures for bioassay testing under laboratory conditions, Bataineh et al. (2006) provided evidence to indicate that oil sands tailings water is dominated by highly persistent, high molecular weight, alkyl-substituted NA isomers. This was based on bioassay testing of tailings water samples from the clarified zone of a storage pond at Syncrude Canada Ltd. (West In Pit Lake). The West In Pit Lake is a biologically active storage pond that receives OSPW and recycles clarified process water back to the facility for use in the extraction process. Bataineh et al. (2006) reported that aging of tailings water is associated with natural biodegradation of lower molecular weight NA compounds that contributes to some of the reported OSPW aquatic toxicity.

Lo et al. (2006) fractionated NA mixtures from oil sands tailings pond water for analysis by the Microtox® bioassay. They observed that NA fractions with a higher proportion of multi-ring structures (i.e., higher molecular weights) exhibited lower toxic potency in the Microtox® bioassay compared to commercial NA. Commercial NAs have lower molecular weight and are more biodegradable.

Han et al. (2008) reported that OSPW-derived NAs from the Syncrude Canada Ltd. West In Pit Lake are more resistant to biodegradation by microorganisms indigenous to this storage pond compared to commercial NA. Using established procedures for bioassay testing under laboratory conditions, Han et al. (2008) observed that $t_{½}$ ranged from 1 to 8 days for a commercial NA solution, whereas $t_{½}$ for OSPW-derived NAs from the West In Pit Lake ranged from 44 to 240 days.

Han et al. (2009) estimated $t_{½}$ for "aged" OSPW-derived NAs on the order of 13 years based on historical behavior of NA in OSPW storage ponds. This estimate was based on initial and current (2008) NA concentrations in two experimental reclamation ponds that were originally filled with OSPW back in 1993 and 1997. One pond was originally filled with OSPW from the Syncrude Canada Ltd. Mildred Lake Settling Basin in 1993 and the other pond was filled with OSPW released from mature fine tailings in 1997. This half-life is ~600 times greater than that observed for commercial NA under bioassay testing (i.e., 1 to 8 days), indicating that OSPW-derived NA compounds appear very resistant to biodegradation.

Headley et al. (2010) compared the combined sorption and biodegradation of Fluka commercial NA (Sigma-Aldrich, Oakville, ON) to OSPW-derived NAs (collected from an oil sands operation in 2005 at Fort McMurray, Alberta) by non-adapted lake biofilms cultivated in a bio-reactor. Fluka commercial NAs are different compared to Athabasca oil sands NAs in that lower molecular weight components are more prevalent. They reported $t_{½}$ values for two groups of Fluka commercial NAs of 7 and 143 days; however, no biodegradation was observed for OSPW-derived NAs. They concluded that the difference between the combined sorption and biodegradation of commercial NA and OSPW-derived NAs might have arisen from three aspects: (1) molecular structure; (2) mass; and (3) presence of sulphur and/or nitrogen inhibiting biodegradation of OSPW-derived NAs by the lake biofilm.

In view of the above discussion, expected conditions of NAs associated with aged OSPW (i.e., relatively strong resistance to biodegradation) indicate that they will be persistent in an aquatic environment.

4.4 Fate in Groundwater

To date oil sands tailings and process affected water (OSPW) have been maintained in active and abandoned open-pit mines or in above grade tailings impoundments, which are constructed using sand-based dykes. Seepage plumes which may form adjacent to tailings impoundments are thought to originate from drainage of the fluid phase of these tailings used in construction of the dykes (Ferguson et al. 2009) and from the bottom of some impoundments. In these settings, OSPW-derived NAs can be introduced to surface water through seepage of oil sands tailings ponds into groundwater and subsequent discharge to surface water. Gervais (2004) investigated three NA plumes in the oil sands region:

- Albian Sands Test Pit plume at the Albian Sands Muskeg River Mine. Investigation of this plume provided the opportunity to evaluate an existing plume of "naturally" NA-rich groundwater from the McMurray Basal Aquifer in a shallow glacial aquifer.
- Process-affected water from the Suncor Energy Inc. (Suncor) holding Pond 2/3. Gervais (2004) stated that process water from the pond had migrated into a semi-confined anaerobic aquifer, probably from dewatering of Dyke 2W at the pond.
- Syncrude Canada Ltd. Mildred Lake Settling Basin.

Gervais (2004) indicated that a decrease of NA concentrations at all three plume sites arose from dispersive dilution; however, evidence existed indicating attenuation was occurring because sorption was not found. Gervais (2004) suggested that aerobic biodegradation may have occurred because of decreases in lower molecular weight NA concentrations in the plumes. Anaerobic biotransformation might have also occurred because decreased sulfate concentrations and methane were observed in the plumes.

Oiffer et al. (2009) delineated the plume of process-affected groundwater at the Syncrude Canada Ltd. site, and studied the potential of anaerobic biodegradation of NAs. They suggested that despite more than 20 years of subsurface residence time, anaerobic biodegradation of NAs within the study area was found to be minimal. The attenuation of NAs also appeared to be

weak, and the precise quantification of attenuation was affected by uncertainties associated with spacing of monitoring wells and heterogeneity of the plume. A low soil sorption coefficient (K_d) of 0.23 mL/g was obtained, which suggested that a relatively small quantity of NAs may have been preferentially adsorbed (Oiffer et al. 2009). However, since the degree of attenuation was limited, it was suggested that physical dynamics of the groundwater flow would be the principal control of transport of groundwater NAs (Oiffer et al. 2009).

Wang and Kasperski (2010) studied the adsorption of commercial NA using a new analytical method. They reported that the existence of cyclic rings may cause the difference in the adsorption of straight-chain versus cyclic NA onto sorbents (i.e., clay with 2.5 wt% adsorbed bitumen), and straight-chain NAs were observed to more readily adsorb given the same pH (Wang and Kasperski 2010).

Previous discussion of OSPW-derived NAs indicates that contact activities with surface water (ingestion and skin contact) represent a plausible way in which human exposure may occur.

5 TOXICOLOGICAL EVIDENCE FOR NAPHTHENIC ACIDS RELEVANT TO HUMAN HEALTH

Defensible use of human health risk assessment requires that – ideally – rigorous data on both exposure and toxicity be available to adequately characterize potential risks of chemicals of concern to human health. Weakness caused by poor data, or absence of data, in either the exposure or effects stages of risk assessment significantly reduces confidence in the overall risk assessment.

Toxicity information of interest for understanding human health risk includes: acute toxicity, subchronic/chronic adverse responses (e.g., weight loss, immunosuppression, etc.), neurotoxicity, developmental and reproductive toxicity, and genetic toxicity (mutagenicity and carcinogenicity).

In early research, MacKinnon and Boerger (1986) conducted acute laboratory bioassays to characterize the effects of OSPW on common aquatic test organisms such as the phosphorescent bacteria *Vibrio fischeri* (15-minute exposures using the Microtox® test) and trout and Daphnia (standard 96-hour static exposures). Fresh OSPW – containing >100 mg/L of NAs – was found to be acutely toxic to these organisms. Additional toxicological data of naphthenic acid compounds are discussed further below.

5.1 Live Animal Acute Toxicity

5.1.1 *Oral*

In vivo (live animal) acute (LD_{50}) studies with rodents indicate that toxicity of NA from crude oil to rats is relatively low: greater than 3 grams per kg body weight. Table 4 summarizes toxicological evidence from acute live animal oral toxicity studies.

Table 4. Toxicological evidence from acute live animal oral toxicity studies of naphthenic acids.

Test Species	NA Source	Details	Reference
rats	crude kerosene and mixed crude oils	oral LD_{50}: • 3 g NA per kg body weight (7% to 93% NA fraction from crude kerosene acids) • 5.2 g NA per kg body weight (65% to 90% NA fraction from mixed crude oils) Death resulted from gastrointestinal disturbances, with mortality peak occurring on third to fourth day after administration. Animals exhibited anorexia, inanition, diarrhea, and asthenia.	Rockhold 1955
rats	crude oil	oral LD_{50}: 3 g NA per kg body weight	Lewis 2004
rats (Wistar) 10 females/dose (3 doses, plus control) and 10 males/dose (1 dose, plus control)	NA extract from OSPW as an aqueous solution (NA was isolated from OSPW collected from the upper (0 to 3 m) clarified zone of the Syncrude Canada Mildred Lake Settling Basin)	acute oral non-LD_{50}: • 3, 30, and 300 mg NA per kg body weight (aqueous solutions containing 55,080 or 5,508 or 550 mg/L NA, respectively) (dosage levels chosen – 3, 30, and 300 mg NA per kg body weight) • reflected 0.5, 5, and 50 times, respectively, a worst-case, single day exposure for wild animals based upon NA concentration of 100 mg/L in water that is ingested at a rate of 60 mL per kg body weight/day Female rats were given a single oral dose of NA at 3, 30 or 300 mg/kg body weight, while male rats received 300 mg/kg body weight. Dosages were chosen to bracket worst-case, environmental exposure scenarios to small mammals. 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. The following effects were seen in the high dose (300 mg NA per kg body weight) groups: • decreased food consumption following dosing • lethargy and mild ataxia (2/10 females, 3/l0 males) • statistically significant increase relative organ weights: ovaries, spleen in females; testes, heart in males • 7 of 10 females and 6 of 10 males exhibited eosinophilic pericholangitis • 6 of 10 males and 2 of l0 females with brain hemorrhage In the mid-dose group, 7 of l0 females and 4 of l0 males had heart lesions.	Rogers et al. 2002a Rogers 2003

NA = naphthenic acids; OSPW = oil sands process water.

Of interest is the acute OSPW-derived NA toxicity study of Rogers (2003) and Rogers et al. (2002a) using Wistar rats. Female rats were given a single oral dose of OSPW-derived NAs at 3, 30, or 300 mg/kg body weight; while male rats received 300 mg/kg body weight. Control animals were given tap water. The dosage levels chosen – 3, 30, and 300 mg NA per kg body weight – reflected 0.5, 5, and 50 times, respectively, a worst-case, single day exposure for mammalian wildlife based upon a NA concentration of 100 mg/L in surface water that is ingested at a rate of 60 mL per kg body weight/day. Effects were observed in the mid- and high-dose groups (Table 4).

5.1.2 *Dermal*

Table 5 summarizes toxicological evidence from dermal (skin contact) and eye acute toxicity studies. Live animal acute studies with rabbits indicate that NAs are judged to cause moderate irritation after skin or eye contact in rabbits on the basis of two studies (BIBRA 1999).

Table 5. Toxicological evidence from acute live animal dermal and eye toxicity studies of naphthenic acids.

Test Species	NA Source	Details	Reference
rabbits, New Zealand White, male/female, 2 per sex	raw NA derived from kerosene administered undiluted	3.16 g NA per kg body weight applied dermally to clipped abraded abdomens of each animal (LD_{50} > 3.16 g per kg body weight) 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. 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. Three 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.	BIBRA 1999
rabbits, New Zealand White, male/female, 3 per sex	raw NA derived from kerosene administered undiluted	0.1 mL NA placed into conjunctival sac of eye of each of six rabbits; lids were held together briefly to insure adequate distribution; untreated eye served as a control The rabbits were observed at 1 and 4 hours, and on days 1, 2, 3, 4, and 7. Material was judged to be moderate irritant. According to Draize chart, 4 to 6 rabbits with positive scores observed at 1, 2, or 3 days.	BIBRA 1999

NA = naphthenic acids.

5.2 Live Animal Subchronic/Chronic Noncarcinogenic Toxicity

Noncarcinogenic toxicity here refers to health effects other than developmental and reproductive toxicity and cancer. Three live animal repeated dose studies have been undertaken to examine chronic/subchronic nongenetic effects of NAs (Table 6). Female Wistar rats in the Rogers (2003) and Rogers et al. (2002a) study were given oral doses of OSPW-derived NAs five days per week over 90 days at 0.6, 6, or 60 mg/kg body weight/day. Control animals were given tap water. The dosage levels chosen – 0.6, 6, or 60 mg NA/kg body weight/day – reflected 0.1, 1,

and 10 times, respectively, a worst-case, daily exposure for mammalian wildlife based upon a NA concentration of 100 mg/L in water that is ingested at a rate of 60 mL per kg body weight/day.

Table 6. Toxicological evidence for chronic/subchronic noncarcinogenic effects from repeated dose toxicity studies of naphthenic acids.

Test Species	NA Source	Details	Reference
mice (male, Wistar) No other experimental details provided in abstract.	no information provided	oral subchronic (30 day): animals given 1,000 mg NA per kg body weight daily Repeated daily administration (30 days) of naphthenic acid at doses of 1,000 mg/kg orally revealed a few cases of: • CNS depression without analgesia and no loss of the corneai reflex • hematological changes • weight loss leading eventually to death due to respiratory arrest • gross morphological changes in the liver and stomach • histomorphological changes in a few selected organs	Pennisi and Lynch, 1977 [meeting abstract]
rats (female, Wistar) 12 animals per dose level; 1 dose/day – Mon. to Fri (5 days/week)	NA extract from OSPW as an aqueous solution (NA was isolated from OSPW collected from the upper (0 to 3 m) clarified zone of the Syncrude Canada Mildred Lake Settling Basin)	oral subchronic (90 day): - 0.6, 6, or 60 mg NA per kg body weight per day (aqueous solutions containing 8,549; 846; or 84.5 mg/L NA, respectively) - controls received water (7.0 mL tap water) (dosage levels chosen – 0.6, 6, and 60 mg NA per kg body weight per day – reflected 0.1, 1, and 10 times, respectively, a worst-case, daily exposure for wild animals based upon NA concentration of 100 mg/L in water that is ingested at a rate of 60 mL per kg body weight/day) 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. The following significant effects were seen in the high dose groups: • decreased food consumption immediately following dosing • severe, clonic seizures lasting 20 seconds (25% of animals) observed after day 40 after which all animals, except one that died, resumed normal activity$^{\Delta}$ • 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 of l2 rats with increased glycogen storage The following effects were seen in the mid-dose group: • severe, clonic seizures lasting 20 seconds (17% of animals) observed after day 40 after which all animals except one that died, resumed normal activity$^{\Delta}$ • 3 of 12 rats with increased glycogen accumulation In the low-dose group, 2 of 12 rats had increased glycogen accumulation $^{\Delta}$ rats in low-dose (8%) and control (17%) groups also demonstrated	Rogers, 2003 Roger et al., 2002a

Test Species	NA Source	Details	Reference
		milder episodes, characterized primarily by muscle twitching	
mice (female) Six-to-eight week old C57BL/6 mice purchased from Charles River Laboratories (Wilmington, MA)	non-metallic commercial naphthenic acids (C-NA), neutral and acid extractable organic fraction of naphthenic acids (OSPW-OF)	Mice were caged in groups of four and fed commercial diet of libitum. At 10 weeks of age, mice were gavaged once per week for up to 8 weeks with 50 mg or 100 mg NA per kg of body weight, with either C-NA or OSPW-OF. Using a daily water intake reference value of 7.8 mL per 30 g of body weight for mice, and an estimated NA concentration of 27 mg/L in an OSPW sample, a 30-g mouse would consume ~1.47 mg NA per week. A dose of 50 mg/kg of body weight NA per week represented a weekly NA intake of 1.5 mg for a 30-g mouse, while a dose of 100 mg/kg body weight represented a weekly intake of 3 mg of NA. Mice were exposed to C-NA or OSPW-OF by gavage so that each mouse would receive exactly the same NA dose based on their weight. The control group for C-NAs were gavaged with distilled water, while the control group for OSPW-OF exposed mice were gavaged with the product of the neutral and acid organic extraction protocol performed using distilled water. Mice were euthanized every week after the onset of exposure, the peritoneal cavity was lavaged to recover peritoneal cells, and the spleen removed for gene expression analysis. Both control and experimental groups consisted of 8 mice for every time point. The expression of different macrophage-activating cytokine genes in the mouse spleen were examined: • non-significant changes in gene expression were observed in the spleen before the 8th week of C-NA or OSPW-OF exposure • 50 mg/kg doses of C-NA or OSPW-OF NA had no effect on gene expression throughout the assay • 100 mg/kg C-NA for 8 weeks resulted in down-regulation of only IL-1 B • 100 mg/kg OSPW-OF NA for 8 weeks resulted in down regulation of IFN1, IL-1 B, and CSF-1 The expression of pro-inflammatory genes in different mouse organs was determined using quantitative PCR (qPCR): • C-NA and OSPW-OF altered the expression of pro-inflammatory genes, inducing either expression down-regulation or up-regulation, depending on the organ examined and time after exposure • the time at which gene expression alterations occurred, and the specific sets of genes whose expression was altered, were very different between animals exposed to C-NA or to OSPW-OF The ability of the mouse peritoneal macrophages to phagocytose yeast cell wall, as a measure of the ability of mice to mount a central function of the innate immune response was examined: • phagocytosis was significantly reduced in animals exposed to C-NA, but enhanced in mice exposed to OSPW-OF, indicating that studies using C-NA may not necessarily reflect the possible effects induced in animals by exposure to OSPW-OF	Garcia-Garcia et al. (2011a,b)

NA = naphthenic acids; C-NA = commercial naphthenic acids; OSPW OF = neutral and acid extractable organic fraction of naphthenic acids.

Effects observed in the high-dose group (Table 6) were:

- Decreased food consumption immediately following dosing.
- Severe, clonic seizures lasting 20 seconds (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.

A more recent study (Garcia-Garcia et al. 2011a,b) examined antimicrobial responses of bone marrow-derived macrophages *in vitro* (including production of reactive oxygen and nitrogen intermediates and phagocytosis, and pro-inflammatory cytokine gene expression *in vivo* and *in vitro* from exposure to commercial naphthenic acids (C-NA) and NAs present in the neutral and acid-extractable organic fraction of OSPW (OSPW-OF).

Garcia-Garcia et al. (2011a) initially showed that both C-NA and OSPW-OF are immunotoxic to bone marrow-derived macrophages *in vitro*. They then examined whether C-NA and OSPW-OF had immunotoxic effects *in vivo*. They stated that in mammals, the spleen is a major immune organ, performing fundamental functions required for efficient anti-bacterial and anti-fungal immune defense.

Garcia-Garcia et al. (2011a) observed that OSPW-OF causes mammalian immunotoxic effects that may impair the ability of an exposed host to defend against infectious disease. Specifically, oral exposure of mice to the neutral and acid-extractable organic fraction of OSPW caused down-regulation in the expression of genes encoding pro-inflammatory cytokines in mouse spleen.

To evaluate whether C-NA are an adequate model to study OSPW toxicity in complex organisms, Garcia-Garcia et al. (2011b) compared the effects of C-NA and OSPW-OF exposure on mice immune mechanisms *in vivo*. They found that C-NA and OSPW-OF altered the expression of pro-inflammatory genes, inducing either expression down-regulation or up-regulation, depending on the organ examined and time after exposure. They also found that the time at which gene expression alterations occurred, and the specific sets of genes whose expression was altered, were very different between animals exposed to C-NA or to OSPW-OF.

Finally, Garcia-Garcia et al. (2011b) examined the ability of mouse peritoneal macrophages to phagocytose yeast cell wall, as a measure of the ability of mice to mount a central function of the innate immune response. They observed that phagocytosis was significantly reduced in animals exposed to C-NA, but enhanced in mice exposed to OSPW-OF, indicating that studies using C-NA may not necessarily reflect the possible effects induced in mammals from exposure to process water from tailing ponds.

No full two-year (chronic) studies of NAs (commercial or OSPW-derived) were identified in the available literature.

5.3 Live Animal Developmental and Reproductive Toxicity

API (2003) summarized results of live animal reproductive testing submitted to the U.S. Environmental Protection Agency under the U.S. *Toxic Substances Control Act*. Ten male and 2 female rabbits (strain unreported) were treated dermally 6 hours/day, 5 days/week with 2 mL (neat) of a commercial calcium naphthenate over a 10-week exposure period prior to males mating in 1984. API (2003) reported that there was 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 a 12 week post-exposure observation period. In the male animals, there were no significant changes in the testes weights.

In 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.

Other information was reported by Rogers (2003) and Rogers et al. (2002b) on results of OSPW-derived NA reproductive testing study on rats. Forty-one 10-week old female Wistar rats were assigned to 3 groups:

- Control (n=14).
- Low-dose corresponding to 6 mg/kg/day (n=13).
- High-dose corresponding to 60 mg/kg/day (n=14).

Twenty-one 8-week old male rats were used for breeding purposes. Female and male animals were exposed by oral gavage each day throughout a 2-week pre-breeding and a 2-week breeding period. For females that became pregnant, dosing was continued throughout gestation.

The high dose reflected 10 times a worst-case exposure scenario for mammalian wildlife drinking water containing OSPW-derived NAs at concentrations comparable to those in tailings pond water. OSPW-derived NA in an aqueous solution was fed daily – with 7 mL of aqueous solution containing either 854.9 mg/L (low-dose) or 8,549 mg/L (high-dose) NAs. These amounts represented exposures equivalent to 6 (low-dose) or 60 (high-dose) mg/kg body weight NA per day. The NAs were isolated from OSPW collected from the upper (0 to 3 m) clarified zone of the Syncrude Canada Ltd. Mildred Lake Settling Basin.

Rogers et al. (2002b) reported the following results:

- Reproductive toxicity testing demonstrated adverse 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/day successfully bore a litter.

- Litter size of the high-dose group (7 pups) was about half the mean of the other groups.
- Total cholesterol of the high-dose group was 30% lower than controls.
- Mating and ovulation were comparable among control and dose groups, while fetal malformations were not apparent in any offspring.

Rogers (2003) and Rogers et al. (2002b) proposed 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.

No other developmental toxicity studies of NAs (commercial or OSPW-derived) were identified in the literature.

5.4 Genetic Toxicity

5.4.1 *In Vitro*

In vitro testing of NAs was conducted by the National Toxicology Program in the 1990s. BIBRA (1999), Brient et al. (1995) and NTP (2008) report that commercial sodium naphthenate was not mutagenic when tested by the Ames mutagenicity test (in *Salmonella typhimurium* strains TA98, TAl00, TAl537, and TA1538) with or without activation. Commercial sodium naphthenate did not induce chromosome aberrations in hamster ovary cells; but it was positive for sister chromatid exchanges (BIBRA 1999, NTP 2008).

5.4.2 *Live Animal – Carcinogenicity*

5.4.2.1 Oral

No standard two-year carcinogenicity studies of naphthenic acids (commercial or OSPW-derived) were identified in the literature.

5.4.2.2 Dermal

API (2003) summarized results of live animal dermal testing submitted to the U.S. Environmental Protection Agency under the U.S. *Toxic Substances Control Act*. Female mice (strain unreported) were treated dermally 2 times/day over a 2-year period with 0.05 mL (neat) of a commercial calcium naphthenate solution in 1987. Clinical observations reported by API (2003) 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 a negative control group, no cutaneous tumors developed at or distant to treated sites. Twelve epidermal and one dermal tumor at treated sites were observed in eight mice that were exposed to the test material. Four of the tumors were benign (i.e., did not exhibit uncontrolled cellular growth) and none of the tumors were malignant (i.e., display uncontrolled

cellular growth). The first of these tumors were reported after 392 days of treatment. No metastatic tumors (i.e., new tumors in another part of the body) were present.

5.5 Discussion of Potential Human Toxicity of Naphthenic Acids

Regulatory agencies (e.g., Alberta Environment and Water) have the responsibility to set limits for chemicals of concern in the environment that may pose public health risks in Alberta. Alberta Environment and Water has established maximum acceptable concentrations of chemicals of concern in exposure media (e.g., air, water, soil) that are protective of human and ecological health.

Traditional toxicity testing of laboratory animals provides most data and evidence used for setting acceptable concentrations in human exposure media and for risk assessment (NRC 2006, 2007). This includes information on possible effects of exposure to a chemical and exposure concentrations at which effects might be observed. However, these animal bioassay testing approaches have inherent uncertainties associated with (NRC 2007, U.S. EPA 2009a): (1) human relevance of laboratory animal studies (species extrapolation), (2) use of high doses in animals to estimate human responses associated with much lower environmental exposures (dose extrapolation), and (3) predicting effects in susceptible populations.

The National Research Council Committee on Toxicity Testing and Assessment of Environmental Agents (NRC 2006, 2007) reviewed established and emerging toxicity testing methods and strategies for chemicals. An outcome of this review was a recommendation that the intensity and depth of toxicity testing of substances – including both *in vitro* and *in vivo* testing – should be based on practical needs. Specifically, NRC (2006, 2007) identified human use, likelihood of human exposure, and scientific questions that need to be answered to support reasonable science policy decisions as key points that should be addressed in developing strategies and employing methods for toxicity testing of chemicals.

NRC (2006, 2007) and U.S. Environmental Protection Agency (2009a) recommended a shift in scientific thinking about using traditional animal bioassay testing approaches towards focusing on *toxicity pathways* in toxicity testing. Toxicity pathways are cellular response pathways that, when sufficiently altered, are expected to result in adverse health effects. This approach emphasizes scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function.

Two important components of the toxicity pathway concept are (U.S. EPA 2009a): (1) extending knowledge of molecular alternations and cell signaling pathways to understand linkages between levels of biological organization, and (2) extending knowledge of *in vitro* and *in vivo* markers relevant to adaptive changes and/or adverse effects. Recently, Garcia-Garcia et al. (2011a,b) focused their investigation on toxicity pathway testing of the extractable organic fraction of OSPW. This fraction represents a complex mixture of chemicals thought to contribute to toxicity of OSPW.

With respect to human toxicological evidence for OSPW-derived NAs, results from several mammalian toxicity studies were based on testing of commercial NA salts (API 2003,

Pennisi and Lynch 1977). It is important to note drawbacks regarding applicability of this type of evidence in representing potential human toxicity of OSPW-derived NAs in surface water.

Levels of NA will be relatively high in fresh OSPW and tailings (i.e., recently produced from the extraction process), but over time aerobic biodegradation of these compounds will limit their buildup even in active process waters and eventually lead to their reduction (Schramm et al. 2000). Differences in biological toxicity are observed when tailings water is allowed to age. Numerous studies have consistently shown that with time, microbial degradation of lower molecular weight NA leads to a decrease in observed aquatic toxicity in tailings water (Clemente et al. 2004, Lai et al. 1996, Lo et al. 2006, MacKinnon and Boerger 1986, Schramm et al. 2000).

Bataineh et al. (2006) indicated that aged tailings water is dominated by highly persistent, higher molecular weight, alkyl-substituted NA isomers. Aging of tailings water encourages natural biodegradation of lower molecular weight NA that are attributed to aquatic toxicity. Commercial NAs – on the other hand – are lower molecular weight NA and are readily biodegradable (Headley et al. 2010). Bataineh et al. (2006) reported that it is these forms that likely contribute to the lion's share of aquatic toxicity posed by OSPW-derived NA in tailings water.

Bataineh et al. (2006) undertook controlled biodegradation studies of commercial (refined) NA (Merichem, Houston, TX) and aged tailings water NAs. Their results showed that oil sands tailings water microorganisms preferentially depleted the least alkyl-substituted fraction of NA and may be responsible for resulting NA profiles (i.e., multi-ring structure NA with higher molecular weights) in aged tailings water. Their observations offered a plausible explanation for why refined (lower molecular weight) NAs were readily biodegraded while no significant degradation occurred for tailings water (higher molecular weight) NAs.

Commercial NAs – such as refined Merichem or Sigma-Aldrich – are typically prepared from petroleum sources that have not undergone extensive biodegradation (Brient et al. 1995), whereas OSPW-derived NA come from bitumen which is considered to be extensively biodegraded petroleum (Han et al. 2008). This finding is consistent with observations that the aquatic toxic potency of tailings ponds water declines with age – i.e., due to a dominance of higher molecular weight, multi-ring structures that are more highly branched and more resistant to microbial degradation.

Therefore, inferring potential human toxicity on the basis of toxicological evidence observed for commercial NA derived from crude oils (Garcia-Garcia et al. 2011a,b) or commercial NA salts (API 2003, Pennisi and Lynch 1977) will not be representative of NA present in aged and reclaimed OSPW. Higher molecular weight, multi-ring NA forms – that are more resistant to microbial degradation and less potent in toxicity to biological organisms – are the forms that would be present in aged and reclaimed OSPW.

5.5.1 *Acute Toxicity*

Boyd et al. (1996) reported that the daily dose which killed 50% of young male albino rats (LD_{50}) after administration of table salt for 100 days was 2.69±0.12 g/kg. Acute toxicity studies

(Table 1) indicate that LD_{50} values of crude kerosene and mixed crude oils containing NAs and naphthenic acid mixtures found within crude oils (i.e., 3 to 5.2 g/kg) are similar to the oral toxicity of table salt. More recent acute toxicity testing in rats (Rogers 2003, Rogers et al. 2002a) revealed that behavioral and histopathological effects resulted from a single administration of OSPW-derived NAs, but primarily at a dosage estimated to be 50 times a worst-case environmental exposure for small mammalian wildlife. This dosage is not realistic for humans in the oil sands region.

5.5.2 *Subchronic/Chronic Noncarcinogenic Toxicity*

In both acute and subchronic (90-day repeated exposure) testing of rats to OSPW-derived NAs, Rogers (2003) and Rogers et al. (2002a) reported that the liver was the major target organ affected. Two blood parameters – amylase enzymes and cholesterol – were observed to be adversely affected at the highest dosage level in the 90-day study, with both effects possibly linked to an impact on liver function. Absolute and relative liver weights were also reported by Rogers (2003) to be higher following exposure in the 90-day study. Lower mean body weights throughout the exposure period and increased relative organ weights (liver, kidney and brain) were also observed at the highest dosage level.

Garcia-Garcia et al. (2011a) undertook weekly repeated (subchronic) exposure testing of mice for eight weeks and observed that the extracted organic fraction of OSPW caused mammalian immunotoxic effects that may impair the ability of an exposed host to defend against infectious disease. Garcia-Garcia et al. (2011b) also observed that toxicity studies using commercial NA may not necessarily reflect the possible effects induced in mammals from exposure to OSPW from tailing ponds.

The Garcia-Garcia et al. (2011a,b), Rogers (2003) and Rogers et al. (2002a) studies are particularly relevant in that NA and other organics extracted from OSPW were used as the test substances. These forms are directly comparable to forms relevant for potential human exposure in surface waters. However, as the number of these studies is limited it is apparent that an understanding of nature of potential noncarcinogenic effects is incomplete.

Finally, no full two-year (chronic) studies of NAs (commercial or OSPW-derived) were identified in the available literature. Uncertainty remains in the understanding of toxicokinetic (fate in the body) and toxicodynamic (mode of action and dose-response) information needed to infer noncarcinogenic human exposure-related responses to NAs and other acid-extractable organics present in aged and reclaimed OSPW.

5.5.3 *Developmental and Reproductive Toxicity*

Reproductive bioassay testing reported by Rogers (2003) and Rogers et al. (2002b) indicated adverse effects in female rats exposed to 60 mg per kilogram body weight per day of OSPW-derived NAs, and provided insight into one possible mechanism of toxic action for these compounds. These results suggested that exposure of females rats to OSPW-derived NAs resulted in impaired fertilization and, following copulation, failure of embryonic implantation.

Rogers (2003) stated that this provided evidence that the reproductive toxicity of OSPW-derived NAs is secondary, and is due to a primary effect on the liver (i.e., cholesterol production).

No other developmental toxicity studies of NAs (commercial NA derived or OSPW-derived) were identified in the available literature for the oral route. With respect to toxicological evidence described above, gaps in knowledge of developmental and reproductive toxicity of naphthenic acids were discussed by the U.S. Environmental Protection Agency. In response to toxicity data presented by API (2003) for NA, the U.S. EPA (2004) concluded that a need existed for undertaking more developmental and reproductive toxicity testing via the oral route and additional genetic testing of NA. Specifically, U.S. EPA (2004) recommended the following toxicity tests:

- OECD 422 test to further investigate the repeated-dose, developmental, and reproductive toxicity endpoints (discussed further below).
- Micronucleus test to further examine a potential genetic toxicity (chromosomal aberration) endpoint (discussed further under Genetic Toxicity – section 6.3.4).

Description of the OECD 422 test – Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

The OECD 422 test comprises a reproduction/developmental toxicity screening test to provide initial information on possible effects on male and female reproductive performance (OECD 1996). Performance indicators include endpoints such as: testis or ovary function, mating behavior, development of conception and birth, either at an early stage of assessing the toxicological properties of test substances (OECD 1996).

In OECD 422, a test substance is administered in graduated doses orally by gavage to several groups of males and females rats (OECD 1996). Males should be dosed for a minimum of four weeks; while females should be dosed throughout the study (approximately 54 days). Normally, mating "one male to one female" should be used in the study. Each group should be started with at least 10 animals of each sex. Generally, at least three test groups and a control group should be used. Dose levels should be selected taking into account any existing toxicity and toxicokinetic data available. The limit test corresponds to one dose level of at least 1,000 mg/kg body weight.

Results of the study should include measurements (weighing, food/water consumption), daily detailed observations (including sensory reactivity to stimuli), and gross necropsy and histopathology (OECD 1996). Findings of the toxicity study should be evaluated in terms of observed effects, necropsy, and microscopic findings. This test does not provide complete information on all aspects of reproduction and development (OECD 1996). In particular, it only offers a means of detecting postnatal manifestations of prenatal exposure, or effects that may be induced during postnatal exposure. Due to selectivity of the end points, and the short duration of the study, the method will not provide evidence for definite claims of no reproduction/developmental effects.

Although a negative result from an OECD 422 test (i.e., absence of reproduction and development effects) does not indicate absolute safety, this type of result offers some reassurance if actual exposures will be clearly less than a dose related to the No Observed Adverse Effect Level in the test (OECD 1996). In addition, such a test provides plausible evidence intended to assist in closing a knowledge gap with respect to characterization of potential human developmental and reproductive effects of OSPW-derived NAs.

The other potential direction to be explored for developmental and reproductive endpoints is *in vitro*/*in vivo* bioassay testing focusing on cellular response pathways, consistent with that recommended by NRC (2006, 2007).

5.5.4 *Genetic Toxicity*

In vitro genetic toxicity testing of commercial sodium naphthenate observed the following (BIBRA 1999, Brient et al. 1995, NTP 2008):

- Negative when tested by the *Salmonella* (Ames) mutagenicity test.
- Negative for chromosome aberrations in hamster ovary cells.
- Positive for sister chromatid exchanges.

No standard two-year carcinogenicity studies of NA (commercial or OSPW-derived) were identified in the available literature for the oral route. With respect to genetic toxicity testing, a battery of *in vitro* and *in vivo* tests have been used by the U.S. National Toxicology Program for identifying carcinogens, for example:

- *Salmonella* (Ames) mutagenicity test (*in vitro*).
- Standardized chromosome aberration test (*in vitro*).
- Mammalian cell mutagenicity test (*in vitro*).
- *In vivo* (live animal) testing to confirm positive results of *in vitro* tests.

Zeiger (1998) reviewed results of the *Salmonella* (Ames) mutagenicity, *in vitro* chromosome aberration, mutations in mouse lymphoma cells, rodent bone marrow micronucleus, and rodent carcinogenicity tests performed by the U.S. National Toxicology Program. Zeiger (1998) found that a positive *Salmonella* (Ames) test result was the most predictive of carcinogenicity, and that the data reviewed did not support using the other tests in addition to the *Salmonella* (Ames) test for predicting carcinogenicity.

Zeiger (1998) stated that the genetic toxicity tests reviewed did not complement each other, and batteries or combinations of the tests were no more predictive of carcinogenicity than the *Salmonella* (Ames) test alone. Zeiger (1998) concluded that if a substance is shown to be mutagenic in the *Salmonella* (Ames) test – i.e., positive – it should be considered a potential rodent carcinogen, unless ancillary information suggested otherwise. Thus, negative findings in the Ames mutagenicity test offers support for not pursuing *in vivo* (live animal) genetic testing for OSPW-derived NAs.

Description of Micronucleus Test

This is an *in vitro* toxicity test that can provide plausible evidence to assist in confirming the absence of potential human genetic toxicity of OSPW-derived NAs. The U.S. EPA (2004) previously indicated a need for additional *in vitro* genetic testing of NAs (*in vitro* micronucleus testing). The *in vitro* micronucleus test provides a simple method to detect genotoxic potential of mutagens. The test is used for detection of substances and samples which can cause structural and numerical chromosomal aberrations (e.g., disruption or breakages of chromosomes and substances which affect division of cells) (Fenech 2000).

Increased micronucleus formation can reflect the formation of chromosomal breaks or incorrect separation of chromosomes during the cell division. The *in vitro* micronucleus test can be used as a replacement method for the standardized *in vitro* chromosomal aberration assay, especially as a screening method as it is cheaper and faster to perform. The *in vitro* micronucleus assay can be performed on different cell lines (e.g., human hepatoma cell line HepG2, Chinese hamster ovarian cells CHO, human lymphocytes, etc.) (Fenech 2000, Kirsch-Volders et al. 1997).

6 FINDINGS AND RECOMMENDATIONS

6.1 General Characteristics of Naphthenic Acid Mixtures in OSPW

There are several important findings with regard to chemical characteristics of naphthenic acid mixtures in oil sand process waters:

- OSPW represents a complex mixture of NAs along with other organic chemicals that can also contribute to potential toxicity of the mixture.
- There is a difference in the distribution of organic compounds and their contribution to potential toxicity of OSPW that is fresh (i.e., OSPW recently produced from the oil sands extraction process) versus OSPW that is allowed to age (i.e., OSPW that has been aged for a number of years in inactive storage ponds or pit lakes). Aged OSPW contains higher molecular weight, multi-ring NAs that have been shown to be more resistant to microbial degradation and less potent in toxicity to biological organisms. An understanding of the forms and composition of NAs and other organic compounds present in fresh and aged OSPW, and the effect of aging and aging environment on this composition, and variation in OSPW composition across oil sands processes is incomplete.

6.2 Human Exposure Evidence

Information on human use and likelihood of human exposure is needed to have a complete understanding of the human health risk of OSPW-derived NAs. These substances are not used by the human population and the potential for human exposure in the oil sands region will arise from their natural presence in surface water or from potential future release of reclaimed OSPW to surface water. Based on the information reviewed, it was found that:

- Direct contact activities with surface water (e.g., ingestion and skin contact) represent a plausible way in which human exposure may occur to OSPW-derived NAs.

For any environmental chemical present in surface water to be of importance via the inhalation pathway, it must be present in substantial concentrations in water (e.g., at least greater than 0.1% by weight) and it must possess properties that enable it to readily partition (volatilize) from water to air. Based on the information reviewed, it was found that:

- Low water-to-air transfer properties and dilute concentrations of aged and reclaimed OSPW-derived NAs provide no meaningful scientific evidence to support the inhalation pathway as being important for potential human exposure.

For any environmental chemical originally present in surface water to be of importance via a secondary pathway (i.e., consumption of fish), it must possess properties that enable it to readily transfer directly from water to fish tissue (bioconcentrate) or be readily taken up along the food chain of fish (bioaccumulate). Based on the information reviewed, it was found that:

- Expected properties of aged OSPW-derived NAs (i.e., low octanol water partition values and apparent rapid depuration) offer no meaningful scientific evidence to support the fish ingestion pathway as being important for potential human exposure to these compounds.

6.3 Toxicological Evidence

Toxicity information of interest for understanding human health risk from chemicals in the environment includes: acute toxicity, subchronic/chronic adverse responses (e.g., weight loss, immunosuppression, etc.), neurotoxicity, developmental and reproductive toxicity, and genetic toxicity (mutagenicity and carcinogenicity).

A general finding of this review is:

- Toxicological evidence observed for commercial NAs derived from crude oils and/or commercial NA salts will not be representative of NAs in aged and reclaimed OSPW. Higher molecular weight, multi-ring NAs, which are more resistant to microbial degradation and less potent in toxicity to biological organisms, are the forms reported to be present in aged and reclaimed OSPW.
- OSPW-derived NAs come from bitumen which is considered to be extensively biodegraded petroleum; whereas commercial NAs are typically prepared from

petroleum sources that have not undergone extensive biodegradation. Therefore, potential human toxicity and corresponding human exposure limits for OSPW-derived NAs should not be inferred from studies of commercial NAs.

6.3.1 *Acute Toxicity*

Acute toxicity studies indicate that crude kerosene and mixed crude oils containing NAs and NA mixtures found within crude oils exhibit similar oral toxicity to table salt. Acute toxicity testing in rats by others revealed behavioral and histopathological effects from a single administration of OSPW-derived NAs, but primarily at dosages estimated to be 50 times a worst case environmental exposure for small mammalian wildlife. This is not a realistic exposure condition that would be applicable for humans in the oil sands region.

6.3.2 *Subchronic/Chronic Noncarcinogenic Toxicity*

The liver was the major target organ affected in both acute and 90-day repeated exposure (subchronic) testing of rats to OSPW-derived NAs. In addition, weekly repeated (subchronic) exposure testing of mice for eight weeks has demonstrated that the extractable organic fraction of OSPW caused mammalian immunotoxic effects that may impair the ability of an exposed host to defend against infectious disease.

A finding of this review is:

- Based upon limited information reviewed, uncertainty remains in the understanding of toxicokinetic (fate in the body) and toxicodynamic (mode of action and dose-response) information needed to infer noncarcinogenic human exposure-related responses to NAs and other acid-extractable organics present in aged and reclaimed OSPW.

A recommendation of this review is:

- There is a need to further examine potential subchronic/chronic toxicity of NAs and other acid-extractable organics present in aged and reclaimed OSPW.

There were no full two-year (chronic) studies of NAs (commercial or OSPW-derived) identified in the available literature.

6.3.3 *Developmental and Reproductive Toxicity*

A reproductive study reported adverse effects in female rats exposed to 60 mg OSPW-derived NA per kilogram body weight per day, and provided insight into one possible mechanism of toxic action for these compounds. This dosage reflected 10 times a worst-case daily environmental exposure for small mammalian wildlife. Results indicated that exposure of females to OSPW-derived NAs at this level resulted in impaired fertilization following copulation, and/or failure of embryonic implantation.

Potential gaps in knowledge of developmental and reproductive toxicity of NAs were evaluated by the U.S. Environmental Protection Agency in response to toxicity data presented by American

Petroleum Institute for naphthenic acids. The U.S. Environmental Protection Agency concluded that a need existed for undertaking more developmental and reproductive toxicity testing via the oral route.

A finding of this review is:

- Based upon limited information reviewed, uncertainty remains in knowledge of developmental and reproductive toxicity of NAs and other acid-extractable organics present in aged and reclaimed OSPW.

A recommendation of this review is:

- There is a need to further examine developmental and reproductive toxicity endpoints of NAs and other acid-extractable organics present in aged and reclaimed OSPW using *in vitro*/*in vivo* bioassay testing focusing on cellular response pathways.

6.3.4 *Genetic Toxicity*

In vitro genetic toxicity testing of commercial sodium naphthenate by others produced the following results: negative when tested by the *Salmonella* (Ames) mutagenicity test; negative when tested for chromosome aberrations in hamster ovary cells; and positive when tested for sister chromatid exchanges. No standard two-year carcinogenicity studies of NA were identified in literature for the oral route. Others have concluded that if a substance is shown to be mutagenic in the *Salmonella* (Ames) test, i.e., positive, it should be considered a potential rodent carcinogen, unless ancillary information suggested otherwise. However, negative findings in the Ames mutagenicity test offers support for not pursuing *in vivo* (live animal) genetic testing for naphthenic acids.

Potential gaps in knowledge of genetic toxicity of NAs were evaluated by the U.S. Environmental Protection Agency in response to toxicity data presented by American Petroleum Institute for NAs. The U.S. Environmental Protection Agency concluded that a need existed for undertaking additional genetic testing of NAs. Specifically, *in vitro* genetic (micronucleus) testing was recommended to further examine a genetic toxicity endpoint for NAs.

A finding of this review is:

- Based upon limited information reviewed, uncertainty remains in the knowledge of genetic toxicity of NAs and other acid-extractable organics present in aged and reclaimed OSPW.

A recommendation of this review is:

- There is a need to further examine genetic toxicity endpoints (including carcinogenic endpoints) of NAs and other acid-extractable organics present in aged and reclaimed OSPW using *in vitro* genetic (micronucleus) testing and/or other suitable tests focusing on cellular response pathways.

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8 GLOSSARY

8.1 Terms

Acute toxicity

Any poisonous effect produced from a single or short exposure (24 to 96 hours) resulting in severe biological harm or death.

Adipsia

Absence of thirst; an abnormal avoidance of drinking.

Asthenia

Weakness. Lack of energy and strength. Loss of strength.

Ataxia

Loss of the ability to coordinate muscular movement.

Bioaccumulation

The accumulation of chemicals in the tissue of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, and pore water in the sediment.

Bioconcentration

A process leading to a higher concentration of a substance in an organism than in the environmental media to which it is exposed.

Chronic toxicity

Long-term toxicity of a substance in small, repeated doses.

Clonic seizures

An abnormality in neuromuscular activity characterized by rapidly alternating muscular contraction and relaxation.

Depuration

The removal of impurities, especially from bodily fluids; purification, cleansing.

Down-regulation

Down-regulation is the process by which a cell decreases the quantity of a cellular component, such as RNA or protein, in response to an external variable.

Eosinophilic pericholangitis

Inflammation of stained cell tissues around the bile ducts.

Gavage

Forced feeding of nutritive material into the stomach by means of a tube.

Histopathology

The study of the microscopic anatomical changes in diseased tissue.

Inanition

Lack of mental or spiritual vigor and enthusiasm. Exhaustion caused by lack of nourishment.

Isomer

Each of two or more compounds with the same formula but a different arrangement of atoms in the molecule and different properties.

Labile compound

Organic compound susceptible to physical, chemical and biological decomposition.

LD_{50}

Standardized measure for expressing and comparing the toxicity of chemicals (dose that kills half, 50%, of animals tested).

Necropsy

An examination and dissection of a dead body to determine cause of death or the changes produced by disease.

Phagocyte

A cell, such as a white blood cell, that engulfs and absorbs waste material, harmful microorganisms, or other foreign bodies in the bloodstream and tissues.

Phagocytosis

The engulfing and ingestion of bacteria or other foreign bodies by phagocytes.

Ptosis

Abnormal lowering or drooping of an organ or a part, especially a drooping of the upper eyelid caused by muscle weakness or paralysis.

Refractory compound

Organic compound resistant to physical, chemical, biological decomposition.

$t_{½}$

Time required for the concentration of an environmental chemical to decrease to half its original value.

Toxicity pathway

Cellular response pathways that, when sufficiently altered, are expected to result in adverse health effects.

Toxicodynamic

The physiological processes for the absorption, distribution, metabolism and excretion of substances foreign to the body (i.e., mode of action and dose-response).

Toxicokinetic

The rate at which a chemical will enter the body and what happens to it once it is in the body (i.e., fate in the body).

Tumor

A swelling of a part of the body, generally without inflammation, caused by an abnormal growth of tissue, whether benign or malignant.

- Cutaneous tumor – tumor of the skin
- Epidermal tumor – tumors of the epidermis (outer layer of the skin).
- Dermal tumor – skin lesion.
- Malignant tumor – neoplasm which exhibits aggressive behavior including anaplasia, invasion, and metastasis.
- Benign tumor – neoplasm which does not exhibit aggressive behavior and grows only at its original site.
- Metastatic tumor – a malignant tumor.

Up-regulation

Up-regulation is the process by which a cell increases the quantity of a cellular component, such as RNA or protein, in response to an external variable.

8.2 Acronyms

AENV	Alberta Environment
API	American Petroleum Institute
CCME	Canadian council of Ministers of the Environment
C-NA	Commercial Naphthenic Acids
K_d	Soil sorption coefficient
K_{ow}	Octanol-water partition coefficient
MEDLINE	Medical Literature Analysis and Retrieval System Online
M.W.	Molecular Weight
m/z	Mass-to-charge ratio
NA / NAs	Naphthenic Acid / Naphthenic Acids
NCEA	National Center for Environmental Assessment
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OSPW	Oil Sands Process Water
OSPW-OF	Oil Sands Process Water – Organic Fraction
OSRIN	Oil Sands Research and Information Network
RAMP	Regional Aquatics Monitoring Program
SEE	School of Energy and the Environment
TOXNET	TOXicology Data NETwork
U.S. EPA	U.S. Environmental Protection Agency

9 LIST OF OSRIN REPORTS

OSRIN reports are available on the University of Alberta's Education & Research Archive at https://era.library.ualberta.ca/public/view/community/uuid:81b7dcc7-78f7-4adf-a703-6688b82090f5. The Technical Report (TR) series documents results of OSRIN funded projects. The Staff Reports series represents work done by OSRIN staff.

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