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Sensory analyses of naphthenic acids
as potential compounds for fish tainting

by

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Abstract

Naphthenic acids (NAs), a group of compounds found in oil sands process-affected waters, have been implicated as a cause of the atypical odors which characterise fish taint. Sensory analyses were undertaken to clarify the role of NAs in fish taint. Triangle test and three-alternative forced choice (3-AFC) methods were used to estimate olfactory detection thresholds of NAs. Due to cognitive advantages, the 3-AFC method was found to be superior for the estimation of olfactory detection thresholds of NAs. 3-AFC analyses by trained panels of two commercial preparations and one oil sands extract of NAs, revealed that the odor detection thresholds and odor profiles of NAs differ markedly depending upon their source. Consumer preference panels revealed no evidence that the taste of fish collected from the Athabasca River was preferred less than the taste of fish from two other water basins in Alberta.

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3-AFC	Three-alternative forced choice
AML	Ascending method of limits
ASTM	American Society for Testing and Materials
C	Carbon
CTPW	Consolidated tailings ponds water
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EPL	End-pit lakes
ESI-FT-ICR MS	ESI Fourier transform ion cyclotron resonance mass spectrometry
FTIR	Fourier transform infrared
GC-MS	Gas chromatography-mass spectrometry
GESAMP	Group of Experts on the Scientific Aspects of Marine Pollution
H	Hydrogen
HCl	Hydrochloric acid
ISO	International Organization for Standardization
M	Molar
Mm	Millimolar
MTBSTFA	N-(tert.-butyldimethylsilyl)-N-methyl-trifluoroacetamide
N	Nitrogen
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NAs	Naphthenic acids
O	Oxygen
PB	Phosphate buffer
QDA	Quantitative descriptive analysis
r	Test-retest reliability coefficient
S	Sulfur
SAGD	Steam assisted gravity drainage
TCA	2,4,6-trichloroanisole
UV	Ultraviolet

Chapter 1
Introduction and Background

Tainting of aquatic species caused by oil and other petroleum compounds such as naphthenic acids (NAs) has been recognized by international organizations such as the Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) since 1977 (GESAMP, 1977). NAs are natural constituents in most petroleum sources, including the bitumen in the oil sands of northern Alberta, Canada (Holowenko et al., 2002). When bitumen is extracted from the oil sands vast amounts of process-affected waters are created, which contain concentrated amounts of NAs (Headley and McMartin, 2004). Although tailings waters cannot be discharged into the water systems, a risk of leakage may still exist. This situation supports the need for studies such as the ones developed in this thesis, to further understand the impact that these compounds may inflict on the environment. Of particular interest to this research is the potential of NAs to cause detrimental effects on the odor qualities of fish, resulting in fish tainting.

1.1 Oil sands and impact on the environment

Canadian oil deposits are one of the world's largest petroleum accumulations, representing 13.4% of proven global oil reserves (CIA, 2008), placing Canada into second position behind Saudi Arabia in terms of proven petroleum supply. Currently, the oil sands industry in Alberta produces 1 million barrels of oil per day (CAPP, 2008a).

The oil industry is the key to Alberta's economy. About 95% of Canada's oil supply is reserved in naturally-occurring heavy crude oil deposits (Jardine and Hrudey, 1988), the majority of which are contained in three main regions in northern Alberta; the Athabasca, Cold Lake, and Peace River deposits (Figure 1.1). Oil sands are deposits of a highly biodegraded, viscous form of non-conventional crude oil; composed of a mixture of sand, clay and high molecular weight petroleum known as bitumen (Koning and Hrudey, 1992). In its naturally occurring viscous state, crude bitumen has the consistency of cold molasses and will not flow unless it is heated or diluted with lighter hydrocarbons (Government of Alberta - Environment, 2008).

There are two main methods used for oil sands extraction: in-situ and open-pit mining. About 82% of Alberta's oil sands reserves are considered to be recoverable by

in-situ methods, the remainder is recoverable by surface mining methods (Chalaturnyk et al., 2002). The extracted oil sands bitumen must be upgraded into synthetic crude oil before it can be used by refineries to produce gasoline and diesel fuels (Charpentier 2009).

In-situ extraction techniques are used for the recovery of deep reservoirs (>75 m below the surface). The deep bitumen deposits are recovered using a process known as steam assisted gravity drainage (SAGD). These deposits are injected with steam through a wellbore. This heating process allows the bitumen to reduce its viscosity and flow to the surface through a producing wellbore while the sand is left in place (CAPP, 2008b; Charpentier et al., 2009). Through in-situ recovery methods no tailings ponds are produced (Government of Alberta - Environment, 2008); however, 2.5 to 4 barrels of water are needed to produce one barrel of bitumen (Alberta Geological Survey, 2009).

Open-pit mining methods are used for shallower deposits (<75m below the surface). After clearing over burden from the deposits, the oil sands are excavated by shovel operations and transported to an extraction plant where warm water and caustic are mixed with the oil sands to help separate the bitumen from the sand (Government of Alberta - Environment, 2008; Charpentier et al., 2009). Surface mining extraction methods require two to five barrels of water to produce one barrel of oil (CAPP, 2008b). The extraction of bitumen from the oil sands is achieved by a method known as "Clark hot water process", where alkali-hot water and steam are used to separate the petroleum from the sand-clay matrix (Koning, 1987). The residual tailings produced by the aforementioned method is a slurry containing water, silt, clay, remaining bitumen, and inorganic and organic compounds such as NAs, which are pumped into large tailings ponds (Koning and Hrudey, 1992; Young et al., 2007; Kavanagh et al., 2009). After long periods of settling, water released from the tailings ponds can be reused in the extraction process. Up to 80% of the water can be recycled. Water obtained from the extraction process is known as oil sands process-affected water.

In recent years, tailings pond waters have become a great concern, because a diversity of the residual compounds found in these process-affected waters are acutely toxic to the aquatic ecosystems, including fish (Koning and Hrudey, 1992; Chalaturnyk et al., 2002). Due to their toxicity, governmental agencies prohibit the direct release of oil sands tailings water to the Athabasca River or any other water course, thus, oil extraction companies have implemented a "zero discharge policy" in which no extraction process-affected waters can be intentionally released into ground or surface water bodies. As a

consequence, the tailings ponds are growing at vast volumes with a net annual growth of up to 30 million m^3 per year (Headley and McMartin, 2004).

Despite the efforts of Alberta's oils sands mining industry and governmental agencies to monitor and avoid the release of tailings into surface and underground water resources, a risk of leakage may still exist. This situation reinforces the need for studies such as the ones described in this thesis to enhance our understanding of the potential negative impact of substances such as NAs on water systems and the aquatic life they support. Of particular interest is the potential of NAs to cause off-odors in fish known as 'fish taint'.

1.2 Naphthenic Acids

Tailings ponds contain a diverse range of residual compounds, among them NAs. This group of compounds is of interest in the study of fish health (Nero et al., 2006; Peters et al., 2007; Young et al., 2007; Young et al., 2008) because they are the most toxic organic compounds found in petroleum refinery wastewaters (Schramm et al., 2000). When oil sands bitumen is recovered, the alkali nature of the water used in the extraction process solubilizes and concentrates the NAs into the tailings ponds aqueous phase (Young et al., 2007) which may then be released into fresh water bodies. NAs are of special interest in the present study, because it is hypothesized that they have the potential to cause detrimental sensory properties in fish (GESAMP, 1977).

NAs are a group of compounds that occur naturally in hydrocarbon deposits (oil sands, bitumen, crude oils and petroleum). They comprise a complex mixture of saturated acyclic, monocyclic and polycyclic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$, where n is the carbon number and Z represents the hydrogen atoms lost as the structures form rings (Figure 1.2) (Headley and McMartin, 2004; Clemente and Fedorak, 2005). Figure 1.2 shows potential structures of some NAs.

Natural concentrations of NAs in the Athabasca River oil sands region are generally below 1 mg L^{-1} (Headley and McMartin, 2004). In contrast, it has been reported that tailings ponds contain NAs at levels in the range of 20-120 mg L^{-1} (Clemente and Fedorak, 2005).

Recently, environmental and regulatory agencies have been paying closer attention to the NAs fraction of the oil sands process-affected waters due to their persistence in the environment and aquatic toxicity at the concentrations found in the

wastewaters of oil sands extraction facilities (Headley and McMartin, 2004). Thus, it has become important to understand the environmental impact of this group of compounds.

In general, the use of the term "NAs" is imprecise. As mentioned above, the term usually applies to mono-carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$. However, the phrase "NAs" has been used for a few decades when referring to acidic materials that are extracted from the oil sands or oil sands process-affected waters after acidification and extraction with an organic solvent such as dichloromethane. These extracted "NAs" have been, and continue to be, quantified by the oil sands industry standard FTIR (Fourier transform infrared) spectroscopy method (Jivraj et al., 1995).

NAs have many commercial uses (Brient et al., 1995) and are sold by various chemical companies. Using a gas chromatography-mass spectrometry (GC-MS) method, St. John et al. (1998) demonstrated that the compositions of NAs from various suppliers differed. Using the same GC-MS method, (Clemente et al., 2003) showed that the compositions of commercial NAs differed from the compositions of "NAs" extracted from oil sands process-affected waters. In addition, "NAs" extracted from several different oil sands process-affected waters were shown to have different compositions (Clemente et al., 2003).

Recent applications of high resolution mass spectrometry have revealed much new information about the components found in the "NAs" extracted from oil sands process-affected waters. For example, when Barrow et al. (2009) analyzed "NAs" from the oil sands area, they found compounds with the formula $C_nH_{2n+Z}O_x$, where x = 2-5. Similarly, Han et al. (2009) detected mono- and di-oxidized NAs (i.e. $C_nH_{2n+Z}O_3$ and $C_nH_{2n+Z}O_4$) in extracts from Syncrude Canada Ltd. oil sands process-affected waters and they observed that the oldest water from experimental reclamation ponds contained more $C_nH_{2n+Z}O_3$ and $C_nH_{2n+Z}O_4$ species than parent acids ($C_nH_{2n+Z}O_2$). In contrast, re-examination of ESI-FT-ICR MS (ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry) data of Scott et al. (2009) showed that in the commercial Merichem preparation >96% of the "NAs" are the parent acids ($C_nH_{2n+Z}O_2$) (Fedorak and co-workers, unpublished results).

From the examples above, it is clear that the compositions of "NAs" differ markedly, depending upon their source. Thus, in the sensory studies presented in this thesis, the source of each "NAs" preparation is clearly identified. These include commercial products from Merichem and Acros (i.e. Merichem NAs and Acros NAs,

respectively) and from the acid extract obtained from Syncrude Pond 9 water (i.e. Pond 9 NAs).

1.3 Fish taint

When fresh, the majority of fish skeletal muscle is highly palatable. The aroma and flavor of fish consists of a complex mixture of several aromatic compounds; the flesh of cooked fish is typically sweet, with a range of naturally occurring flavors which can vary widely depending upon the species, physiology (e.g. amount of body fat), dietary habits, environment, water sources (i.e. freshwater and saltwater fish) and the season in which they are caught (Högnadóttir, 2000; Davis et al., 2002).

Unfortunately, there are occasions when fish acquire atypical odors and flavors that arise from exposure to substances dissolved in their aqueous environment. Water-soluble substances are easily absorbed into fish, and when they reach a concentration at which they can be detected sensorially (odor detection threshold) the fish quality is altered (Davis et al., 2002).

According to the GESAMP, tainting in an aquatic organism is defined as “a foreign flavor or odor in the organisms induced by conditions in the water to which the organisms are exposed" (GESAMP, 1982). Tainting of aquatic species can be caused by different sources, including aquatic microorganisms, by-products of marine algae, chemical contaminants in the water, onset of enzymatic degradation, oxidation of lipids and handling and storage (Höfer, 1998; Tucker, 2000; Wilkes et al., 2000). However, the majority of fish tainting incidents have occurred in extremely polluted waters, after coastal tanker accidents (Höfer, 1998) and from industrial wastewaters (Persson, 1984).

For a food product to be considered as tainted, the tainting agent must be positively recognized by meeting or exceeding the taste and/or odor detection threshold, and it needs to be characterised as detrimental to the flavor and/or odor quality of the product (Saxby, 1996). In general, any off-flavor or odor atypical to those naturally occurring in any food product are undesirable; as these uncharacteristic sensory features can generate serious problems for retailers, manufacturers and ingredient suppliers (Kilcast, 2003). Fish taint or even the fear of taint can cause severe economic losses to fisheries and the local economy (Tidmarsh et al., 1985), because this issue can cause product rejection from consumers, loss in revenue, loss in resources and expensive litigation proceedings. Moreover, the isolation and identification of the sources of taint

can be expensive and time consuming. In the Athabasca River region of northeastern Alberta, walleye, northern pike and lake whitefish are depended upon by the First Nations people and other local populations for nutrition and income, thus the possibility of fish taint becomes of great concern.

An unpleasant odor or flavor in food might be perceived by consumers as a sign of a physical health hazard related to contamination (Wilkes et al., 2000); however, many chemical species can be detected at concentrations far below those that would adversely affect consumers' health (Young et al., 1996). Thus, flavor is not necessarily an indication of toxicity but of quality.

There are several factors that play an important role in how we perceive and respond to food taint. Food taints can often be detected at sub-parts per million levels, and as low as parts per trillion. Nevertheless, the concentration at which a substance can be detected varies considerably among individuals due to the nature of the different chemical species but also to genetic and cognitive differences, and personal experiences (Saxby, 1996).

1.3.1 Petroleum compounds as a source of fish taint.

Incidences of tainting of aquatic species caused by oil and other petroleum compounds have been reported as a result of petroleum, fuel and refinery wastewaters being discharged into fresh water bodies and marine systems. Petroleum off-flavors occasionally develop in wild fish when waters are contaminated with odorous water pollutants by accidental spills of diesel fuel or gasoline, from boats, refinery effluents, losses from pipelines and municipal and industrial discharges (GESAMP, 1982). Several compounds related to the oil and refinery industry have been implicated in fish tainting incidents. According to the GESAMP (1982) the principal components of crude oil that cause taint include phenols, dibenzothiophenes, NAs, mercaptans, tetradecanes and methylated naphthalenes.

The pathways by which the uptake of tainting compounds occur vary depending on the fish species, the water sources (i.e. freshwater or saltwater), the habitat, the food source and the nature of the compound (National Research Council (U.S.) Steering Committee for the Petroleum in the Marine Environment Update, 1985). For example, for freshwater fish species, the primary uptake of petroleum compounds is through the gills, followed by intake through the alimentary canal during feeding. For marine fish, the main

source of hydrocarbon intake is through the alimentary canal because they are required to drink vast volumes of seawater for osmoregulation (Persson, 1984).

Feeding habits also influence the uptake of foreign compounds in fish (Leppänen, 1995; Law and Hellou, 1999; Li et al., 2009; Viñas et al., 2009). Lake whitefish (*Coregonus clupeaformis)* and walleye (*Sander vitreus*, formerly *Stizostedion vitreum*), two of the most important commercial and recreational fish species in Alberta (Government of Alberta - Sustainable Resource Development, 2009), have different feeding habits. Lake whitefish are bottom feeders and walleye are predators inhabiting the entire water body, thus it is expected that the absorption of tainting compounds should be different (Koning, 1987).

A significant correlation exists between bioaccumulation of aromatic hydrocarbons and the lipid content in the muscle tissue of fish. In general, higher percentages of hydrocarbons are observed in lipid-rich fish than in lipid-poor fish. This might be due to the affinity between hydrocarbons and lipids in the animal's muscle tissue (National Research Council (U.S.) Steering Committee for the Petroleum in the Marine Environment Update, 1985; Poels et al., 1988; Johnsen and Lloyd, 1992; Zhou et al., 1997; Davis et al., 2002; Percival et al., 2008). According to Koning (1987), lake whitefish, a lipid-rich fish, may be more vulnerable to petroleum tainting compounds in comparison to a much leaner species such as walleye.

1.3.2 Review of previous incidents of petroleum-based fish taint in Alberta

Incidences of petroleum-based fish tainting in Alberta have been documented as early as 1950. Early that year, anglers reported that rainbow trout (*Oncorhynchus mykiss*) caught in the Bow River downstream from Calgary, Alberta had an obnoxious "oily" flavor. This taint incident was associated with petroleum refinery wastewater discharges (Krishnaswami and Kupchanko, 1969). A preliminary study conducted in 1958 by the Alberta Department of Health, revealed that exposing rainbow trout to various dilutions of effluents similar to those found in a local refinery induced an oily flavor in the flesh of the fish after an exposure time of 24 to 48 h. In a subsequent study, Krishnaswami and Kupchanko (1969) confirmed the occurrence of an oily flavor in rainbow trout exposed to diluted petroleum refinery wastewaters.

Jardine and Hrudey (1988) reported that the Athabasca River commercial fishery was closed in 1982 after a series of spill accidents at one of the oil sands plants during the winter of 1981-1982 resulted in the release of hydrocarbons into the Athabasca River

under ice conditions. At that time, there were reports of walleye and whitefish with "petroleum-like off-odors and off-flavors", prompting the exploration of potential compounds responsible for this odor and flavor impairment. Thus, Jardine and Hrudey (1988) investigated the concentrations at which specific oil sands wastewater chemicals could cause fish tainting. Samples were prepared by spiking the homogenized flesh of walleye with a wide concentration range of the following compounds: naphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, benzothiophene, dibenzothiophene, 2,3,5-trimethylnaphthalene, p-xylene and 2-5-dimethylphenol (Jardine and Hrudey, 1988). Boiled ten gram samples of spiked fish flesh were presented to a trained sensory panel composed of 11 screened panelists and evaluations were performed by the Consistent Series Threshold Method. Panelists were given 12 samples for each replication, 6 of which were tainted at different concentrations, and 6 of which were controls. Panelists were asked to evaluate each sample compared to a reference, first by smelling, and then, if no odor was perceived, by tasting and expectorating the samples. Jardine and Hrudey (1988) found that detection threshold values ranged from 0.09 mg kg^{-1} to 12.2 mg kg^{-1} for benzothiophene and 2,6-dimethylnaphthalene, respectively. Moreover, they found a relationship between detection threshold values and the compound's molecular weight and vapor pressure. As molecular weight increased, the detection thresholds increased whle vapor pressure appeared to be inversely related to the compound's detection threshold (Jardine and Hrudey, 1988).

Since Jardine and Hrudey's (1988) study, oil sands tailings waters have been used in three studies to assess their potential to taint fish (Diversified Research Laboratories Limited, 1992; Golder Associates Ltd., 1996; LeBlanc et al., 2000). Results of two of these studies have shown that dilutions of these tailings waters could cause fish tainting.

Diversified Research Laboratories Limited (1992), (Toronto, ON), conducted five sensory tests with rainbow trout, utilizing fish provided by Syncrude. These fish were raised in water from various tailings ponds, as well as water from Mildred Lake and Beaver Creek Reservoir. The research group performed two successive triangle tests to determine the perceivable difference between samples using an in-house panel of 12 individuals. To prepare samples, the fish were first filleted and boiled in sealed bags, before being flaked into a composite sample which was then portioned into 30-mL cups and presented to the panel for tasting. After identifying the odd sample, panelists were asked to indicate which sample they preferred. With 95% confidence intervals, it was

determined that fish raised in pond 1 were perceptibly different from those raised in ponds 3 and 4 and Mildred Lake. However, fish from pond 1 were not perceptibly different than those from ponds 5 and 6 and Beaver Creek Reservoir. Furthermore, there was no difference in preference among the samples. Although Diversified Research Laboratories (1992) found differences in perception between ponds 1 and ponds 3 and 4 and Mildred Lake, these conclusions appear to be in error. When the raw data presented in the Diversified Research Laboratories (1992) report were checked against statistical tables to obtain the "Critical Number of Correct Responses in a Triangle Test" (Meilgaard et al., 1999) no statistical differences are observed. Thus re-analysis of the data indicates that no difference could be detected among the fish samples from any of the water sources. Moreover, even though chemical analysis of the waters' compositions were reported, no specific details of the waters' source and composition were presented, and no details on the exposure experiments were provided, limiting the understanding of the possible differences among the samples (Diversified Research Laboratories Limited, 1992).

Golder Associates (1996) in conjunction with Diversified Research Laboratories conducted fish flavor impairment studies to evaluate rainbow trout exposed to water from different sources at Suncor Energy Inc. and with different quality conditions. The exposure tanks contained 0.5% Tar Island dyke water, 0.5% refinery effluent water, Athabasca River water (laboratory exposures), Athabasca River water (field exposures) and a time zero water control (laboratory tap water). Samples were prepared as per the American Society for Testing and Materials (ASTM D3696-89, 1989), and were assessed using eight double triangle difference tests and double overall preference ranking. Panelists were instructed to chew the sample, expectorate it, and then perform the evaluation. Combining both triangle tests, at a 99% confidence interval, it was found that fish exposed to 0.5% Tar Island dyke water and 0.5% refinery effluent water had a perceptibly different taste than those exposed to lab Athabasca River water and field Athabasca River water, but not time zero water. Even though fish exposed to Athabasca River water in the field were not perceptibly different from time zero water, those that were exposed to Athabasca River water in the laboratory were. For the preference ranking evaluations, it was found that fish exposed to time zero water were the most preferred, whereas those exposed to 0.5% refinery effluent water were least preferred (Golder Associates Ltd., 1996). These findings seem peculiar, because there was no perceptible difference found between these two treatments. As in the aforementioned study, the

chemical composition of these water treatments were not described, and no reasoning was given for the results obtained, making the interpretation of results difficult. Furthermore, no chemical analysis of the fish tissue was presented.

A third study was conducted by Leblanc et al. (2000) at the PEI Food Technology Center in Charlottetown, Prince Edward Island. In this research, hatchery-raised rainbow trout were exposed to consolidated tailings ponds waters (CTPW) from each of Syncrude, Suncor and Albian Sands Energy Inc. at concentrations of 10%, 1%, 0.1% and 0.01% or 0% (as control) (v/v). Dechlorinated Fort McMurray municipal drinking water was used to prepare dilutions. Fourteen panelists from the Athabasca region were screened and trained prior to evaluations. Fillets were minced into a homogenous mixture and cooked using a microwave oven. Samples were assessed for odor and flavor using a 10 point difference-from-control test. For the Syncrude samples, it was found that the 10% sample was significantly different ($p \leq 0.05$) in flavor from the control and the 1% samples, and that the 1% sample was significantly different ($p \leq 0.05$) in aroma from the 0.1 % and control samples. Overall, samples were described as having a strong, fishy, oily and sweet aroma and flavor. A significant difference ($p \leq 0.05$) in aroma and flavor was observed for the 0.1%, 1% and 10% Suncor samples when compared to the control. These samples were described as being oily, fishy, metallic, sweet, bitter, musty and sour. For the Albian Sands samples, the aroma of the 1% and 10% samples were significantly different ($p \leq 0.05$) from the control, while the flavor of the 0.1%, 1% and 10% samples were significantly different ($p \leq 0.05$) from control. These samples were described as sour, oily, strong and fishy with sweet, bitter and muddy notes (LeBlanc et al., 2000). Leblanc et al. (2000) provided an insight into the differences in flavor and aroma perception when fish is exposed to different CTPW concentrations, showing the potential of tailings water to induce fish taint. Unfortunately, the chemical composition of the CTPW were not provided, raising the question of which tailings pond water components might be responsible for fish tainting.

Industrial activity around the Athabasca River region, such as oil sands operations, may have an impact on the perceived sensory qualities of aquatic species (Diversified Research Laboratories Limited, 1992; Golder Associates Ltd., 1996; LeBlanc et al., 2000). However, there is no documentation that fish caught near the oil sands operations have an off-flavor during times when there have not been accidental releases of process-affected waters by the extraction and upgrading plants in the Athabasca River region. Fish in the Athabasca oil sands area of Alberta are naturally

exposed to bitumen. Erosion by the Athabasca River and its tributaries has led to the exposure of bitumen-containing outcrops in some river valleys. Conly et al. (2002) provide a geological cross-section that illustrates how this erosion cuts into the oil sands bearing stratum. These outcrops were the first indication of the presence of oil sands, long before the oil sands industry started. Thus, if fish tainting in this river system is a problem, it may be difficult to identify whether the potential source of the taint is the oil sands operations or natural outcrops of oil sands (Conly et al., 2002).

To the best of my knowledge there are no recent official records of tainted fish in the oil sands region of the Athabasca River, nonetheless, concerns from regulatory and First Nations groups have arisen, because anecdotal reports suggest an off-flavor in fish caught near the oil sands operations. The specific compounds responsible for causing off-flavors and off-odors in fish around the oil sands area have not been determined with certainty.

1.4 Taint assessment

Sensory testing has proven to be the most reliable and sensitive methodology for the assessment of food taint (Davis et al., 2002). The human sense of smell is far more sensitive than instrumental chemical analyses; the human nose is 10- to100-fold more sensitive than the most sensitive gas chromatography, which would detect approximately 10^9 molecules per millilitre (Meilgaard et al., 1999). Taint can be perceived by the senses of smell and taste. However, because the sense of smell is responsible for 80% of flavor perception (McGinley et al., 2000) and the ingestion of NAs has the potential to cause harmful effects on animals (Rogers et al., 2002), the studies completed for this thesis focused on the assessment of taint by olfaction. Thus, a description of the physiology of the sense of smell is essential.

1.4.1 Olfaction

Flavor perception is a complex combination of taste, aroma, chemical response and texture, but also cognitive and psychological effects (Meilgaard et al., 1999; Högnadóttir, 2000; Baigrie, 2003). The sense of smell is the most complex and unique in structure and organization of all five senses. It is not only the main source of flavor sensation, but it is also one of the important tools of our mechanism of defence by creating an aversion response to malodors and irritants (McGinley et al., 2000).

Odorants are small molecules, generally less than 1 kDa. Odors are typically comprised of several hundred odorants carried in an air stream which itself contains many other odorants. Although only few odorants are the key contributors to certain odors, our perception as a whole depends on the interaction of all of them (Lawless and Heymann, 1998; Högnadóttir, 2000).

We perceive odors when the volatiles of a product that are sniffed (voluntarily or otherwise) enter the nasal passage and reach the olfactory receptors in the roof of the nasal cavity; either directly through the nose (orthonasal route) or indirectly via the retronasal path when swallowing or exhaling (Meilgaard et al., 1999; Högnadóttir, 2000; Kilcast, 2003). Olfactory receptors are comprised of approximately ten to twenty-five million olfactory cells, which constitute the olfactory epithelium. With sufficient stimulus, the olfactory epithelium triggers electrical impulses via olfactory nerves to the olfactory bulb in the brain (McGinley et al., 2000). Odor receptors are easily saturated, thus when panelists perform odor evaluations it is necessary to wait a few seconds between sample evaluations to allow the olfactory receptors to reset (Kilcast, 2003).

A variety of chemical analyses can be performed to assess the composition of olfactory taint including; gas and liquid chromatography, gas chromatography-mass spectrometry (GC-MS), Ultraviolet (UV), infrared absorption and the electronic nose (Tidmarsh et al., 1985; Berna et al., 2008). Although chemical analyses aid in the assessment of taint by providing precise results and high detection sensitivity, considerable challenges are experienced where complex mixtures (e.g. mineral oil, fuel, oil, petrol) of taint compounds are present and when tainting is caused by compounds in which volatiles occur at the lower limits of analytical detection (Höfer, 1998). Thus, chemical analyses are seen as less practical in comparison to sensory analytical techniques. Chemical analyses are best used to determine the possible compounds responsible for taint and to confirm concentrations of contaminants (Davis et al., 2002). The final judgment about the presence of taint in a food product relies on human assessors (Reilly and York, 2001; Davis et al., 2002).

1.5 Sensory evaluation techniques for taint assessment

There are several sensory evaluation techniques that aid in the assessment of taint. The choice of a specific sensory evaluation method depends on many factors, such the quantity of product available, the information desired, and the sensory capabilities of

the panel. Thus, there is no universal sensory test used for the evaluation of all taints (Jardine, 1988, Meilgaard et al., 1998).

Analytical sensory procedures are widely used in the assessment of taint. "Descriptive tests" characterize and quantify the perceived intensities of specific sensory attributes. "Difference or discrimination tests" are used to determine if there are perceivable differences between samples with similar sensory attributes and can be used to determine the threshold of a substance. Threshold estimation is used to determine the concentration at which a compound suspected of causing taint can be perceived by an individual. When analytical sensory techniques are used, trained human assessors are used as analytical instruments (Lawless and Heymann, 1998).

Descriptive analysis is used for the assessment of fish taint to create a product profile of the perceptual differences between tainted and non-tainted samples or food products. The profiling of a food product generates objective information on the qualitative and/or quantitative properties of its individual sensory attributes, including appearance, odor, flavor and texture (Meilgaard et al., 1999). This technique requires the use of highly trained human assessors because individuals must be capable of detecting, describing and quantifying the perceived sensory characteristics of samples with consistent and accurate terms known as "descriptors" (Lawless and Heymann, 1998; Meilgaard et al., 1999). Descriptors for a specific food or beverage are often grouped in categories or compiled in a wheel format, such as the well-known wine aroma wheel (Noble et al., 1987). Sensory wheels have been used in environmental and fish tainting studies to describe off-odors and off-flavors in catfish (Van der Ploeg, 1991), drinking water (Suffet et al., 2004), wastewater biosolids (Suffet et al., 2009), and air (McGinley et al., 2000).

Discrimination tests are widely used in the assessment of food taint to ascertain if sensory differences in products or stimuli can be perceived between tainted and non-tainted food products. Heras et al. (1992) conducted discrimination tests to evaluate the tainting potential of the water-soluble fraction of light crude oil in Atlantic salmon (*Salmo salar*). The fish were exposed to three different concentrations of the hydrocarbon, and an experienced panel evaluated the cooked samples by a series of duo-trio difference tests. The flavor of the exposed fish was significantly different from the control ($p<0.01$), even when the fish were exposed to low concentrations for a short time. Redenback (1997) used triangle tests to evaluate flavor impairment of fish exposed to pulp and paper mill effluents and observed a significant difference in taste between

effluent-exposed fish and control ($p<0.05$). However, the most common method of assessing food taint by sensory discrimination tests is through threshold estimations, because threshold values give an objective reference point above which consumer perception and preference may be affected (Prescott et al., 2005).

1.5.1 Threshold assessment

Thresholds are the limits of the sensory capacities of an individual or a group of individuals. Generally, threshold is defined as "the concentration in a specified medium that is detected or recognized by 50% of a specified population" (Saxby, 1996; Meilgaard et al., 1999; Kilcast, 2003). For any compound, there are several threshold measurements that can be assessed, such as absolute or detection threshold, recognition threshold and difference threshold (Meilgaard et al., 1999). For food taint assessment, the focus is usually the estimation of detection thresholds, which are defined as the minimum physical intensity of a stimulus needed to give rise to a sensation (Saxby, 1996).

All threshold determinations are based on the ascending method of limits (AML), where the quantity of physical stimulus to be tested is presented in an ascending concentration series of successive discrete steps until there is a change in response, i.e. until the subject perceives a sensation. In this method, assessors are forced to choose among alternative samples at each concentration step (forced choice), even if they haven't detected any differences (Lawless and Heymann, 1998).

Sensory threshold testing has been broadly used for the assessment of taint because with this methodology it is possible to measure the sensitivity of an individual or group of individuals to specific stimuli. Furthermore, it is possible to measure the ability of a chemical species to evoke sensory responses, factors which will determine the concentration at which a suspected tainting compound can be perceived (ISO, 2002).

Thresholds are not absolute values, but rather are estimates due to the variations among and within individuals (Lawless and Heymann, 1998). The ability of panelists to detect an odor or flavor varies greatly as a result of the broad range of random variation in individual physiological and psychological aspects (Saxby, 1996) and in factors such as state of hunger, mood, alertness, attention, fatigue, health, gender and menstrual cycle (Meilgaard et al., 1999). Therefore, when estimating the threshold of a compound the group average threshold is determined to quantify the biological activity of the stimulus (Lawless and Heymann, 1998). Due to the wide range of individual variation in

perception it is essential to control those variables that would affect sensory evaluations, such as experimental variables, training, environment and sample preparation.

Training has a great impact on panelist performance; it has been reported that panelists' sensitivity and memory can improve, producing precise and consistent reproducible measurements (Lawless and Heymann, 1998). Training can lower a panelist's threshold as much as 1000-fold (Meilgaard et al., 1999). During training, panelists must become knowledgeable regarding test procedures, sample exposure techniques (sniffing, expectoration, swallowing), length of exposure, order of sampling, and the substance being tested to generate familiarity with the sensory properties of the compound of interest. After training, panelists should be prepared to participate in the assessment with no further instructions (Meilgaard et al., 1999).

When estimating thresholds, panelists are used as analytical instruments, thus they must practice objective evaluations, leaving aside personal preferences towards the sample to be assessed. Moreover, to determine if an individual is qualified to participate in a trained sensory test panel, it is essential to screen assessors for sensory acuity and use those subjects who have above average sense of smell and/or taste (Saxby, 1996). These subjects can be selected through clinical olfactory assessments such as the of *n*-butanol threshold (Cain et al., 1988). To participate in the trained panels described in this thesis, candidates were required to detect 4 parts per million *n*-butanol and to describe, detect and identify low concentrations of Merichem NAs in phosphate buffer (PB) (0.5 mg L^{-1}).

Based on the literature, the most common sensory methods used for the assessment of taints by means of threshold estimation are the triangle test (Poels et al., 1988; Whitfield et al., 1988; Redenbach, 1997; Prescott et al., 2005; Mazzoleni and Maggi, 2007) and three-alternative forced choice method (3-AFC) (Davis et al., 1992; Annor-Frempong et al., 1997; Davis et al., 2002; Howgate, 2004; Galvan et al., 2007; Kennison et al., 2007) . Internationally recognized organizations such as the GESAMP (1989; 2002) and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1987) have recommended the use of the triangle test to determine thresholds when assessing the effects of environmental contaminants on the odor and flavor of exposed aquatic species. However, ASTM E1432-04 (2004), ASTM E679-04 (2004) and ISO 13301 (2002) sensory evaluation protocols recommended the use of the 3-AFC method to determine the threshold of a substance. Although the triangle test and 3-AFC methods diverge only slightly in design and instructions given to panelists, it has been suggested that these variations have a significant impact on the final outcome of the

results, as this difference influences the proportion of correct responses when panelists perform sensory evaluations (Frijters, 1981; MacRae, 1995). This contradiction in recommended methods shows the necessity of comparing the performance of the triangle test and 3-AFC method for the estimation of detection thresholds of compounds implicated in fish tainting.

1.5.2 Triangle test for threshold assessment

The triangle test is widely used in discrimination testing to determine if there are any detectable sensory differences between two products with similar composition (Meilgaard et al., 1999). Thus it is used in taint studies to establish whether or not a detectable difference exists between tainted and non-tainted samples, without recognition of the nature of those differences (Davis et al., 1992).

In the triangle test, the panelists are presented with three randomized coded samples, two of which are identical (either A-treatment material or B-control) and one different (A or B, respectively). The panelists' task is to identify the odd sample, which may be tainted or not (Davis et al., 1992; Lawless and Heymann, 1998). Samples should be counterbalanced across all panelists within the six possible presentation orders ABB, BAB, BBA, AAB, ABA and BAA (Lawless and Heymann, 1998). The triangle test results are analyzed using a probability table to compare the number of correct responses to the probability expected by chance alone of correctly identifying the odd sample ($p=1/3$) (Meilgaard et al., 1999). When detection thresholds are to be evaluated, triangle test sample sets are presented to panelists with the stimulus of interest in ascending concentration. The threshold of a substance is determined as the lowest concentration where a significant ($p \leq 0.05$), number of panelists correctly identify the odd sample according to probability tables (Meilgaard et al., 1999), as described above.

For example, Redenback (1997) reviewed investigations of the effects of pulp and paper mill effluents on the taste and odor perception of exposed wild fish. In these reports, a series of triangle test were used to determine the concentrations at which the exposed fish were deemed to be tainted. Redenback (1997) reported that tainting occurred in rainbow trout, eulachon (*Thaleichthys pacificus)*, Dolly Varden char (*Salvelinus malma*) and sockeye salmon (*Oncorhynchus nerka)* at concentrations below 0.08% ($v\ v^{-1}$).

Prescott et al. (2005) used triangle tests to determine the detection thresholds for 2,4,6-trichloroanisole (TCA) in white wine (2.1parts per trillion). The authors related the

detection threshold to the concentration at which white wine consumers would begin to reject wine containing TCA (3.1 parts per trillion). In another study, Mazzoleni and Maggi (2006) evaluated the effect of wine styles on the detection threshold of TCA in red and white wine. Using a triangle test, it was determined that TCA detection thresholds depended on the wine style.

1.5.3 Three-alternative forced choice (3-AFC) for threshold assessment

The 3-AFC test is a variation of the family of multiple-AFC methods and is used in the assessment of thresholds (Lawless and Heymann, 1998). In this method, assessors are presented with three randomized, coded samples, two of which contain the control or diluted sample, and one of which contain the stimulus of interest. The panelists' task is to choose the sample containing the target stimulus with the previous knowledge that "only one" sample has the stimulus under study. Samples sets should be counter balanced across all panelists, within the three possible presentation orders, ABB, BAB, BBA to avoid positional bias (Lawless and Heymann, 1998).

In the 3-AFC method, the panelists evaluate the difference among samples to find the sample with the sensory stimulus that is stronger. This guideline was established after Frijters (1981) observed that sensory adaptation occurred when assessors evaluated sensory differences by triangles, which had an impact on the discrimination capabilities of assessors.

When detection thresholds are determined by the 3-AFC method, the stimulus is presented to panelists according to the AML. The detection threshold of an individual is the lowest concentration of three consecutive correctly identified samples. The detection threshold of a group of individuals is the geometric mean of the group's individual thresholds (Lawless and Heymann, 1998).

1.5.4 Triangle test vs. 3-AFC test

As described in the preceding sections, both the triangle test and 3-AFC method are commonly used for threshold estimation, including taint assessment (Davis et al., 1992; Davis et al., 2002). The differences in panel performance between the triangle test and the 3-AFC were first observed by Byer and Abrams (1953) when they performed discrimination tests of aqueous solutions of quinine sulfate and dextrose. In their study, panelists evaluated the different solutions by the triangle test, and approximately half of the assessors were unable to correctly identify the odd stimulus. However, when the same

panelists were asked to identify the weakest or strongest stimulus, individuals made the correct decision (Byer and Abrams, 1953). This finding was known as “the paradox of discriminatory nondiscriminators” (Gridgeman, 1970). Based on the theories of Thurstone, Frijters stated that the paradox was unreal, as the difference in performance was due to the less complex decision-making strategies used by panellists in the 3-AFC (Frijters, 1979). This behavior has also been observed and analyzed by several researchers on several food products (Stillman, 1993; MacRae, 1995; Masuoka, Hatjopoulos, O'Mahony, 1995; Rousseau and O'Mahony, 1997) and serves as the basis of the greater advantages the 3-AFC offers in comparison to the triangle test when determining thresholds.

Although both sensory tests have the same probability of panellist selection of the correct response by chance (1/3) and only differ in the instructions provided to the assessors and the presentation of the stimulus (see section 1.5.2 and 1.5.3), the 3-AFC method generates a larger proportion of real identifications. This is due to the different cognitive processes undertaken when panelists perform sensory discrimination tasks (Rousseau, 2001). In the 3-AFC test, the nature of the differences in the samples is specified; for example, the panelist will know that the stimuli of two samples will be weak while the third one will be strong (O'Mahony, 1995). In the triangle test, the panelist does not know the specific nature of the differences; thus, individuals are required to discriminate among the sensory differences between samples (Dessirier and O’Mahony 1999). The difference in performance does not rely on the fact that the assessors are given more information to complete the 3-AFC, but on the fact that panelists undertake different optimal decision rules when performing sensory discrimination tasks (Rousseau, 2001). In other words, when panelists perform a discrimination task such as the triangle test, a mental evaluation of the different perceptual intensities between the three samples is made. This process may be confusing to panelists because finding the odd sample requires a comparison and estimation of differences of the three samples. The 3-AFC method only requires the correct perception of the strongest intensity of the three stimuli. There is greater variability in judging differences than in judging intensities, thus panelists perform better in the 3-AFC methodology due to the simplicity of the task and the lower variability when making decisions (Lawless and Heymann, 1998)

In summary, several analytical sensory procedures can be used to evaluate fish taint. For example, discrimination testing has been widely used to determine differences

between a sample suspected of being tainted and a control, and in the estimation of the detection threshold of a substance with the potential of causing taint. The GESAMP (1989) and the ECETOC (1989) have suggested the use of the triangle test to estimate detection thresholds of substances related to fish taint. However, organizations such as the ISO (ISO 13301, 2002) and the ASTM (ASTM E679-04 and E1432–04, 2004) have recommended the use of the 3-AFC to determine thresholds. This contradiction reinforces the need to study the performance of the triangle test and the 3-AFC method to determine odor detection thresholds of environmental contaminants such as NAs, to assess the role that these group of compounds may have in the perceived sensory properties of fish.

1.6 Research objectives

NAs are the most water-soluble organic compounds in oil sands process-affected waters and have been suggested as a cause of fish taint. Therefore, the overall purpose of the present research was to determine by sensory evaluation techniques the role of this group of compounds as a source of fish taint. The objectives of the present research were as follows:

- To compare the triangle test and the 3-AFC test for estimation of the olfactory detection threshold of NAs (Chapter 2)
- To determine the odor detection threshold of commercial and oil sands process-affected water NAs by analytical sensory methods (Chapter 3).
- To determine if a consumer sensory panel can distinguish between wild fish collected from a water basin near the oil sands and wild fish collected from two other water basins in Alberta (Chapter 4).

1.7 Figures

Figure 1.1 Location of the Alberta oil sands deposits (Government of Alberta - Environment, 2008)

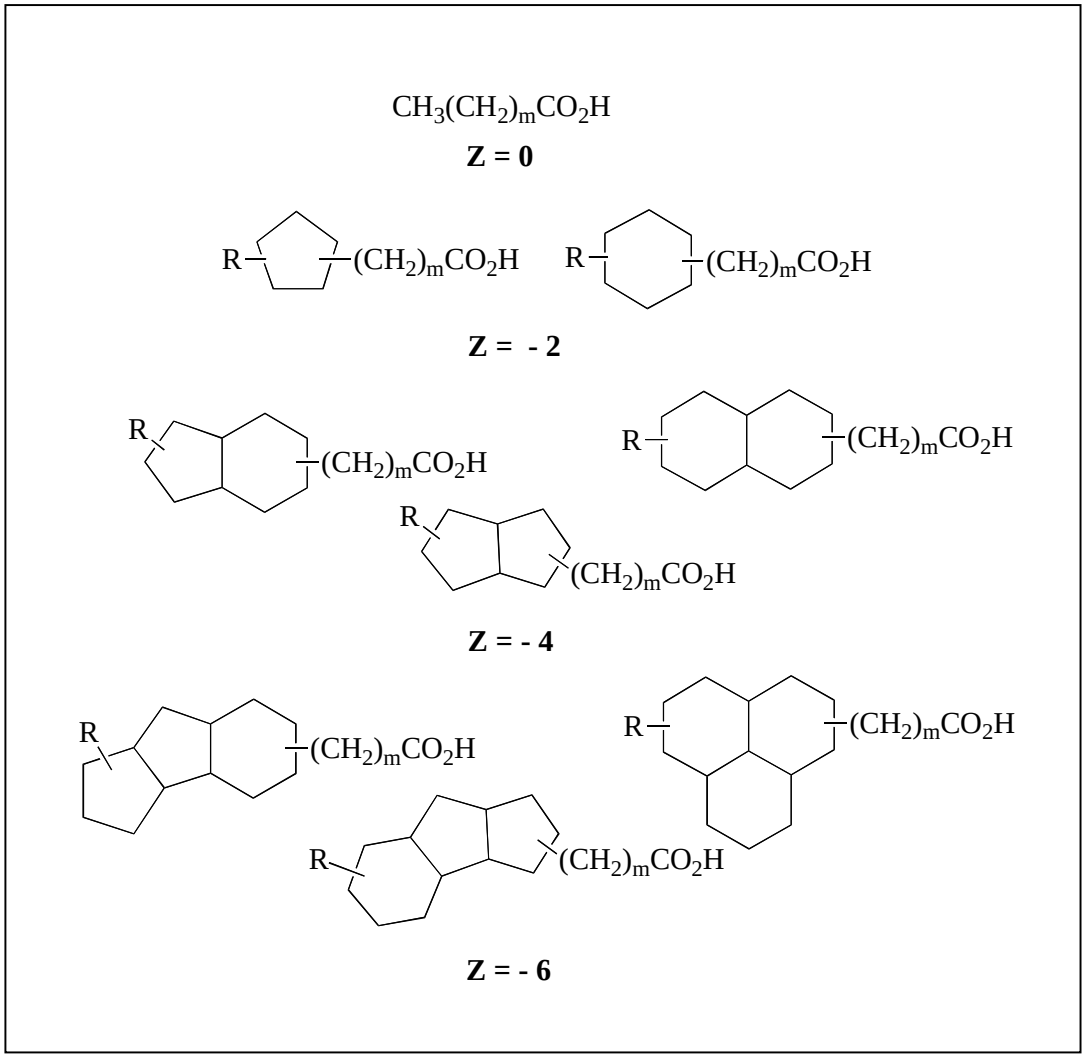

Figure 1.2 Structures of some NAs with the general formula $C_nH_{2\ n+Z}O_2$, where $m \geq 0$ and R is alkyl (Holowenko et al., 2002).

1.8 References

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Chapter 2

Comparison of the triangle test and 3-alternative forced choice method for the evaluation of fish taint odor detection thresholds

2.1 Introduction

Water-soluble substances are easily absorbed into fish, and when they reach a concentration at which they can be detected sensorially, the fish quality can be altered (Davis et al., 2002). According to the Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) tainting in aquatic organisms is defined as “a foreign flavor or odor in the organisms induced by conditions in the water to which the organisms are exposed" (GESAMP, 1982). Flavor impairment of aquatic species can be caused by compounds from many different sources, including aquatic microorganisms, by-products of marine algae, chemical contaminants in the water, onset of enzymatic degradation, oxidation of lipids and handling and storage (Höfer, 1998; Tucker, 2000; Wilkes et al., 2000). However, the majority of fish tainting incidents have occurred in extremely polluted waters and after coastal tanker accidents (Höfer, 1998).

Petroleum off-flavors occasionally develop in wild fish when waters are contaminated by accidental spills of diesel fuel or gasoline, from boats, refinery effluents, losses from pipelines and municipal or industrial discharges (GESAMP, 1982). Several compounds related to the oil and refinery industry have been implicated in fish tainting (Davis, 2002; Höfer, 1998; Motohiro, 1983). According to the GESAMP (1982), the principal components of crude oil that have caused fish tainting include phenols, dibenzothiophenes, naphthenic acids (NAs), mercaptans, tetradecanes and methylated naphthalenes. NAs are compounds of interest in the study of fish health and contamination (Nero et al., 2006; Young et al., 2007; Young et al., 2008) because they are water-soluble, toxic organic compounds found in petroleum refinery wastewaters and oil sands process-affected waters (Schramm et al., 2000). NAs are a group of carboxylic acids that occur naturally in petroleum deposits (oil sands, bitumen, and crude oils). They comprise a complex mixture of saturated acyclic, monocyclic and polycyclic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$, where n is the carbon number and Z represents the hydrogen atoms lost as the structures form rings (Clemente and Fedorak, 2005; Headley and McMartin, 2004). It has been hypothesized that NAs have the

potential to taint fish, although there are no current sensory studies published on the detection threshold of NAs.

A variety of chemical analyses can be performed to assess taint, such as steam distillation, gas chromatography, gas chromatography-mass spectrometry (GC-MS) and the use of the electronic nose (Berna et al., 2008; Tidmarsh et al., 1985). Although these analyses provide precise results with high detection sensitivity, they still face significant challenges where complex mixtures of tainting compounds (e.g. mineral oil, fuel, oil, petrol) are present and when taint is caused by compounds in which volatiles occur at the lower limits of analytical detection (Höfer, 1998). Chemical analyses should only be used to determine the possible compounds responsible for taint and to confirm concentrations of contaminants (Davis et al., 2002). Because taint is a sensory experience, the final judgment about its presence in a food product relies on human panelists (Davis et al., 2002; Reilly and York, 2001). To study tainting, it is necessary to identify the compound that is the source of the off-flavor or off-odor and estimate its detection threshold. Analytical sensory techniques, such as the triangle test and the three-alternative forced choice (3-AFC) method, are most widely used for taint assessment for threshold estimation (Annor-Frempong et al., 1997; Davis et al., 1992; Davis et al., 2002; Galvan et al., 2007; Howgate, 2004; Prescott et al., 2005; Redenbach, 1997). The detection threshold of a substance is the minimum concentration necessary to produce a sensory response (Kilcast, 1995) and it can potentially provide a reference point above which consumer`s perception and preference may be affected (Prescott et al., 2005). Detection threshold values are not absolute, but rather they are estimates because of the great variations in perception among and within individuals (Lawless and Heymann, 1998).

In the food industry the triangle test is traditionally used in discrimination tests to determine sensory differences in products with similar composition (Meilgaard et al., 1999). Although the triangle test is not conventionally employed for the assessment of detection thresholds, it has been broadly used for the assessment of taints because it allows investigators to establish whether or not a detectable difference exists among samples without recognizing the nature of those differences (Davis et al., 1992). In the triangle test, panelists are presented with three randomized, coded samples, two of which are identical and one which is different. The panelists' task is to identify the odd sample, which may be tainted or not. The 3-AFC method (a variation of the triangle test) is widely used for the assessment of thresholds. In the 3-AFC test, the panelist is presented with three randomized, coded samples, two of which are the control and the third contains

the substance under study. The panelists' task is to indicate the sample with the stronger sensory stimulus (Davis et al., 1992; Lawless and Heymann, 1998), with the previous knowledge that only one sample has the stimulus.

The triangle and 3-AFC methods diverge slightly in design and instructions and it has been suggested that these variations have a significant impact on the final results, as these differences influence the proportion of correct responses when panelists perform sensory evaluations (Frijters, 1981; MacRae, 1995). International agencies, such as the American Society for Testing and Materials (ASTM E679-04, ASTM E1432–04) and the International Organization for Standardization (ISO 13301), have suggested the use of the 3-AFC method to assess thresholds. However, Poels et al. (1998) and the GESAMP (2002) (based on the ECETOC, 1987; GESAMP, 1989) recommend the use of the triangle test for threshold determinations associated with fish taint. This contradiction shows the necessity for the establishment of a standard method for the assessment of sensory thresholds that pertain to fish tainting.

The primary objective of this study was to compare the performance of two analytical sensory methods, the triangle test and the 3-AFC method, to establish a sensory detection threshold protocol for the olfactory assessment of fish taint. The secondary objective was to determine the odor detection threshold of a commercial (Merichem) NAs preparation. In the present study, taste evaluations were not performed, due to the plausible toxicity of NAs (Rogers et al., 2002). Instead, odor detection thresholds were evaluated for the assessment of taint in fish, as the sensory flavor experience when consuming a food product is mainly due to the volatile compounds present in the food, perceived by the sense of smell (Meilgaard et al., 1999).

2.2 Materials and methods

2.2.1 Chemicals and fish

Refined Merichem NAs were a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX). Fish samples used to perform the odor panels were walleye (*Sander vitreus,* formerly *Stizostedion vitreum*) fillets purchased from a local fishmarket and stored at -20 °C prior to use.

2.2.2 Sensory methods

Odor detection thresholds of NAs were determined in PB (pH 8) and in walleye fish. The former was intended as a preliminary evaluation, with the objective of familiarizing the panelists with the olfactory attributes of NAs and to obtain the odor detection threshold for NAs in a simple system. Thresholds were determined by both the triangle test (GESAMP, 2002) and the 3-AFC method. Panels were replicated the following day to avoid olfactory fatigue and to compare reliability of both sensory tests (e.g. NAs in PB were evaluated by the triangle test, 24 h later the test replicate was assessed). Approval for the study was obtained from the Human Research Ethics Board in the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta.

2.2.3 Panelists

The olfactory sensory panels consisted of nine or ten non-smoking, trained individuals, aged 18 to 39 years. The majority of panelists were females. Panelists were recruited from the general University of Alberta population. Panelists were screened for superior olfactory acuity by a series of clinical olfactory assessments (Cain et al., 1988) and their ability to detect and identify low concentrations of Merichem NAs (0.5 mg L^{-1}) in PB. Before performing sensory evaluations, individuals were trained and familiarized with the test method and the odor properties of Merichem NAs. Panelists were required to be free of colds, fragrances and were asked not to eat or drink 1 h prior to the assessments because these factors may affect odor evaluations. Panels were performed in special sensory testing rooms equipped with individual booths and white light.

2.2.4 Sample preparation and sensory evaluation of Merichem NAs in PB

For the sample preparation of NAs in PB, appropriate volumes of a stock solution of 100 mg Merichem NAs L^{-1}, dissolved in 0.1 M NaOH, were added to 40-mL capped glass vials (Fisher Scientific, Nepean, ON). The solutions were then brought up to 10 mL with room temperature (~21 °C) PB, pH 8. The vial contents were mixed by manual agitation. Each sample concentration was given a randomly selected three-digit code to blind the sample identity. Sample sets were presented to the panelists in ascending concentration order using a randomized block design within sample sets to avoid positional bias. Participants took short sniffs of the vial contents. To ‘zero’ their noses

and avoid olfactory carry-over panelists sniffed room temperature water and waited 20 s between samples.

2.2.4.1 Odor threshold assessment of Merichem NAs in PB by triangle test

Preliminary odor sensory panels were performed (data not shown) to establish the concentration range for the evaluation of Merichem NAs in PB. Six concentrations of Merichem NAs in PB were assessed for detection threshold determination; 0.25, 0.5, 1, 2, 3 and 5 mg NAs L^{-1}, with 0 mg NAs L^{-1} used as the control. Samples were prepared and presented to participants as described above. Each panelist received three samples, two of which were identical (either control or spiked) and one which was different (either control or spiked), along with a questionnaire and a glass of Brita®-purified water. Panelists were asked to identify which of the three samples they perceived to be different. The triangle test was 'forced choice', thus if the participant was unsure, one sample had to be selected. Detection thresholds were determined using a p=1/3 probability table at $\alpha<0.05$ by comparing the number of positively identified responses at each concentration with the total number of panelists ($\alpha<0.05$) (Meilgaard et al. 1991).

2.2.4.2 Odor threshold assessment of Merichem NAs in PB by 3-AFC test

Twelve concentrations of Merichem NAs in PB were used for 3-AFC testing; 0.00063, 0.00125, 0.0025, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2 and 3 mg NAs L^{-1}, with 0 mg NAs L^{-1} used as the control. Samples were prepared and presented to participants as described above. Each panelist received a questionnaire, a glass of purified water and three samples, two of which had the control solution (PB) and the third had the NAs solution. The panelist's task was to identify which of the three samples was perceived to contain NAs. Again the test was 'forced choice'. A panelist's threshold was determined as the lowest Merichem NAs concentration of three successive correct evaluations. The mean threshold for the panel was determined by calculating the geometric mean of all panelists' thresholds (Meilgaard et al., 1999).

2.2.5 Sample preparation and sensory evaluation of Merichem NAs in fish

Walleye fillets were thawed overnight for 16 h in a refrigerator at <4 °C and then cut into approximately 1-cm cubes. Flesh from all fillets was mixed to create a composite. Fish flesh composite (300 g) was homogenized in a food processor (Power Pro II, Black and Decker, Hunt Valley, MD) on a continuous low setting for approximately 30 s.

Appropriate volumes of 50 mM sodium bicarbonate (1 mL $NaHCO_3$ per 30 g fish flesh) and a suitable portion of a stock solution of 4.0 g Merichem NAs L^{-1}, dissolved in 50 mM sodium bicarbonate, were mixed in a test tube and vortexed (Vortex Genie 2 Mixer, Fisher Scientific) for 5 s. This solution was added to the homogenized fish by mixing in the food processor for 30 s. After stopping to scrape down the sides of the bowl and mix the sample with a silicone spatula, the sample was homogenized again for approximately 30 s, transferred to a glass bowl and mixed by hand. After the homogenization process, nine 20-g portions were wrapped individually in heavy duty aluminum foil as per ASTM E1810-96 (2004) and steamed in a food steamer (Vitacuisine Steamer, T-fal, Canada) for 8 min, or until the internal temperature reached 70 °C. Samples were allowed to cool to room temperature, unwrapped and placed in 120-mL Styrofoam cups and covered with un-vented lids.

2.2.5.1 Odor threshold assessment of Merichem NAs in fish by the triangle test

Ten NAs-spiked samples were prepared with the following concentrations; 0 (only sodium bicarbonate), 0.5, 1, 2, 4, 6, 8, 10, 12, 16 and 20 mg Merichem NAs kg^{-1} fish. Concentration ranges for the evaluation of NAs in fish were established in previous sensory panels (data not shown). Fish samples with only sodium bicarbonate were used as control. Sample blinding, presentation, evaluation and statistical analysis were identical to the triangle test evaluation of the Merichem NAs in PB described above.

2.2.5.2 Odor threshold assessment of Merichem NAs in fish by the 3-AFC test

For the assessment of the odor detection threshold of NAs in fish by the 3-AFC, nine spiked samples were prepared with the following concentrations; 0.05, 0.08, 0.2 0.3, 0.5, 0.8, 1, 1.3 and 1.7 mg Merichem NAs kg^{-1} fish. Fish samples with only sodium bicarbonate were used as control. Sample preparation, blinding, presentation, evaluation and statistical analysis were identical to the 3-AFC evaluation of the NAs in PB described above.

2.2.6 Statistical methods to determine odor detection thresholds and test-retest reliability

Panels were performed in duplicate and results from replications were analyzed both independently to examine each method's reliability, and pooled (Golder Associates Ltd., 1996; Jardine and Hrudey, 1988; Redenbach, 1997) to estimate odor detection

thresholds of NAs in PB and in fish. For the triangle test, the probability of the pooled correct results was compared to the probability table "Critical Number of Correct Responses in a Triangle Test" (Meilgaard et al., 1999). For the 3-AFC test, the geometric mean of the best estimate individuals' threshold was calculated. Test-retest reliability coefficients (*r*) were determined to evaluate the reliability (Daly and Bourke, 2000) of the triangle test and the 3-AFC method.

2.3 Results

2.3.1 Odor detection thresholds of Merichem NAs in PB and fish

Appendix A provides detailed results from the various odor panels. The estimated odor detection thresholds of Merichem NAs in PB determined using the triangle test were 2 mg L^{-1} and 0.5 mg L^{-1} from panels 1 and 2, respectively (Table 2.1). Inconsistent threshold values were acquired when Merichem NAs in fish were assessed by the triangle test. Two detection thresholds, 2 mg kg^{-1} and 20 mg kg^{-1} ($p<0.05$), within the testing of panel 1 were determined on the first evaluation day (Table 2.1), whereas the estimated threshold obtained for the replicate was 16 mg kg^{-1} ($p<0.05$). In comparison, very different and concise detection thresholds of Merichem NAs were determined by the 3-AFC method. Detection thresholds of 0.05 and 0.03 mg L^{-1} were estimated in PB, whereas thresholds of 0.86 and 0.41 mg kg^{-1} were obtained in fish (Table 2.1).

When the data were pooled, the estimated detection thresholds of Merichem NAs in PB assessed by the triangle test and the 3-AFC method were 0.5 mg L^{-1} ($p<0.05$) and 0.04 mg NAs L^{-1}, respectively. The pooled odor detection threshold for Merichem NAs in fish evaluated by the triangle test was determined to be 10 mg kg^{-1} ($p<0.05$), whereas the detection threshold evaluated by the 3-AFC method was 0.57 mg kg^{-1} (Table 2.1).

2.3.2 Tests reliability of triangle and 3-AFC tests

According to Albrecht et al. (2008) the validity of an olfactory test is a function of its reliability, which can be measured by correlating the scores of a test that has been administered to a group of subjects at two different times. This is known as the test-retest reliability coefficient (*r*) and Fig. 2.1 shows the determination of these coefficients. The *r* values for the triangle and 3-AFC protocols are presented in Table 2.1.

2.4 Discussion

Higher detection thresholds were observed in fish compared to the thresholds detected in PB with each method. From the pooled results (Table 2.1), the detection threshold of Merichem NAs in fish was 20 times higher than those recorded in PB using the triangle test. When thresholds were assessed using pooled data from the 3-AFC method (Table 2.1), a difference factor of 14 was found for the detection threshold of NAs in fish in comparison to PB. The differences in thresholds are likely due to the complexity of odor evaluation in fish flesh. PB is a simple aqueous medium, whereas fish flesh is complex. Performing evaluations in a complex food system such as fish is a more complicated task in comparison to evaluating odors in PB. In the former, the panelist faces a more challenging assignment because the subject must isolate the stimulus of interest from the background volatiles present in fish flesh.

It was interesting to observe that in general, when panelists evaluated a fish sample by the triangle test, frequently they were unable to correctly identify the odd stimulus (Fig. 2.1B). However, when the same panelists were asked to identify the odd sample from using the 3-AFC method, which includes the knowledge that only one of the samples contains the stimulus, individuals more frequently made the correct decision (Fig. 2.1D). This behavior was previously observed and analyzed by several researchers (Stillman, 1993; MacRae, 1995; Masuoka et al., 1995; Rousseau and O'Mahony, 1997) and served as the basis for the establishment of the greater advantages that the 3-AFC offers in comparison to the triangle test when determining thresholds. Even though both sensory tests have the same probability of success (1/3), the 3-AFC method generates a larger proportion of real identifications, which is due to the different cognitive processes (i.e. different optimal decision rules) undertaken when panelists perform sensory discrimination tasks (Rousseau, 2001). According to Dessirier and O'Mahony (1999) and Rousseau (2001), the 3-AFC method is more efficient because of its advantageous cognitive strategies. In the 3-AFC method, panelists seek the sample with the highest *intensity* on a sensory continuum. In comparison, the triangle test requires the panelists to compare the sensory *differences* between samples (Dessirier and O'Mahony 1999). When panelists perform a discrimination task such as the triangle test, a mental evaluation of the different perceptual intensities between the three samples is made. This process may be confusing to panelists because finding the odd sample requires a comparison and

estimation of intensities of the three stimuli. The 3-AFC method requires only the correct perception of the strongest of the three stimuli. There is greater variability in judging differences than in judging intensities, thus panelists perform better in the 3-AFC method due to the simplicity of the task and the lower variability when making decisions (Lawless and Heymann, 1998).

Despite the relative cognitive simplicity of the 3-AFC method over the triangle test, Davis et al. (1992) reported no difference between the two methods when assessing odor detection thresholds of diesel fuel in fish by a trained sensory panel. They found no difference in test sensitivity as the thresholds obtained from both methods were similar (Davis et al., 1992). The results presented in the aforementioned study showed some incongruities in their method and data analysis. For example, the concentration ranges used in both sensory methods were not incremented in a constant progression of stimulus; as well, the stimuli presented to panelists were not incremented in equal ratios, leaving large gaps between concentrations. Furthermore, the concentrations presented are those of diesel in the exposure tanks. the assumption of a 100% uptake of the compound by the fish and equal distribution of the diesel in the fish flesh is not accurate, because when exposures are performed *in-vivo*, it is expected that the concentration of the compound in fish tissue will be higher than that in the aqueous environment (Howgate, 2004) due to bioconcentration and that the concentrations in specific tissues will vary depending on biological aspects, such as the tissue's lipid content (Howgate, 2004; Percival et al., 2008; Zhou et al., 1997). Moreover, chemical analyses to confirm the concentration of diesel in the samples evaluated were not presented and a correlation analysis of the tests was not performed, which is essential when comparing the performance of two methods applied to one specific problem (Doty et al., 1995).

In one of the sensory panels in this study, a bimodal threshold (2 mg kg^{-1} and 20 mg kg^{-1}) was generated when NAs in fish were evaluated by the triangle test (Table 2.1), whereas only one detection threshold (0.86 mg kg^{-1}) was found when NAs in fish were evaluated by the 3-AFC method, suggesting that the triangle test might not be an appropriate method for the evaluation of Merichem NAs thresholds. Overall, the pooled odor detection threshold values of NAs obtained by the 3-AFC method were almost 10-fold lower than those obtained by the triangle test, in both PB (0.04 and 0.5 mg L^{-1}) and fish (0.57 and 10 mg L^{-1}). Moreover, the r values obtained from the 3-AFC method (r = 0.98 and r = 0.65; Fig. 2.1) were much higher in comparison to those obtained from the

triangle test (r = 0.59 and r = - 0.17 Fig. 2.1), supporting the superior reliability of the 3-AFC method.

The results of this study showed that the 3-AFC method is a superior protocol compared to the triangle test when assessing contaminants related to fish taint. The 3-AFC method provided more efficient cognitive strategies when panelists made their evaluations (Rousseau, 2001), leading to more stable estimates of thresholds during both sets of tests. This is reflected in the threshold and reliability values obtained by the two sensory techniques.

The odor thresholds reported in this paper are specific for this commercial preparation of refined Merichem NAs, and these concentrations must not be considered to represent all NAs. St. John et al. (1998) demonstrated that the compositions of NAs from various commercial sources differ from one another. Similarly, Clemente et al. (2003) showed that the compositions of commercial NAs differed from the compositions of NAs extracted from oil sands process-affected waters. The differences in composition of these complex mixtures known as NAs will likely influence the odor thresholds of various preparations. In future work, we will use the superior 3-AFC method to evaluate the odor thresholds of NAs preparations from various sources.

2.5 Tables

Table 2.1 Odor detection thresholds for duplicate individual panels and pooled panels of Merichem NAs in PB and walleye fish determined by the triangle test and 3-AFC method. Numbers of correct responses from panelists for each test are given in Appendix A.

	Individual test results						**Pooled results**	
	Triangle Test			3-AFC			Triangle Test	3-AFC
	Panel 1	Panel 2	*r* [a]	Panel 1	Panel 2	*r*	Panels 1 & 2	Panels 1 & 2
NAs in PB (mg L^{-1})	2	0.5	0.59	0.05	0.03	0.98	0.5	0.04
NAs in Fish (mg kg^{-1})	2 & 20[b]	16	- 0.17	0.86	0.41	0.65	10	0.57
Ratio, (Fish ÷ PB)							20	14

[a] *r* = Test-retest reliability coefficients
[b] Two threshold concentrations were detected

2.6 Figures

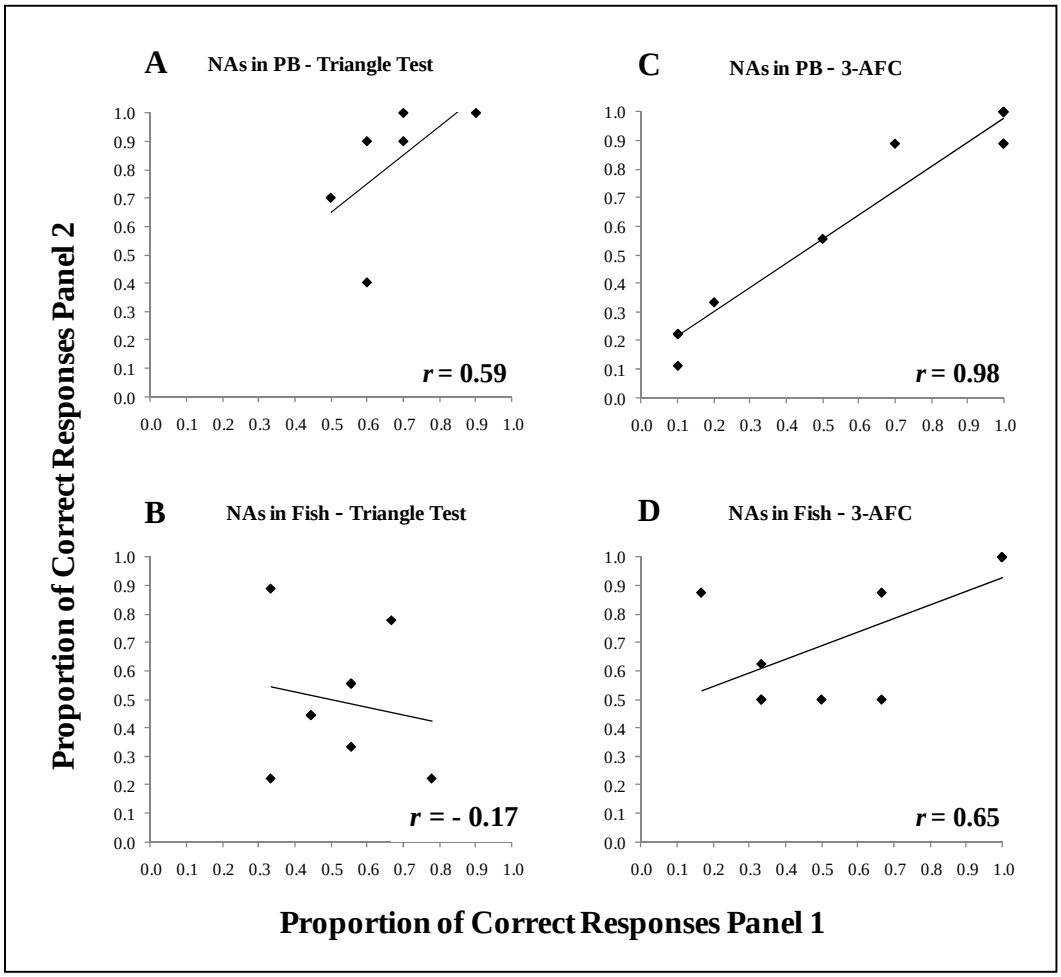

Figure 2.1 Test-retest reliability coefficients, *r*, for the olfactory detection thresholds of Merichem NAs in PB and fish evaluated by two sensory methods. NAs in PB by triangle test (A), NAs in fish by triangle test (B), NAs in PB by 3-AFC method (C), and NAs in fish by 3-AFC method (D).

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Chapter 3

Odor detection thresholds of naphthenic acids from oil sands process-affected water and from commercial sources

3.1 Introduction

The oil sands in northeastern Alberta are considered to be one of the largest petroleum reservoirs in the world (Government of Alberta - Energy, 2009). Oil sands are deposits of a highly biodegraded, viscous form of non-conventional crude oil; composed of a mixture of sand, clay and high molecular weight petroleum known as bitumen (Koning and Hrudey, 1992). Generally, the bitumen can be extracted from the oil sands by a method known as "Clark hot water process", where alkali-hot water and steam are used to separate the petroleum from the sand-clay matrix (Schramm et al., 2000).

The residual wastewater produced by this method must be captured and held in storage ponds due to the toxicity of compounds such as NAs, which are harmful to many aquatic organisms (Clemente and Fedorak, 2005). Despite the efforts of Alberta's oils sands mining industry to avoid the release of tailings into surface and underground water resources, a risk of leakage still exists. For example, an upset at one of the oil sands plants during the winter of 1981 to 1982 caused the release of hydrocarbons into the Athabasca River (Jardine and Hrudey, 1988). This situation reinforces the need for studies to enhance our understanding of the potential negative impact of substances such as NAs on water systems and the aquatic life they support. Furthermore, information of the effects that wastewaters may have on the aquatic environment will become essential when creating regulations for future tailings ponds reclamation (Clemente and Fedorak, 2005).

NAs are natural compounds found in most hydrocarbon deposits, including oil sands, bitumen and crude oils. When oil sands bitumen is recovered, the alkali nature of the water used in the extraction process solubilizes and concentrates the NAs into the tailings ponds aqueous phase (Holowenko et al., 2002). It has been reported that process-affected waters contain NAs at concentrations in the range of 20 to120 mg L^{-1} (Clemente and Fedorak, 2005).

In general, the use of the term "naphthenic acids" is somewhat imprecise. This term usually applies to mono-carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$. Various companies sell products called naphthenic acids. These acids have many commercial uses, for example, they improve water resistance and adhesion of

concrete, preserve wood and textiles, promote rubber vulcanization and stabilize vinyl resins (Brient et al. 1995). Using a gas chromatography-mass spectrometry (GC-MS) method, St. John et al. (1998) demonstrated that the compositions of naphthenic acids from various suppliers differed. Using the same GC-MS method, Clemente et al. (2003) showed that the compositions of commercial naphthenic acids differed from the compositions of "naphthenic acids" extracted from oil sands process-affected waters. In addition, "naphthenic acids" extracted from several different oil sands process-affected waters were shown to have different compositions (Clemente et al., 2003).

Previous studies have been conducted to examine the toxicity of NAs (reviewed in Clemente and Fedorak, 2005) and their detection in fish (Young et al., 2007; Young et al., 2008). Moreover, NAs have been suspected of causing 'fish taint', which is defined as "a foreign flavor or odor in the organisms induced by conditions in the water to which the organisms are exposed" (GESAMP, 1989). Anecdotal reports have suggested an off-odor in fish caught near the oil sands operations in the Athabasca River in northeastern Alberta, Canada; however, the identity and source of the tainting compound has not been discovered. It has been hypothesized that NAs have the potential to cause tainting in fish (GESAMP, 1977), but this has not been proven.

Chemical analyses can help determine the possible compounds responsible for taint and to confirm levels of contaminants (Davis et al., 2002). For example, Young et al. (2007) developed a protocol to detect NAs in exposed fish and reported that NAs can be detected in fish by GC-MS at concentrations as low as 1 µg NAs g^{-1} fish.

However, because taint is a sensory experience, the presence of taint should be determined by human assessors. Sensory threshold testing has long been used as a method of assessing the minimum concentration at which a compound can be detected sensorially (Kilcast, 1995) and can provide a reference point above which consumers' perception and preference for a food product may be affected (Prescott et al., 2005). The 3-Alternative Force Choice (3-AFC) test is an internationally recognized protocol used to determine the best estimate of human sensory threshold for a given substance (Meilgaard et al., 1999; ISO 13301, 2002; ASTM E1432-04, 2004; ASTM E679-04, 2004). In this analytical sensory protocol panelists are provided with sample sets in an ascending concentration of the stimulus. Each set is composed of three samples, two of which are 'control', and the third one containing the stimulus to be studied. The panelist's task is to choose the sample containing the target stimulus, with the previous knowledge that only one sample in each set has the stimulus under study (Lawless and Heymann, 1998). In the

current research, detection thresholds of NAs were assessed only by olfaction, due to the potential toxicity of this group of compounds (Rogers et al., 2002).

Previously reported studies have shown that NAs from different sources have differences in their chemical structure. To the best of our knowledge, no studies have been conducted to analyze the human-perceived sensory properties of this group of compounds. However, to better understand the potential role of NAs in fish tainting, information such as the detection thresholds and odor qualities of this group of compounds are essential. Thus the objectives of this study were (1) to characterize NAs from two commercial sources (Merichem and Acros) and NAs extracted from Syncrude pond 9 water, (2) to determine the olfactory detection thresholds of these NAs in phosphate buffer (PB) pH 8 and in fish, and (3) to characterize the odor qualities of the various sources of NAs.

3.2 Materials and Methods

3.2.1 Fish and chemicals

Fish samples used to perform the odor panels were walleye (*Sander vitreus*, formerly *Stizostedion vitreum*) fillets purchased from a local fishmarket and stored at -20 °C prior to use.

Refined Merichem NAs were a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX), Acros NAs were purchased from Acros Organics (Geel, Belgium) and, as outlined below, pond 9 NAs were extracted from one of the oil sands tailings pond water (SCL 9) provided by Syncrude Canada Ltd (Fort McMurray, AB).

3.2.1.1 Extraction of NAs from Pond 9 tailings water

Syncrude Pond 9 water was used as a source of aged oil sands NAs. This 4-ha experimental reclamation pond was constructed in 1993 when it was filled with 50,000 m^3 of tailings process water from Mildred Lake Settling Basin (Siwik et al., 2000; Han et al., 2009). No mature fine tailings were placed in this pond, and no fresh process water has been added to Pond 9 since it was established. Water from Syncrude's Tailings Pond 9 was received in August, 2009 and stored at 4 °C in white, plastic pails prior to extraction.

NAs were extracted from 40 L of Pond 9 water using the following procedure. Each 1-L of portion of water was acidified to pH ≤ 2 with 2 mL with HCl and extracted with three 50-mL portions of dichloromethane. The combined dichloromethane extracts from the water extractions were left to evaporate at room temperature (20 °C) in a fume hood. The extracts from the 40 1-L extractions were combined and the final concentrated extract was transferred to 2-dram vials and dried under nitrogen. The resulting residue weight of the Pond 9 NAs was determined. The 40 L of Pond 9 water yielded a total residue of 638 mg.

In order to generate a control extract that mimicked the storage of tailings pond water in plastic buckets, 40 L of reverse osmosis water was stored in white, plastic buckets. The water was kept in the buckets for 3 days at room temperature, and then transferred to 4 °C storage. The NAs from the control water were extracted and concentrated as outlined in the paragraph above. The 40 L of control water yielded a total residue of 2.6 mg.

The control samples for the threshold determination of Pond 9 NAs were spiked with approximately the same percentage of total control residue as the Pond 9 highest test sample. For example, the highest Pond 9 concentration tested in the PB trials was 8 mg L^{-1}. For one 10 mL sample, there would be 0.08 mg of Pond 9 residue. From the total 40 L extracted, 638 mg were recovered, therefore, the 10 mL sample vial contained 0.013% of the total extracted material. Controls were spiked with 0.1 mL of a 5 mg L^{-1} stock solution. Each control sample vial contained 0.5 x 10^{-3} mg, which is 0.019% of the total control residue.

3.2.2 Sensory methods

The odor detection thresholds of NAs were determined in PB (pH 8), and in walleye fish. The former was intended as a preliminary evaluation, with the objective of familiarizing panelists with the olfactory attributes of NAs and the sensory testing method, and to obtain the odor detection threshold for NAs in a simple aqueous system. As reported in Chapter 2, the three-alternative forced choice (3-AFC) test was found to be superior to the triangle test for the determination of the olfactory detection threshold of NAs, thus, the 3-AFC method (ISO 13301, 2002) was used for this study. Sensory panels for PB and for fish, for each NAs source, were performed on different days to avoid olfactory fatigue. Approval for this study was obtained from the Research Ethics Board

of the Faculty of Agricultural, Life and Environmental Sciences at the University of Alberta (Edmonton, AB).

3.2.3 Panelists

The trained olfactory sensory panels consisted of nine to ten non-smoking individuals, aged 18 to 39 years; the majority were female. Panelists were recruited from the general University of Alberta population and were screened for superior olfactory acuity by a series of clinical olfactory tests (Cain et al., 1988) and their ability to detect and identify low concentrations of Merichem NAs in PB (0.5 mg L^{-1}). Supra-threshold concentrations (concentration range evidently above the detection threshold (Lawless and Heymann, 1998)) of the odorant in both PB (pH 8) and fish were used for training to familiarize panelists with the panel methods and the odorant they were to detect. Panelists were required to be free of colds and fragrances, and were asked not to eat or drink 1 h prior to the assessments as these factors may affect odor evaluations. Panels were performed in special sensory testing rooms equipped with individual booths and white light. Evaluations of the NAs in the specified media to determine detection thresholds were repeated twice. Panelists received remuneration for participation.

3.2.4 Olfactory detection threshold of NAs in PB

The Acros, Merichem, and Pond-9 NAs solutions were prepared by adding appropriate volumes of a concentrated stock solution of 100 mg of NAs dissolved in 50 mM sodium bicarbonate to enough PB (pH 8.0; 1860 mL of 0.1 M NaOH mixed with 2000 mL of 0.1 M KH_2PO_4) to make one liter of each concentration. All stock solutions were held at room temperature in airtight Pyrex containers. For each test concentration series, 10-mL portions of the NAs-PB solution were added to 40-mL glass vials (Fisher Scientific; Ottawa, ON) for the olfactory evaluations. The vial contents were mixed by manual agitation.

Preliminary odor sensory panels were performed to establish the concentration range for each source of NAs. The concentrations used to determine the odor threshold of Merichem NAs in PB were 0.00063, 0.00125, 0.0025, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2 and 3 mg L^{-1}. Similarly, eight concentrations of Acros NAs (0, 0.5, 1, 2, 3, 4, 5, 6, and 7 mg L^{-1}) and seven concentrations of Pond 9 NAs (0, 0.1, 0.5, 1, 2, 4, 6, and 8 mg L^{-1}) were used. Each PB control for the Pond 9 NAs test was spiked with 0.1 mL of a 5 mg control residue per liter stock solution in 0.1 M NaOH.

For the sensory evaluations, each panelist received a questionnaire, a glass of purified water and three samples, two of which contained the control solution (PB) and the third the NAs solution. Panelists were informed that only one sample out of the three contained the stimulus. Sets of samples were presented with increasing concentrations of NAs. Each sample was given a randomly selected three-digit code in order to blind the sample identity. The panelist's task was to identify which of the three samples contained NAs. The test was 'forced choice', thus the participant had to choose one sample from the three presented. Additionally, panelists were provided with an odor descriptor wheel (Appendix B) for environmental odors (McGinley et al., 2000) to evaluate the 'odor character' of each NAs source. Panelists were asked to provide as many descriptors as possible of each NA at each concentration step.

3.2.5 Olfactory detection threshold of NAs in steamed fish

Frozen walleye fillets were thawed overnight for 16 h. Three hundred grams of fish flesh were homogenized in a food processor (Power Pro II, Black and Decker, Hunt Valley, MD) on a continuous low setting for approximately 30 s. Appropriate volumes of 50 mM sodium bicarbonate (1 mL $NaHCO_3$ per 30 g fish flesh) and a suitable portion of a stock solution of NAs, dissolved in 50 mM sodium bicarbonate, were mixed in a test tube and vortexed (Vortex Genie 2 Mixer, Fisher Scientific) for 5 s. Acros and Merichem NAs to be spiked in fish were prepared from a stock solution of 4000 mg NAs L^{-1} and the Pond 9 NAs amounts added were based on a stock solution of 2000 mg NAs L^{-1}. This solution was added to the homogenized fish by mixing in the food processor for 30 s. After stopping to scrape down the sides of the bowl and mix the sample with a silicone spatula (Starfrit, Nashua, NH), the sample was homogenized again for approximately 30 s, transferred to a glass bowl and mixed by hand with a spatula.

After the homogenization process, 20-g portions were wrapped individually in heavy duty aluminum foil as per ASTM E1810-96 (2004) and steamed in a food steamer (Vitacuisine Steamer, T-fal, Canada) for 8 min, or until the internal temperature reached 70 °C. Samples were then allowed to cool to room temperature, unwrapped, placed in 120-mL Styrofoam cups and covered with un-vented lids.

For Merichem NAs in fish, nine spiked samples were prepared with the following concentrations; 0 (only sodium bicarbonate), 0.05, 0.08, 0.2 0.3, 0.5, 0.8, 1, 1.3 and 1.7 mg Merichem NAs per kg fish. Similarly, seven concentrations of Acros NAs (3, 6, 9, 12, 15, 18 and 21 mg kg^{-1}) and seven concentrations of Pond 9 NAs (6, 10, 14, 16, 18, 20 and

22 mg kg^{-1}) were used. For Merichem and Acros NAs evaluations, control fish samples contained only sodium bicarbonate. For Pond 9 NAs evaluations, control fish samples contained 1.0 mL of a 40 mg control residue per L stock solution in 50 mM sodium bicarbonate, added to 23.5 mL of $NaHCO_3$ for 420 g of fish flesh. Sample blinding, presentation, and evaluation were identical to the sensory method described for the assessment of NAs in PB.

3.2.6 Sensory data analysis

A panelist's best estimate odor detection threshold was determined as the lowest NAs concentration of three successive correct identifications of the NAs sample in a set of three samples. The detection threshold of each source of NAs was estimated by calculating the geometric mean of the panelists' best estimate thresholds (ISO 13301, 2002). The odor detection thresholds of each source of NAs in each testing media were pooled from the two replicate panels. To compare significant differences between thresholds (α=0.05), estimated thresholds were normalised by log-transformation (Daly and Bourke, 2000). Single factor analysis of variance was used to compare differences among the detection thresholds of the three sources of NAs. Tukey's test was used to locate the differences between the thresholds, as appropriate.

3.2.7 Chemical analyses of NAs

Elemental analyses to determine the proportions of C, H, N, and S in the commercial NAs and the Pond 9 NAs were performed using a Carlo Erba EA 1108 elemental analyzer by the analytical lab in the Department of Chemistry at the University of Alberta. The O content was calculated by difference, assuming the samples contained only C, H, N, S, and O. The combined Pond 9 extract was analyzed by Fourier transform infrared spectroscopy (FTIR) to determine the concentration of NAs in the extract (Scott et al. 2008). The commercial NAs and the Pond 9 NAs were analyzed by GC-MS using the method described by (Young et al., 2008) to collect the total ion chromatograms and to produce three-dimensional plots of the distribution of various acids in the NAs preparations (Holowenko et al., 2002).

3.3 Results and Discussion

3.3.1 Odor detection thresholds of NAs by a trained panel

The odor detection thresholds of Acros, Merichem and Pond 9 NAs in PB, as evaluated by the trained panelists, were 1.5 mg L^{-1}, 0.04 mg L^{-1} and 1.0 mg L^{-1}, respectively (Table 3.1). The differences detected between thresholds for NAs from three different sources, using the same testing method, illustrates the complexity of working with NAs.

The odor detection thresholds of Merichem NAs and Pond 9 NAs in steamed fish are presented in Table 3.1. The detection threshold of Acros NAs was not formally derived and it was estimated to be above 21 mg Acros NAs per kg fish, which was the highest concentration of NAs tested in this medium. Panelists consistently showed difficulties in detecting the Acros NAs in the steamed fish. Usually, higher concentrations of a stimulus would be evaluated until an odor detection threshold is generated; however, it is unlikely that higher concentrations of NAs would be found in the environment. The odor detection threshold of Acros NAs in fish is above the concentrations used in this study. Based on the analysis of variance it was determined that only the detection threshold for the Merichem NAs in PB was significantly different than the other two sources.

The odor detection thresholds of NAs were higher in fish in comparison to the thresholds detected in PB (Table 3.1). For example, the detection thresholds of Pond 9 NAs in fish were 12 times higher than those observed in PB. Odor evaluations in fish flesh are a more complex task compared to PB, because in the former, panelists must isolate the odors of the compound of interest (NAs) from those of the fish.

The olfactory detection thresholds for Merichem, Acros and Pond 9 NAs were significantly different from each other in both media. According to the results obtained from the Tukey's test, the Merichem NAs detection threshold was significantly less ($p<0.05$) than for Acros and Pond 9 NAs. Moreover, the odor detection threshold of each source of NAs estimated in fish was 12 to 15 times greater than in PB (Table 3.1).

3.3.2 Odor descriptors of three sources of NAs in PB and steamed fish

Panelists were presented with an environmental odor descriptor wheel (McGinley et al., 2000) and they were asked to describe the 'odor character' of the NAs as they were making their threshold evaluations. Odor descriptors were collected at the detection

thresholds and supra-threshold concentrations of NAs in both PB and fish to compare the odor attributes among the three sources of NAs at two different concentrations.

Although the general descriptor categories were similar for all three NAs (chemical and earthy), the specific odor qualities and the frequency each was mentioned, were more variable among the different sources of NAs (Table 3.2). For example, at their respective detection thresholds, the Pond 9 NAs were reported to smell like tar, gasoline or solvent, whereas the majority of panelists described the Acros and Merichem NAs as having a plastic or gasoline smell, respectively. The secondary odor qualities of each of the three sources of NAs were also variable. Acros NAs were comprised of floral and fruity odor notes, the Pond 9 NAs were floral and earthy smelling, and Merichem NAs were described as having an earthy undertone.

The odor qualities of NAs changed at the higher concentration. The odor qualities of the high concentration of Acros NAs were described with a greater number of chemical descriptors. The Merichem and oil sands NAs were also reported to have a stronger chemical component at the supra-threshold concentration; however, their secondary odor profile changed. Merichem NAs presented earthy and fragrant undertones at the higher concentration, whereas the previously floral secondary odors in the oil sands NAs were absent and medicinal qualities were mentioned at supra-threshold concentrations.

As in the PB evaluations, differences among the odor attributes of the three different NAs in fish were provided by panelists (Table 3.2). Although the general descriptor categories of the samples were similar in fish, the nature of the category and the frequency with which they were identified were more variable. It appeared to be more difficult for the panelists to generate descriptors of the fish samples spiked with NAs than PB.

For the steamed fish evaluations, the odor qualities of the three different NAs preparations was described as chemical in nature. At the respective detection thresholds, both the Merichem and Pond 9 NAs were reported to smell of gasoline; however, the Pond 9 NAs also smelled of tar (Table 3.2). Both Merichem and Pond 9 NAs were described with earthy secondary odor characteristics. The Acros NAs were primarily reported as having oil and plastic odors to them, with medicinal secondary odor components.

At the supra-threshold concentrations evaluated in fish, the intensity and number of odor descriptors increased when panelists evaluated the Merichem and oil sands NAs

(Table 3.2). Both NAs maintained their earthy odors, while their chemical descriptors changed. For example, the odor character of Merichem NAs were reported as smelling more of gasoline and oil in the steamed fish, in comparison to the varnish and gasoline combination detected in the oil sands NAs-spiked fish. Acros NAs were not evaluated at supra-threshold concentrations because a formal detection threshold was not generated.

3.3.3 Chemical characterizations of NAs used in this study

The extraction of Pond 9 water yielded was a dark brown viscous oil. The mass of residual oil from 40 L of Pond 9 water was 638 mg. Based on this gravimetric result, the concentration of the NAs in Pond 9 was 16 mg L-1. The concentration of naphthenic acids in Pond 9 water determined by FTIR analysis of this extract was 15.6 mg L^{-1}. Based on similar FTIR analyses, Siwik et al. (2000) reported NAs concentrations between 27-74 mg L^{-1} (mean 45.6 mg L^{-1}) in Pond 9 water, and Han et al. (2009) reported NAs concentration of 31 mg L^{-1} in this water. A routine NAs analysis of a Pond 9 water sample in June 2009 by Syncrude, gave a NAs concentration of 20 mg L^{-1} (T. Penner, Syncrude Canada Ltd., personal communication). The results from 2000 to present demonstrate the gradual decrease in NAs concentrations as a result of aging in Pond 9.

In order to prepare solutions for dosing PB and fish for the sensory threshold determinations, the initial concentration of NAs in Pond 9 water determined by FTIR (15.6 mg L^{-1}) was used. This reflects all of the acid-extractable material in the Pond 9 water that may potentially cause taint,

Table 3.3 summarizes the elemental analyses of the NAs preparations used in this study. The Merichem and Acros NAs have essentially the same composition, and are devoid of N and S. To ensure the elemental analysis method would detect S, a sample of Merichem NAs was spiked with 3-methylbenzothiophene. Analysis of this sample detected the presence of S (Table 3.3). Unlike the commercial NAs, the Pond 9 NAs extract contained N and S. In addition, the calculated amount of O was approximately double the amounts of O in the other NAs samples (Table 3.3).

Recent applications of high resolution mass spectrometry have revealed much new information about the components found in the NAs extracted from process-affected waters in relation to those that are commercially available. For example, Han et al. (2009) detected mono- and di-oxidized NAs (i.e. $C_nH_{2n+Z}O_3$ and $C_nH_{2n+Z}O_4$) in extracts from Syncrude oil sands process-affected waters. Similarly, Barrow et al. (2009) analyzed NAs from the oil sands area, they found compounds with formula $C_nH_{2n+Z}O_x$, where x = 2-5. In contrast, re-examination of ESI-FT-ICR MS data of Scott et al. (2009) showed that in the

commercial Merichem preparation >96% of the "NAs" are the parent acids ($C_nH_{2n+Z}O_2$) (Fedorak and co-workers, unpublished results). From these examples, it is clear that the elemental compositions of NAs differ markedly, depending upon their source. The higher O content in the Pond 9 NAs (Table 3.3) is consistent with the recent high resolution mass spectrometry data which show the presence of highly oxygenated NAs in oil sands process-affected waters.

GC-MS analyses of the three NAs preparations are shown in Fig. 3.1. These total ion chromatograms show that each preparation is distinctly different from the other two preparations. The Merichem and Pond 9 NAs show an unresolved hump, which is typical of NAs as shown in previous studies (St. John et al., 1998; Scott et al., 2005). The Merichem NAs (Fig. 3.1A) show more individual peaks emanating from the hump compared to the Pond 9 NAs (Fig. 3.1C) In contrast, the Acros NAs (Fig. 3.1B) give a markedly difference total ion chromatogram.

To help visualize the difference among various NAs preparations, Holowenko et al. (2002) converted data from total ion chromatograms of NAs into three-dimensional plots based on the general formula $C_nH_{2n+Z}O_2$. The three-dimensional plots for the Merichem, Acros, and Pond 9 NAs are given in Fig. 3.2 showing the distribution of acid according to carbon number (n) and Z values. Like the total ion chromatograms (Fig. 3.1) the three-dimensional plots showed that these three NAs preparations were very different. One very obvious difference was that the three-dimensional plot of the Pond 9 NAs showed the presence of acids with carbon numbers of 22 to 30 (Fig. 3.2C). These were absent in the Merichem NAs (Fig. 3.2A) and scarce in the Acros NAs (Fig. 3.2B).

Clemente et al. (2004) hypothesized that the so called C22+ acids that appeared in the three-dimensional plots were due to presence of hydroxy-naphthenic acids which were derivatized with the addition of two *tert*-butyldimethylsilyl moieties. One moiety reacts with the carboxylic acid group and the other reacts with the hydroxy group. This would yield a higher molecular mass product that is detected as a C22+ naphthenic acid. Using GC-high resolution MS, Bataineh et al. (2006) confirmed the presence of two *tert*-butyldimethylsilyl moieties on the derivatized naphthenic acids that appear as the so called C22+ acids in the three-dimensional plots. The occurrence of these C22+ acids suggests that the NAs in the Pond 9 extract should be more highly oxygenated than the commercial NAs. The elemental analyses summarized in Table 3.3 showed that there was a higher proportion of O in the Pond 9 NAs than in the commercial NAs. These results showed that the three sources of NAs differed in their chemical composition.

3.3.4 Elemental differences among three sources of NAs

Another elemental difference between the Pond 9 NAs and the two commercial sources is the presence of sulfur in the analyzed sample. Sulfur is often associated with odorous compounds; however, it cannot be inferred that this is the reason for the differences in odor threshold or odor quality characteristics. Pond 9 naphthenic acids, which contained approximately 1% sulfur in the elemental analysis (Table 3.3), were not characterized with typical sulfur odors, such as rotten eggs or sewage, by trained panelists. As well, the detection threshold (1.0 mg L^{-1}) was in between the detection threshold of the other two naphthenic acid sources. In addition to the lower estimated detection threshold of Merichem naphthenic acids (0.04 mg L^{-1}), trained panelists generally reported that the intensity of the Merichem samples was stronger and the acids were more distinct smelling than the other naphthenic acids sources. Comparatively, the Acros naphthenic acids had a much higher odor detection threshold (1.5 mg L^{-1}), and were reported to be almost pleasant smelling in comparison to the Merichem samples. Acros and Merichem naphthenic acids did not contain sulfur in their respective elemental analyses.

Although Merichem, Acros and Pond 9 NAs represent a single family of compounds, this study has shown that their chemical and sensory profiles differ. There are mixtures of compounds derived from the same family that are known to have different sensory characteristics, such as vanilla and wine varietals. This has been observed in the odor qualities of vanilla, one of the most important flavorings worldwide. The flavor profile of vanilla varies according to country of origin and the method by which it is produced. For example, the aroma of Bourbon vanilla has been described as richly smooth, spicy and sweet, whereas Mexican vanilla has a sharp, slightly pungent aroma, with woody flavor notes (Reineccius, 2006). McCormick & Company, Inc. (2009) provide complete flavor-profile spider plots of vanilla from different sources. Vanilla flavor is composed of a wide range of different volatile components; however, its principal aromatic constituent is vanillin. Although the chemical structure of natural (methyl vanillin) and synthetic (ethyl vanillin) vanillin diverge slightly, and even though they have a similar vanilla-like odor, they have different odor detection thresholds in water, 0.02 (Matheis, 2007) and 0.065 ppm (Calkin and Jellinek, 1994), respectively.

Another example of similar products with different flavor profiles are those of wine from different regions. Several studies have been conducted to assess the odor and flavor profiles of wine in relationship to viticultural regions, including Malbec wines

from Argentina (Goldner and Zamora, 2006), Chardonay wines from Canada, California, Australia and France (Schlosser et al. 2004), Riesling wines from different areas in the Canadian Niagara Peninsula (Douglas et al., 2001) and young Mencía wines from northwest Spain (Vilanova and Soto, 2006). Odor profiles from these studies revealed that the wines had unique flavor and odor characteristics, in accordance with the winemaking techniques and the geographical area where the grapes were grown (Parr et al., 2007). This is due to differences in climate and soil characteristics of each region (Goldner and Zamora, 2006).

3.4 Conclusion

Trained panel odor detection thresholds of NAs are different between sources, as well as being expectedly lower in PB compared to fish. Just as elemental analyses showed some differences in chemical composition, descriptive sensory analysis revealed that the three different sources of NAs have different odor profiles. Thus, it is important to consider that the odor qualities and detection thresholds of NAs depend upon their source and chemical composition. Therefore, a particular preparation of NAs should not be considered to represent all NAs and commercial preparations of NAs may not represent NAs in oil sands process-affected waters.

3.5 Tables

Table 3.1 Odor detection thresholds of different sources of NAs in PB and steamed fish, evaluated by the 3-AFC method.

	Odor Detection Threshold		
	Merichem	**Acros**	**Pond 9**[A]
NAs in PB* (mg L^{-1}) (n=10, 9, 10)	0.04[a]	1.5[b]	1.0[b]
NAs in Fish* (mg kg^{-1}) (n=9, 10, 10)	0.6[a]	>21[†c]	12[b]
Ratio, (Fish ÷ PB)	15	>14	12

[a] NA extracted from Syncrude Pond 9. Concentrations based on FTIR analysis of extracted material.

[†] Not formally derived. It is estimated to be above 21 mg Acros NAs per kg fish

[*] Detection thresholds within a row with different superscripted letters are statistically significantly different ($p \leq 0.05$).

Table 3.2 Comparison of odor descriptors generated by a trained panel (n=10) for three sources of NAs in PB and in steamed fish at the olfactory detection threshold and supra-threshold concentrations. Odor descriptor wheel for environmental odors shown in Appendix B.

		Acros		**Merichem**		**Pond 9**	
	Medium	**Category**	**Descriptors**[a]	**Category**	**Descriptors**	**Category**	**Descriptors**[a]
Detection threshold[b] **concentration**	**PB**	**Chemical**	Plastic (7), Oil (4), Gasoline (3)	**Chemical**	Gasoline (6), Oil (6), Plastic (4), Solvent (4)	**Chemical**	Tar (2), Gasoline (2), Solvent (2)
		Floral	Fragrant (2)	**Earthy**	Woody (4), Earthy (3)	**Earthy**	Woody (2)
		Fruity	Sweet (2)			**Floral**	Fragrant (2)
	Steamed fish	**Chemical**	Oil (4), Plastic (2)	**Chemical**	Gasoline (2)	**Chemical**	Gasoline (2), Tar (2), Plastic (1), Varnish (1)
		Medicinal	Chlorinous (2)	**Earthy**	Musty (2), Peat-like (2)	**Earthy**	Peat-like (1)
Supra-threshold[c] **concentration**	**PB**	**Chemical**	Plastic (9), Oil (4), Solvent (4), Gasoline (3)	**Chemical**	Gasoline (7), Oil (5), Solvent (1)	**Chemical**	Gasoline (6), Tar (2), Varnish (2)
		Floral	Fragrant (3)	**Earthy**	Woody (2)	**Earthy**	Peat-like (3), Musty (2) Woody (2)
		Fruity	Sweet (2)	**Floral**	Fragrant (3)	**Medicinal**	Disinfectant (2), Vinegar (1)
	Steamed fish			**Chemical**	Gasoline (5), Oil (4), Turpentine (2)	**Chemical**	Varnish (3), Gasoline(2), Plastic (2)
				Earthy	Musty (2)	**Earthy**	Peat-like (2)

[a] Bracketed numbers denote frequency of responses by panelists

[b] Odor detection threshold refers to the group geometric mean of the lowest concentration of three consecutive correct identifications of NA containing samples.

[c] Supra-threshold concentration refers to a concentration above the detection threshold (Lawless and Heymann, 1998).

Table 3.3 Elemental analysis, by percent, of three sources of NAs.

NAs	C	H	O[a]	N	S
Merichem	73.63	9.01	17.36	0	0
Acros	73.40	9.64	16.96	0	0
Merichem + Sulfur[b]	73.28	8.59	17.25	0	0.88
Pond 9 Extract	59.71	6.82	32.18	0.24	1.06

[a] Calculated by difference.
[b] A 50 mg sample of Merichem NAs was spiked with 3 mg of 3-methylbenzothiophene to serve as a positive control for the presence of sulfur.

3.6 Figures

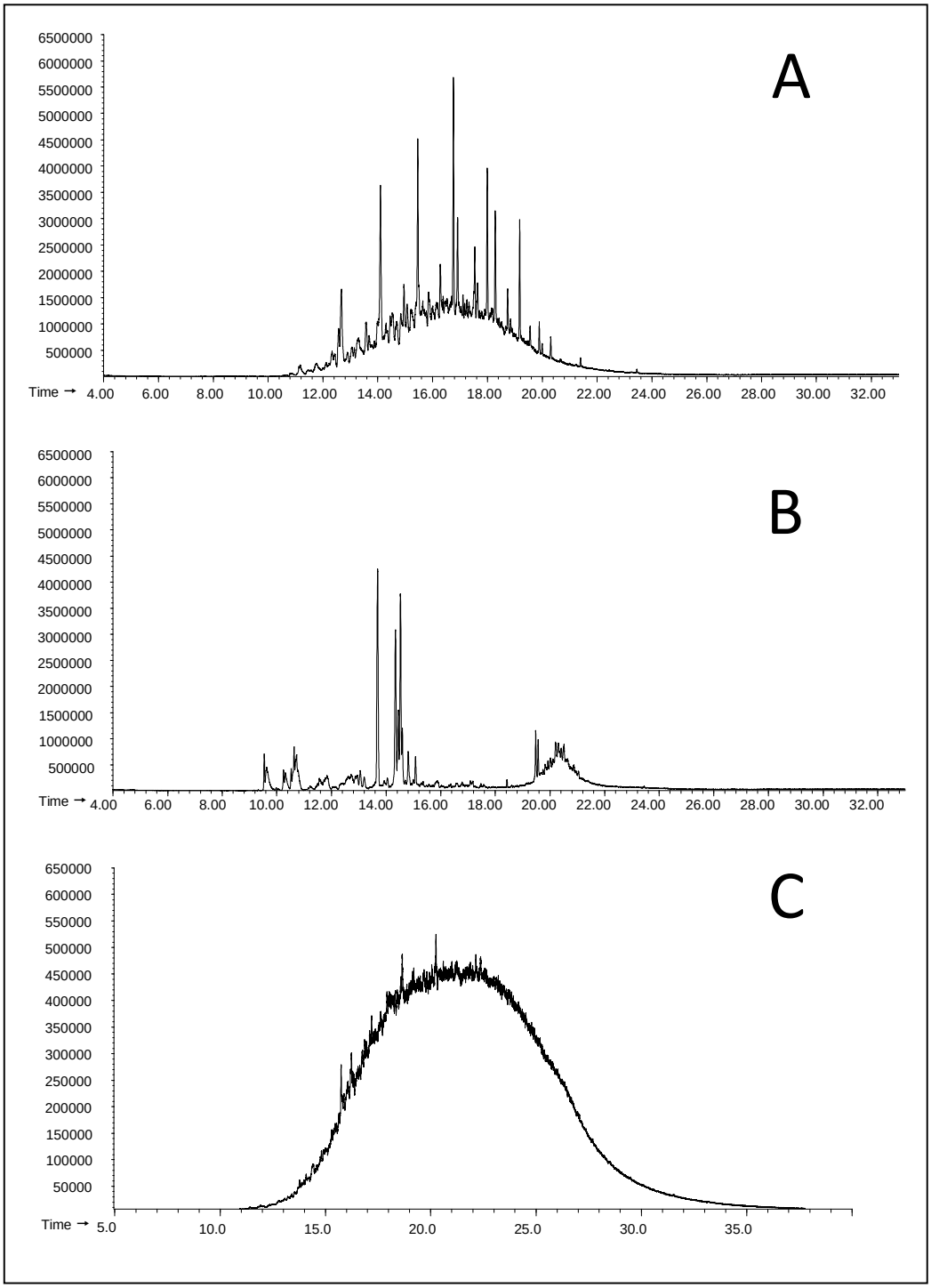

Figure 3.1 Merichem (A), Acros (B) and Pond 9 extracted (C) NAs total ion . chromatograms generated from GC-MS analysis of MTBSTFA derivatized.

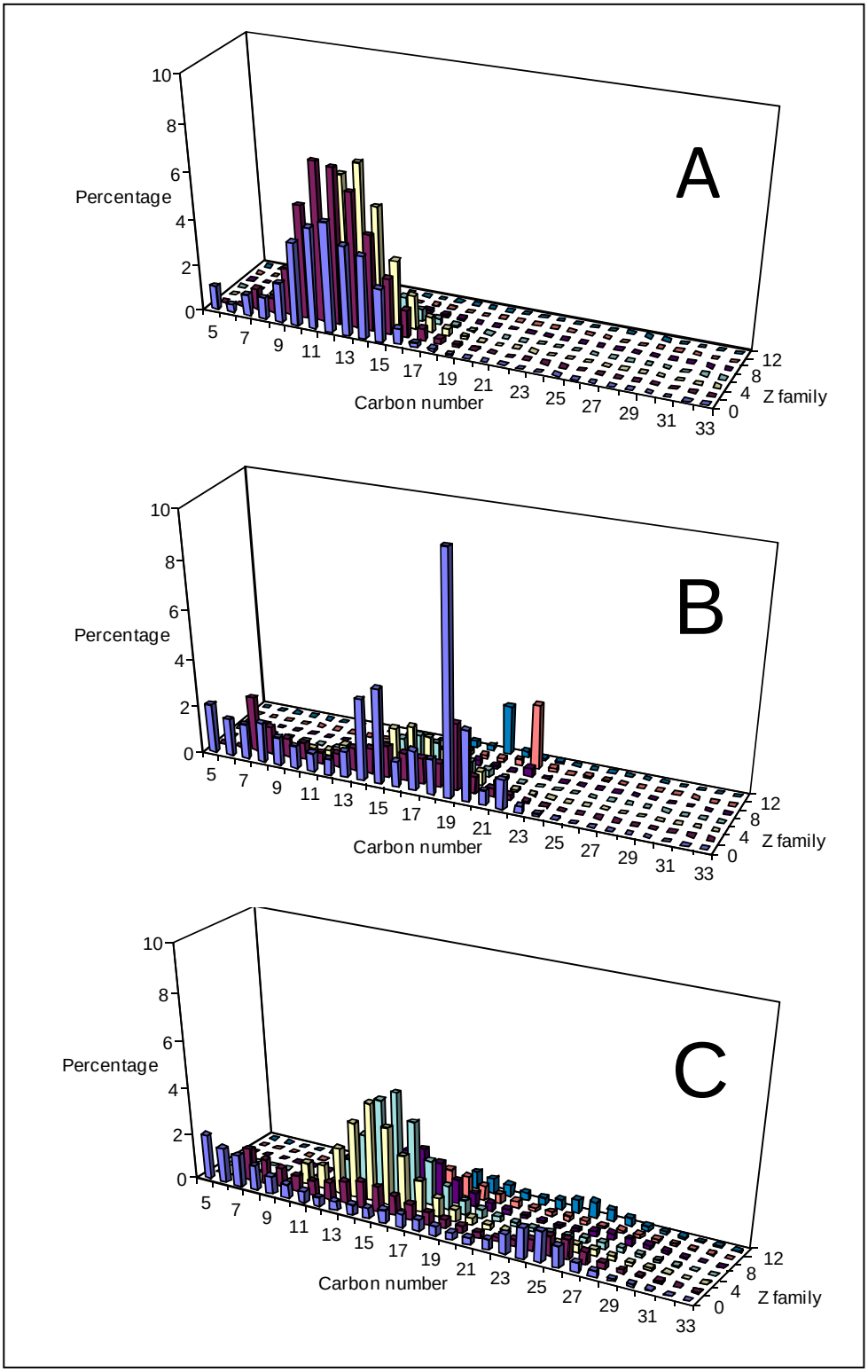

Figure 3.2 3D plots of Merichem (A), Acros (B) and Pond 9 extracted (C) NAs.

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Chapter 4

Consumer sensory panel evaluations of three fish species from three river basins in Alberta, Canada

4.1 Introduction

The Athabasca oil sands deposit in northeastern Alberta, Canada is one of the largest petroleum deposits in the world (Government of Alberta, 2009). This deposit contains highly biodegraded, viscous, tar-like petroleum known as bitumen. Erosion by the Athabasca River and its tributaries has led to the exposure of bitumen-containing outcrops in some river valleys. Conly et al. (2002) provide a geological cross-section that illustrates how this erosion cuts into the oil sands bearing stratum. These outcrops were the first indication of the presence of oil sands.

Full-scale surface mining and bitumen extraction activities began in 1967, and for about 35 years, two major oil sands companies operated just north of Fort McMurray, Alberta. Initially, both operations were on the west bank of the Athabasca River, but now mining has expanded to the east side of the river, directly across from the original site. Over the past decade, many international petroleum companies have invested in the oil sands industry, and new mining and extraction operations have recently started or are being planned. Many of the leases are adjacent to rivers in this area.

In general, the extraction of bitumen from the mined Athabasca oil sands is done using a dilute caustic solution in water which produces tailings consisting of water, sand, clay, and residual bitumen. These tailings are held in vast settling ponds, and the clarified cap water is recycled into the extraction process. Under current regulations, none of these tailings waters can be released into the Athabasca River system because of the toxicity of some components in the process affected waters.

The Athabasca River flows north into Lake Athabasca (Figure 4.1), which supports a commercial fishery. Jardine and Hrudey (1988) reported that the commercial fishery was closed in 1982 after an upset at one of the oil sands plants during the winter of 1981 to 1982 caused the release of hydrocarbons into the Athabasca River. At that time, fish were reported to have “petroleum-like off-flavors” (Jardine and Hrudey, 1988). Since then, oil sands tailings waters have been used in several studies to assess their potential to taint fish (Koning and Hrudey, 1992; Diversified Research Laboratories, 1992; Golder Associates Ltd., 1996; LeBlanc et al., 2000). Results of these studies,

summarized by Rogers et al. (2007), have shown that dilutions of oil sands process affected waters cause fish tainting that can be detected by a sensory panel.

There are anecdotal comments in the media (e.g. Köhler, 2007) and on the Internet that report off-flavor or off-odor in fish caught near the Athabasca oil sands. However, there is no clear documentation that wild fish taken from natural waters near the Athabasca oil sands operations are tainted during times of normal operation of the surface mining, extraction and upgrading plants in the Fort McMurray region. Thus, we sought to determine if a sensory panel of consumers could distinguish between wild fish collected from near the Athabasca oil sands and wild fish collected from two other Alberta locations in different river basins distant from the Athabasca oil sands. We hypothesized that if tainted fish were common near the Athabasca oil sands, a sensory panel would judge fish from the Athabasca oil sands region to be the "least liked" of the fish samples from the three sampling locations. The three main species of fish eaten by people in the Athabasca region are walleye (*Sander vitreus*, formerly *Stizostedion vitreum*), northern pike (*Esox lucius*), and lake whitefish (*Coregonus clupeaformis*) (Brenda Miskimmin, Summit Environmental Consultants Ltd, personal communication). Thus, these were the three species of fish collected from three different Alberta river basins for presentation to a sensory panel.

4.2 Materials and Methods

4.2.1 Sampling locations

All of the wild fish used in this study were collected in September 2008. Figure 4.1 shows three of the river basins in Alberta, Canada and the locations from which the fish were gathered. Fish were obtained from the Athabasca River at a few different locations near the oil sands deposits and mining operations as part of the Regional Aquatics Monitoring Program (RAMP) in Alberta. Fish were also collected from McGregor Lake and Buck Lake during Fall Index fish netting by the Province of Alberta, as part of the Sustainable Resource Development Ministry's Fish and Wildlife Fisheries Management program. McGregor Lake is in the South Saskatchewan River Basin, and Buck Lake is in the North Saskatchewan River Basin (Mitchell and Prepas, 1990). These two locations are more than 450 km from Fort McMurray.

4.2.2 Fish collection and preparation

Wild walleye, northern pike, and lake whitefish were collected from each location. Personnel from RAMP provided gutted, whole fish collected from the Athabasca River. These fish were stored on ice in coolers and transported to the University of Alberta. Whole fish from McGregor Lake and Buck Lake were gutted immediately in the field before placing them in individual plastic bags. The bagged fish were stored on ice in coolers for transportation to the University of Alberta. At the University, the fish were kept on ice in coolers that were stored at 4 °C. Rodríguez et al. (1999) reported that acceptable freshness is maintained for up to 6 days when gutted fish are stored on ice. All of our fish were filleted in a food-grade facility within 6 days of being caught. Fish fillets were rinsed with cold tap water, placed in plastic bags, and frozen for use in sensory studies 4 months later. Table 4.1 summarizes the numbers and masses of fillets of each fish species collected at each sample location.

4.2.3 Consumer Sensory Panel Evaluation

Three consumer panels were conducted; one for each of the fish species (walleye, northern pike or lake whitefish). Steamed fish samples were presented to sensory panel participants to determine if there was any preference difference among fish from the three geographic sources. The consumer sensory panelists were untrained and had to be over 18 years of age and have consumed the fish species within the past year. Panel sizes ranged from 40 to 44 individuals.

For the sample preparation, fish fillets were thawed overnight in a refrigerator at <4 °C. On the evaluation day, fish samples were prepared by cutting the fish fillets in approximately 1-cm cubes. Cubes of fish were mixed to create a composite sample for each fish species from each sample location. Fish samples were prepared as per the standard for fish presentation (ASTM E1810–96, 2004); composites containing approximately 20-g portions of fish flesh were individually wrapped in heavy duty aluminum foil and steamed in a food steamer (Vitacuisine Steamer, T-fal, Canada) for 8 min, or until the internal temperature reached 70 °C as measured by a digital thermometer. Samples were kept warm in the food steamer under the warming setting and held for a period of no more than 30 min. When the panelist was ready to perform the evaluation, the sample was placed in a 120-mL Styrofoam cup and covered with un-vented lid.

A rank preference test (Meilgaard et al., 1999) was performed in order to determine if there was an overall preference among the fish samples. Participants received a tray with three samples of a single species of steamed fish, each one from a different river basin in Alberta, along with a questionnaire, three forks, a glass of purified water and two unsalted crackers. Panelists were asked to taste each sample from left to right; cleansing their palates with unsalted crackers and purified water before and between samples. Samples were presented in a randomized block design. Sample identities were blinded with three-digit codes.

Each panelist's task was to numerically rank the samples from the "most liked" (1) to the "least liked" (3). The data collected from the rank preference test were analyzed by Friedman's Test for Ranked Data to determine if there was a significant difference ($p<0.05$) in liking of fish from the three different water basins. In addition, panelists were asked to write a response to the question "what in particular did you like or dislike about the fish samples?". These comments were tabulated for each fish species from each location.

Along with the ranking, each participant was requested to complete a brief demographics questionnaire which asked their gender, their age, how often they consumed one of these fish species, and where they obtained these fish. These responses were coded numerically and analyzed by frequency.

The consumer sensory panel protocol was approved by an institutional human ethics review board.

4.3 Results and Discussion

In total, 12 fish were collected from the Athabasca River, 16 fish were taken from Buck Lake, and 22 fish were collected from McGregor Lake (Table 4.1), and composite samples of each species were presented to the consumer sensory panelists. Lake whitefish from Buck Lake were preferred significantly more ($p<0.05$) than lake whitefish from McGregor Lake (Table 4.2). This may be due to the texture of the steamed fish; panelists generally indicated that the fish from McGregor Lake was "very soft" in comparison to the fish from the other two regions, which participants described with comments such as the "meat was nice and firm". The lake whitefish from the Athabasca River were not different in preference from whitefish from Buck Lake or McGregor Lake. No significant

difference ($p<0.05$) in preference was found among the walleye or among the northern pike from the Athabasca River, McGregor Lake and Buck Lake (Table 4.2).

The consumer panel participants were nearly evenly divided between the genders, and as expected at a university, tended to be younger than 35 years of age (Table 4.3). All participants consumed the fish at least once per year, and often more frequently. Most of the consumed fish were caught by the participants or obtained from friends or relatives.

All 127 participants provided descriptors to support their liking or disliking of the fish samples. Approximately one-half (63 of 127) of the panelists commented on the texture of the fish samples that were steamed rather than cooked with a traditional method such as frying. In total, there were 372 descriptors provided. One hundred and thirty-two selected flavor descriptors from the panelists are presented in Table 4.4. The chosen descriptors ignore remarks about texture, focusing on comments concerning taste of the composite fish samples. These descriptors were chosen to summarize all of the negative comments (e.g. “unnatural–not like fish”, “little bit dirt tasting” and “odd–slight plastic”) and some of the positive comments (e.g. “best taste”, “most flavor” and “nice flavor!”).

Typical of anecdotal comments, Köhler (2007) reported that “fish pulled from the Athabasca River downstream of the oil sands taste of gasoline”. Referring to lake whitefish taken from the Athabasca River near the oil sands operations in the current study, two of the descriptors provided by participants were “fuel-like (oil/tar)” and “petroleum or gasoline” (Table 4.4). One comment about the walleye from this sampling location was “fish oil flavor/slight oil/tar flavor” and one comment about the northern pike from this source was “oil/tar flavor” (Table 4.4). However, this type of statement was not limited to fish from the Athabasca River. For example, one participant reported “a fuel-like flavor” in walleye from Buck Lake and one participant reported a “grease-like aftertaste” from walleye from McGregor Lake. The most common negative comments about fish from McGregor Lake were “muddy” flavors in all three fish species, and an “earthy” taste in walleye and northern pike (Table 4.4).

The oil sands industry is not allowed to release process affected waters into the Athabasca River or it tributaries. Nonetheless, natural erosion of the Athabasca oil sands yields a chronic input of compounds from the oil sands into the neighboring waters. Barton and Wallace (1979) wrote that “organisms living in these streams are continuously exposed to low levels of hydrocarbons in the water and on the substrate” and these authors commented that “the bed of the stream resembles fine-grained asphalt pavement”.

The latter comment refers to a portion of the Steepbank River, a tributary of the Athabasca River, that was sampled by Barton and Wallace (1979). Conly et al. (2002) also showed the presence of oil-sands derived sediments in the Athabasca River.

Headley et al. (2001) collected sediment samples from three tributaries flowing into the Athabasca River. These tributaries were the Ells River, the MacKay River and the Steepbank River. The lower reaches of each of these rivers cut into the McMurray Formation, which harbors the Athabasca oil sands. Analyses of the sediments from the lower reaches showed the presence of polycyclic aromatic hydrocarbons and their alkylated analogues. Headley et al. (2001) concluded that these were "predominately from petrogenic sources likely from oil sands derived sediments". The natural process of erosion and deposition of oil sands and bitumen in the river sediments may be a reason why a few of the consumer panelists provided descriptors such as "fuel-like (oil/tar)".

We intentionally collected fish from the Athabasca River near two oil sands mining and extraction operations because the possibility of fish being affected by these operations was higher in this region. However, fish are highly mobile as illustrated by data collected from the RAMP fish tagging program. For example, a walleye that was captured from the Athabasca River near the mouth of the MacKay River was tagged by RAMP in May 2005. In October 2006, this tagged fish was caught by an angler fishing in the Slave River near Fort Smith, North West Territories, Canada (Figure 4.1), approximately 400 km from where the fish was tagged (Dwayne Latty, Alberta Sustainable Resource Development, Fish and Wildlife Division, personal communication). Golder Associates Ltd. (2004) provide additional information of fish movements in parts of the Athabasca River system. If fish tainting in this river system is a problem, it would be difficult to identify a potential source of the taint (e.g. oil sands operations or natural outcrops of oil sands) because of the mobility of the fish species.

One limitation of this investigation is the relatively small numbers of fish collected from the Athabasca River that were used for the consumer sensory panel study (Table 4.1). If only a small proportion of fish in this river are tainted, our small sample size may not have included a tainted fish. Alternatively, we may have caught a tainted fish, but in the preparation of the composite samples for the consumer panels, the taint may have been diluted by untainted fish in the mixture, making it difficult for the panelists to detect the taint. Nonetheless, the results from our consumer panel evaluations indicate that taint is not prevalent within the three studied fish species populations in the Athabasca River.

4.4 Conclusions

This is the only rigorous study to assess the taste of wild fish caught from near the Athabasca oil sands. We hypothesized that if tainted fish were common near the Athabasca oil sands, a sensory panel would judge fish from this region to be the "least liked" of fish collected from three different river basins in Alberta. However, this was not the case for any of the three fish species tasted by a consumer sensory panel of ≥ 40 participants who had previous experience consuming these fish species. Thus, despite unsubstantiated comments that fish from near the Athabasca oil sands taste 'bad', there was no statistically significant indication from our consumer preference ranking study to suggest that the taste of the fish from the Athabasca River was preferred less than the taste of fish from two other water bodies in Alberta.

4.5 Tables

Table 4.1 Summary of the numbers each fish species and mass of fillets from the three different sources.

Species		Athabasca River	Buck Lake	McGregor Lake
Lake whitefish	Number of fish caught	6	3	7
	Total mass of fillets (kg)	3.3	2.8	2.9
Walleye	Number of fish caught	3	8	8
	Total mass of fillets (kg)	1.9	2.0	3.3
Northern pike	Number of fish caught	3	5	7
	Total mass of fillets (kg)	2.2	2.7	3.7

Table 4.2 Average ranks and rank sums of preference testing of three species of fish from three different river basins in Alberta (n = number of consumer sensory panelists).

		Athabasca River	Buck Lake	McGregor Lake
Lake whitefish (n = 40)				
	Average Rank	2.1	1.6	2.4
	Rank Sum*	83 [ab]	63 [a]	94 [b]
Walleye (n = 44)				
	Average Rank	2.0	2.0	2.0
	Rank Sum*	87 [a]	89 [a]	88 [a]
Northern pike (n = 43)				
	Average Rank	1.9	2.0	2.0
	Rank Sum*	84 [a]	87 [a]	87 [a]

* Rank sums within the same row followed by different letter are significantly different ($p < 0.05$).

Table 4.3 Demographics of consumer panel participants (n = umber of consumer sensory panelists).

		Lake whitefish (n = 40)	Walleye (n = 44)	Northern pike (n = 43)
Gender	Female	20	23	18
	Male	20	21	25
Age range	18-26	22	24	21
	27-35	10	10	12
	36-44	3	5	4
	45+	5	5	6
Consumption	Once per month	13	14	13
frequency	More than one per month	5	4	4
	Once per year	22	26	26
Source of fish*	Grocery store	10	9	8
	Farmers market	1	1	1
	Fish market	1	4	1
	Caught it myself	19	22	18
	Friends and relatives	16	19	19
	Other	1	2	4

* Some panelists obtained fish from more than one source.

Table 4.4 Flavor descriptors provided by consumer sensory panelists who tasted composite fish samples of each species from three different sampling locations.

Athabasca River	Buck Lake	McGregor Lake
Lake whitefish		
Off flavor	Dirt-like flavor	Odd - Slight plastic
Plasticky or chemical	Slight aftertaste	Very aquarium like
Unnatural – not like fish	Fresh mild flavor	Very fishy taste
Fish taste is more pleasant	Best flavor	Smokey
Petroleum or gasoline	Slight aftertaste	Stronger fishy taste
Strange flavor	Good taste	Muddy taste
Plasticky	Very good	Good
Pepperish	Like silty lakewater	Mild, not too fishy
Off smell and flavor	A bit grassy	Little flavor
Best taste	Tasted good	Taste was good
Very nice aftertaste	Some nice fat/oil good flavor	Mild acceptable flavor
Mild flavor – Nice!	Nice flavor	Not much flavor
Good taste	Most flavor	Acceptable flavor
Nicest taste	Neutral tasting	Ok taste
Good flavor	Stronger flavor	Most bland flavor
Flavorful	Slight aftertaste – did not like	Like cod liver oil
Fuel-like (oil/tar)	Strange taste experience … muddier	Very fishy odor & taste – did not like!
Walleye		
Tangy flavor	Slightly off taste	Tastes a bit like dirt
“Laky” taste	Lake-like flavor	Grease-like aftertaste
Like it came from a slough	Tasted a little rubbery	Musty or muddy flavor
Normal fish flavor	Little swampy	Slightly muddy
Clean taste	Fresh flavor	Mild but good flavor
Best taste	Slightly bland	Very sharp taste
“Swampy” taste/fatty	A fuel-like flavor	Earthy
Bit of a funny taste	Most flavor	Not much flavor
No distinct flavor	Taste is excellent	Least flavor
Very nice flavor	This was the best	Very clean taste
Not much taste	Most flavor	Bland taste
Delicate taste	Flavorful rich taste	Nice flavor
Fish oil flavor/slight oil/tar flavor	First taste was some-what off	Taste similar to smell of murky lake water

Table 4.4 Continued

Athabasca River	Buck Lake	McGregor Lake
Northern pike		
Nice taste	Little bit dirt tasting	Bland tasting
Unpleasant metallic tasting	Aftertaste I didn't like	A little muddy tasting
Best taste	Flavorful	Taste was good
Lack of flavor	Doesn't taste as good	Lake-like flavor
Oil/tar flavor	Very little flavor	Good flavor
Quite good flavor	Clean taste	Sharp laky taste
Very good	Very bland	Taste was good
Plastic flavor	Very good	Somewhat bland
Smells like chemicals	Slight chemical taste	All together unpleasant
Best taste	Flavor too strong	Nice flavor!
Somewhat sweet	Nice flavor	Best
Unpleasant soapy aftertaste	Lots of flavor	Slight soapy aftertaste
Full flavor	Ok flavor	Earthy
Rich full flavor	Not much taste	Slightly unpleasant aftertaste

4.6 Figures

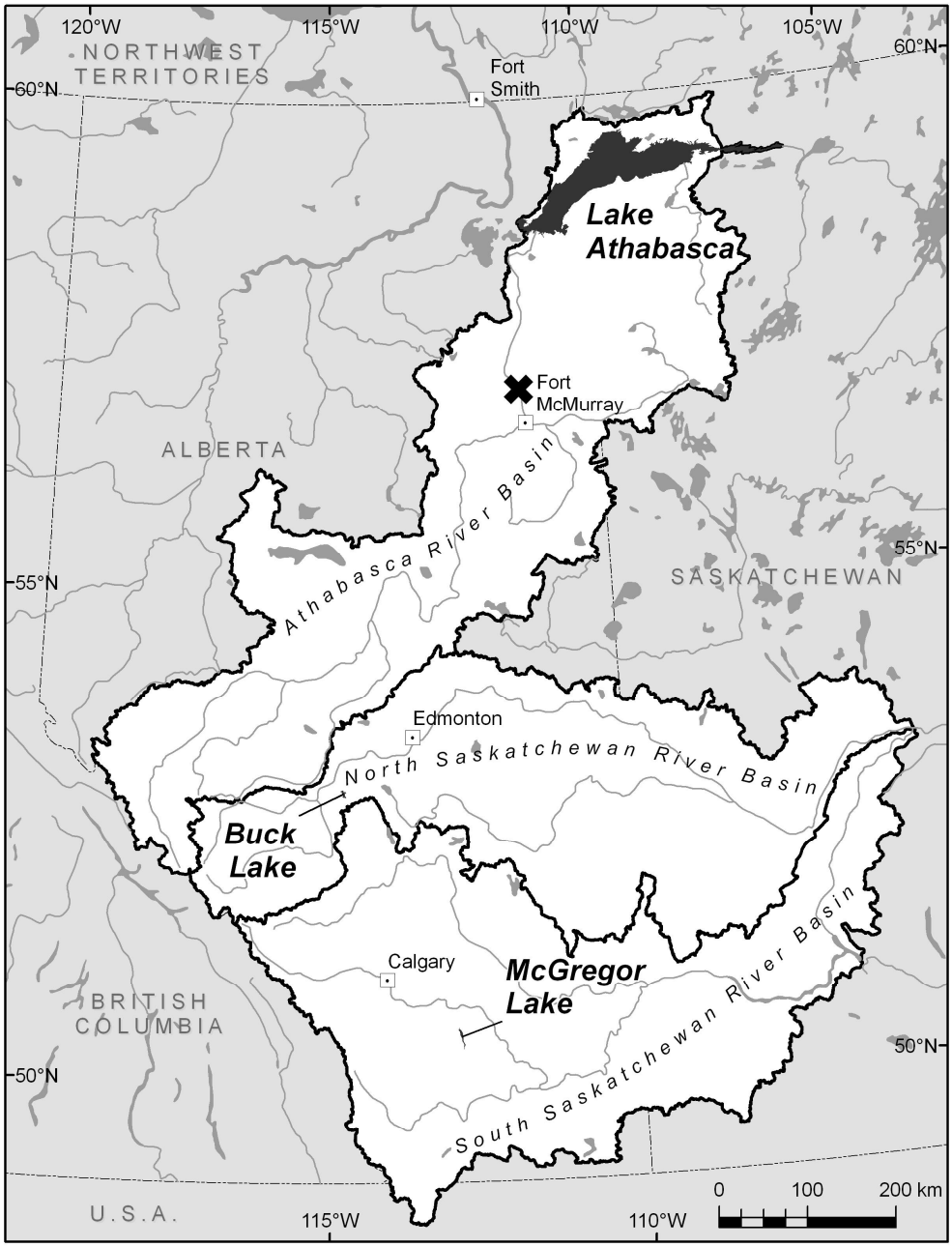

Figure 4.1 Map of Alberta with river basins and sampling lakes.
X represents the oil sands recovery operations and fish collecting location on Athabasca River

4.7 References

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Chapter 5
Summaries, Conclusions and Future Recommendations

5.1 Summaries and Conclusions

Fish can easily absorb water-soluble substances from the environment in which they are exposed. When these compounds reach concentrations at which they can be detected sensorially, the odor and flavor of the fish can become impaired (Davis et al., 2002); this is known as fish taint.

Petroleum off-flavors can potentially develop in wild fish when waters are contaminated by municipal or industrial discharges, losses from pipelines, or accidental diesel fuel or gasoline spills, from boats and refinery effluents (GESAMP, 1982). According to the GESAMP (1982), the principal components of crude oil that have caused fish tainting include phenols, dibenzothiophenes, naphthenic acids (NAs), mercaptans, tetradecanes and methylated naphthalenes. Of particular interest to this thesis, is the potential of NAs as a source of fish taint.

NAs are a complex mixture of saturated acyclic, monocyclic and polycyclic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$, where n is the carbon number and Z represents the hydrogen atoms lost as the structures form rings (Headley and McMartin, 2004; Clemente and Fedorak, 2005). This group of compounds occurs naturally in most hydrocarbon deposits, including oil sands.

Oil sands are deposits of a highly biodegraded, viscous, tar-like form of crude oil; composed of a mixture of sand, clay and high molecular weight petroleum, known as bitumen (Koning and Hrudey, 1992). The recovery of bitumen from oil sands can be achieved through a caustic hot water extraction method. During this process vast volumes of residual wastewater are produced, which are accumulated into large holding ponds known as oil sands tailing ponds. This residual water contains inorganic and organic compounds, among them NAs, which are acutely toxic to the aquatic ecosystems (Headley and McMartin, 2004; Clemente and Fedorak, 2005). The alkali nature of the water used for bitumen extraction enhances the release of NAs into the aqueous phase of tailings ponds (Young et al., 2007). Under current operating licenses, oils sands companies must comply to the “zero discharge” policy in which no extraction process-affected waters can be intentionally released into ground or surface water bodies.

However, despite the efforts of Alberta's oils sands mining industry to monitor and avoid the release of tailings into surface and underground water resources, a risk of leakage may still exist. This situation reinforces the need for studies such as the ones described in this thesis to enhance our understanding of the potential negative impacts of substances such as NAs on water systems and the aquatic life they support. Although previous studies have been conducted to examine the toxicity of NAs (reviewed by Clemente and Fedorak, 2005) and their effects (Peters et al., 2007) and detection in fish (Young et al., 2007), to the best of my knowledge, no sensory studies have been conducted to analyze the sensory properties of this group of compounds and the role they play in fish tainting. The goal of the research described in this thesis was to elucidate the effect of NAs on the odor properties of fish.

5.1.1 Chapter 2: Comparison of the triangle test and 3-alternative forced choice method for the evaluation of fish taint odor detection thresholds

Odor detection thresholds for a commercial (Merichem) NAs preparation in phosphate buffer (PB) (pH 8) and in steamed walleye fish were determined by trained sensory panels (n=9 or 10) following two discrimination sensory methods, the triangle test (GESAMP, 2002) and the three-alternative forced choice (3-AFC) (ISO 13301, 2002). It was deemed necessary to compare these methods to establish a standard method for the assessment of sensory thresholds that pertain to fish tainting as each method is recommended by a well-regarded international standards agency.

Panels were performed in duplicate and results from replications were analyzed both independently to examine the methods' reliability, and pooled (Jardine and Hrudey, 1988; Golder Associates Ltd., 1996; Redenbach, 1997) to estimate odor detection thresholds. Additionally, test-retest reliability coefficients (r) were determined to compare the reliability (Daly and Bourke, 2000) of both the triangle test and the 3-AFC method. The estimated odor detection thresholds for Merichem NAs in PB determined by the triangle test and 3-AFC were 0.5 mg L^{-1} and 0.04 mg L^{-1}, respectively. The detection threshold for Merichem NAs-spiked fish evaluated by the triangle test was 10 mg kg^{-1}, whereas the estimated threshold using the 3-AFC method was 0.6 mg kg^{-1}. Test-retest reliability coefficients for the triangle and 3-AFC protocols were, r = 0.59 (NAs in PB) and r = - 0.17 (NAs in fish), and r = 0.98 (NAs in PB) and r = 0.65 (NAs in fish), respectively. Lower detection thresholds and higher reliability coefficients were obtained

using the 3-AFC test in both PB and fish. This is due to the different cognitive processes (i.e. different optimal decision rules) undertaken when panelists perform sensory discrimination tasks (Rousseau, 2001). The 3-AFC is based on the intensity of the stimuli; for example, panelists seek the sample with the highest *intensity* on a sensory continuum. Comparatively, the triangle test requires the panelists to compare the sensory *differences* between samples (Dessirier and O'Mahony 1999). In other words, a mental evaluation of the different perceptual intensities between the three samples is made. This process can be confusing to panelists because finding the odd sample requires a comparison and estimation of intensities of the three stimuli. On the other hand, the 3-AFC method requires only the correct perception of the strongest of the three stimuli. There is greater variability in judging differences than in judging intensities, thus panelists perform better in the 3-AFC method due to the simplicity of the task and the lower variability when making decisions (Lawless and Heymannn, 1998). The use of the 3-AFC methods was recommended for future studies of the evaluation of NAs in fish.

5.1.2 Chapter 3: Odor detection thresholds of naphthenic acids from oil sands process-affected water and from commercial sources

NAs, compounds found in oil sands process-affected waters, have been proposed as a cause of fish taint. To investigate this hypothesis, odor detection thresholds of NAs from an oil sands process-affected water and from two commercial sources were determined in two media by trained sensory panels (n= 9 to 10) using a 3-AFC method. The estimated odor detection thresholds for Merichem, Acros and Pond 9 NAs in PB (pH 8), were 0.04, 1.5 and 1.0 mg L^{-1}, respectively, while the odor detection thresholds for Merichem, Acros and Pond 9 NAs-spiked fish, were 0.6, >21 and 12 mg kg^{-1}, respectively. The 'odor qualities' of each source of NAs were characterized using an odor descriptor wheel for environmental contaminants (McGinley et al., 2000). Descriptors provided by panelists showed that in general the odor qualities of the three sources of NAs came from the 'chemical' and 'earthy' categories, however, the specific odor qualities among all three NAs were different. For example, when samples were described at their respective detection thresholds, the Pond 9 NAs were reported to smell like tar, whereas the majority of panelists described the Acros and Merichem NAs as having a stronger plastic or gasoline odors, respectively. Elemental analyses of the three NAs sources showed that the composition differed among the three. For example the Pond 9

extract contained sulfur and nitrogen impurities, which were not detected in the commercial preparations. In addition, GC-MS analyses of the three sources of NAs showed that each preparation is distinctly different from the other two preparations.

This study revealed that the three different NAs studied have different sensory profiles as well as some differences in their chemical composition, indicating that NAs are complex mixtures. The odor detection thresholds of the three preparations of NAs were different and the odor profile among the three sources of NAs was unique for each. From this research it can be concluded that a particular preparation of NAs should not be considered to represent all NAs and that commercial preparations of NAs may not represent oil sands waters tainting potential.

5.1.3 Chapter 4: Consumer sensory panel evaluations of three fish species from three river basins in Alberta, Canada

There have been anecdotal comments that the fish caught in the Athabasca River north of Fort McMurray have an off-flavor or off-odor, implying that the oil sands operations are the source. This study was performed to determine if the sensory qualities of wild fish caught near the Athabasca oil sands were less preferred than those of fish collected from two other river basins in Alberta. Consumer sensory panels of 40 to 44 participants tasted steamed samples of each of three fish species (walleye (*Sander vitreus*), northern pike (*Esox lucius*), and lake whitefish (*Coregonus clupeaformis*)) from three different water sources in Alberta (the Athabasca River, Buck Lake, and McGregor Lake). The majority of the consumer panel participants were younger than 35 years of age, and were nearly evenly divided between the genders. All participants consumed the fish at least once per year.

No significant difference in preference was found among the walleye or among the northern pike from the Athabasca River, McGregor Lake and Buck Lake. Lake whitefish from Buck Lake were preferred significantly more than lake whitefish from McGregor Lake, which may have been due to a softer texture of the steamed fish from McGregor Lake in comparison to the fish from the other two regions, which participants described as "… nice and firm". The lake whitefish from the Athabasca River were not different in preference from whitefish from Buck Lake or McGregor Lake.

Descriptors of the taste and odor of all fish species from all locations were both positive and negative. "Oil/tar flavor" comments were not limited to fish from the Athabasca River. For example, one participant reported "a fuel-like flavor" in walleye from Buck Lake and one participant reported a "grease-like aftertaste" from walleye from McGregor Lake. The most common negative comments about fish from McGregor Lake were "muddy" flavors in all three fish species, and an "earthy" taste in walleye and northern pike.

This study showed no statistically significant indication from the consumer preference rankings that the sensory properties of the fish from the Athabasca River was preferred less than the taste of fish from two other water bodies in Alberta.

5.1.4 Conclusions

The oil sands mining operations in northeastern Alberta are continuously expanding, and as the recovery of bitumen from oil sands grows, the production of process-affected waters does as well. Therefore, it is essential to understand the impacts that waste waters may have in the environment, particularly on the water resources and the life they support. The purpose of this research was to determine if NAs, a group of compounds found in process-affected waters, may influence the sensory properties of fish and play a role in fish taint.

Generally, tainting of marine and freshwater organisms by petroleum derived compounds has been assessed following guidelines recommended by internationally recognized institutions, such as the GESAMP (1989, 2002), the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1987) and the ASTM E1810-96 (2004). These guidelines advise the use of the triangle test to determine the effects of water-related contaminants on the odor and flavor of exposed fish, whether or not they are deemed to be unpleasant. The GESAMP (1989, 2002) and the ECETOC (1987) also recommend the use of triangle test to estimate the detection thresholds of chemical compounds suspected of inducing fish taint. However, the sensory protocols recommended by these organizations should be reconsidered. The use of the triangle test to determine detection thresholds is not an adequate sensory tool. The results obtained in Chapter 2 showed that the 3-AFC method is a more reliable and sensitive protocol in comparison to the triangle test when estimating olfactory detection thresholds of

contaminants related to fish taint. Lower odor detection thresholds and higher reliability coefficients where determined when the evaluations were performed using the 3-AFC method. Chapter 2 provides the foundation for the establishment of a standard protocol to evaluate the role that environmental contaminants, such as NAs, may have in fish tainting.

Chemical and sensory characterization of three sources of NAs, two commercial (Merichem and Acros) and one extracted from oil sands tailings pond water (Pond 9), showed that this group of compounds are complex mixtures. St. John et al. (1998) determined that the composition of commercial NAs from various sources differ from one another. Moreover, Clemente et al. (2003) demonstrated that the composition of commercial NAs differed from that of NAs extracted from oil sands process-affected waters. This research has demonstrated that odor detection thresholds and sensory profiles of NAs differ markedly depending upon their source.

Commercial preparations of NAs have been widely used to study NAs aerobic biodegradation (Clemente et al., 2004), chemical composition (Clemente et al., 2003) and effects of these acids in fish development (Peters et al., 2007). However, the findings of this study highlight the significance of using NAs extracted from process-affected waters when evaluating the role that this group of compounds may have on the sensory properties of fish, because commercial preparations of NAs may not represent oil sands waters tainting potential.

Even though there have been anecdotal reports of an off-flavor or off-odor in fish caught near the Athabasca oil sands, data obtained from the sensory consumer panels undertaken in this study revealed that the flavor of fish caught from the Athabasca River near oil sands mining and extraction operations, was not preferred less in comparison to fish caught from two other water basins in Alberta, the McGregor Lake and the Buck Lake. Overall, the current research provides for the first time information on the sensory characteristics of NAs. Moreover, it enhances our understanding of the possible impact that NAs may have in the sensory properties of fish.

A long-term plan of the Athabasca oil sands industry is the final reclamation of the disturbed land and water resources. Research is ongoing to create artificial lakes known as end-pit lakes (EPL), in which process-affected water along with surface and ground water will be incorporated into an oil sands mine pit (Westcott, 2007). One of the purposes of EPL is to create natural, healthy and sustainable aquatic ecosystems. However there is concern about the potential for fish tainting in the EPLs (Westcott,

2007). Thus, the detection thresholds estimated in this research are valuable information for the oil sands industry and government agencies, because these thresholds will serve as a future reference when creating regulations on the permitted concentrations of NAs in the environment and the future EPL.

5.2 Sensory methodological considerations and future recommendations

The sensory methods used in this research are recommended to assess the role that an environmental contaminant may have in fish tainting. However some methodological considerations should be taken to enhance the efficiency of the sensory techniques used in this thesis.

The first consideration concerns panelists' training for the evaluation of the sensory attributes of NAs. Generally, when creating a detailed profile of the sensory attributes of a product, panelists are provided with a broad selection of reference standards, such as chemicals, spices and ingredients that represent the sensory attributes of the stimulus to be evaluated (Lawless and Heymann, 1998). In our studies, when the odor attributes of NAs were assessed, panelists were not trained with standard references, and only an odor wheel with descriptors related to environmental contaminants were provided. This was done to obtain information about the profiles of NAs and to elucidate any differences in the odor profiles among the different preparations of NAs. Training panelists with standards tends to be expensive and time consuming (Lawless and Heymann, 1998), and because the generation of a 'detailed sensory profile' of NAs was not the focus of this thesis, standards were not provided to panelists. However, if a detailed sensory profile of NAs is required in the future, 'Quantitative Descriptive Analysis' (QDA) is recommended, an analytical sensory method in which panelists receive intensive training. In QDA it is essential to train panelists on the use of a variety of reference samples with different intensities, so panelists can relate the descriptors provided to the sensory characteristics of each descriptor and then their respective intensity. QDA can provide detailed information about the odor profiles of different sources of NAs and the sensations associated with them. Furthermore, it can be used to generate a profile of the specific odor characteristics that NAs impart to fish. Lawless and Heymann (1998) give detailed guidelines on the technique of descriptive analysis.

A second consideration is the range of concentrations used when evaluating NAs detection thresholds. The American Society for Testing and Materials (ASTM E679-04,

2004) recommends increasing the stimulus to be evaluated in a homogenic geometric progression of concentration steps, preferably by a dilution factor of two or three. According to Jardine and Hrudey (1988), increasing the concentration in a constant factor of two will aid in the generation of more accurate and reproducible odor detection thresholds. In the studies performed in this thesis, the dilution series presented to panelists were increased in an arithmetic continuum of discrete steps instead of a geometric progression, because the variations among trained panelists are expected to be lower in comparison to a general population. Furthermore, the concentration ranges were determined in a series of preliminary "in-house" assessments which reduced the concentration scale steps evaluated. If the detection threshold of NAs was to be determined by a general population, then it is recommended to increment the stimulus in discrete geometric concentration steps.

For future studies it is recommended that wild fish be exposed to aged oil sands process-affected water in the field. A difference test such as the triangle test should be perform by a trained panel to determine if a detectable difference would be perceived between the flesh of fish that has been exposed and a control.

The sensory work performed in this thesis determined the olfactory detection thresholds of NAs. To determine if NAs from oil sands process-affected water have the potential to induce fish taint, future studies are required. Wild fish caught in the vicinity of oil sands operations should be analyze with GC-MS as per Young et al. (2008), to estimate the concentration of NAs in their flesh. Then, the odor detection threshold of Pond 9 NAs should be compare to the concentration of NAs found in the wild fish. Only then it would be possible to conclude if NAs from process-affected water can taint the fish in the Athabasca River.

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APPENDIX A

Odor Panel Results

Table A1. Number of correct identifications at each concentration for the evaluation of NAs in PB by triangle tests (n = number of panelists).

NAs in PB (mg/L)	Panel 1[a] (n = 10)	Proportion of correct responses	Panel 2[b] (n = 9)	Proportion of correct responses	Pooled results for Panels 1 and 2[c] (n=19)
0.25	6	0.6	4	0.44	10
0.5	5	0.5	6	0.67	11
1	6	0.6	8	0.89	14
2	7	0.7	8	0.89	15
3	7	0.7	9	1.0	16
5	9	0.9	9	1.0	18

[a] = at least 7 correct responses needed to obtain statistical significance ($p \leq 0.05$).
[b] = at least 6 correct responses needed to obtain statistical significance ($p \leq 0.05$).
[c] = at least 11 correct responses needed to obtain statistical significance ($p \leq 0.05$).
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Table A2. Number of correct identifications at each concentration for the evaluation of NAs in PB by 3-AFC tests (n = number of panelists).

NAs in PB (mg/L)	Panel 1 (n = 10)	Proportion of correct responses	Panel 2 (n = 9)	Proportion of correct responses	Pooled results for Panels 1 and 2 (n=19)
0.000625	1	0.1	1	0.11	2
0.00125	1	0.1	2	0.22	3
0.0025	1	0.1	2	0.22	3
0.005	1	0.1	2	0.22	3
0.01	2	0.2	3	0.33	5
0.05	5	0.5	5	0.56	10
0.1	7	0.7	8	0.89	15
0.25	10	1	8	0.89	18
0.5	10	1	8	0.89	18
1	10	1	9	1.0	19
2	10	1	9	1.0	19
3	10	1	9	1.0	19

Table A3. Number of correct identifications at each concentration for the evaluation of NAs in fish by triangle tests (n = number of panelists).

NAs in fish (mg/L)	Panel 1[a] (n = 9)	Proportion of correct responses	Panel 2[a] (n = 9)	Proportion of correct responses	Pooled results for Panels 1 and 2[b] (n=18)
1	3	0.33	2	0.22	5
2	7	0.78	2	0.22	9
4	4	0.44	4	0.44	8
6	4	0.44	4	0.44	8
8	5	0.56	3	0.33	8
10	5	0.56	5	0.56	10
12	5	0.56	5	0.56	10
16	3	0.33	8	0.89	11
20	6	0.67	7	0.78	13

[a,] = at least 6 correct responses needed to obtain statistical significance ($p \leq 0.05$).
[b] = at least 10 correct responses needed to obtain statistical significance ($p \leq 0.05$).

Table A4. Number of correct identifications at each concentration for the evaluation of NAs in fish by 3-AFC tests (n = number of panelists).

NAs in fish (mg/L)	Panel 1 (n = 6)	Proportion of correct responses	Panel 2 (n = 8)	Proportion of correct responses	Pooled results for Panels 1 and 2 (n=14)
0.05	3	0.5	4	0.5	7
0.08	4	0.67	4	0.5	8
0.2	2	0.33	4	0.5	6
0.3	2	0.33	5	0.62	7
0.5	2	0.33	4	0.5	6
0.7	1	0.17	7	0.87	8
0.8	4	0.67	7	0.87	11
1	6	1.0	8	1.0	14
1.3	6	1.0	8	1.0	14
1.7	6	1.0	8	1.0	14

APPENDIX A

Odor Descriptor wheel

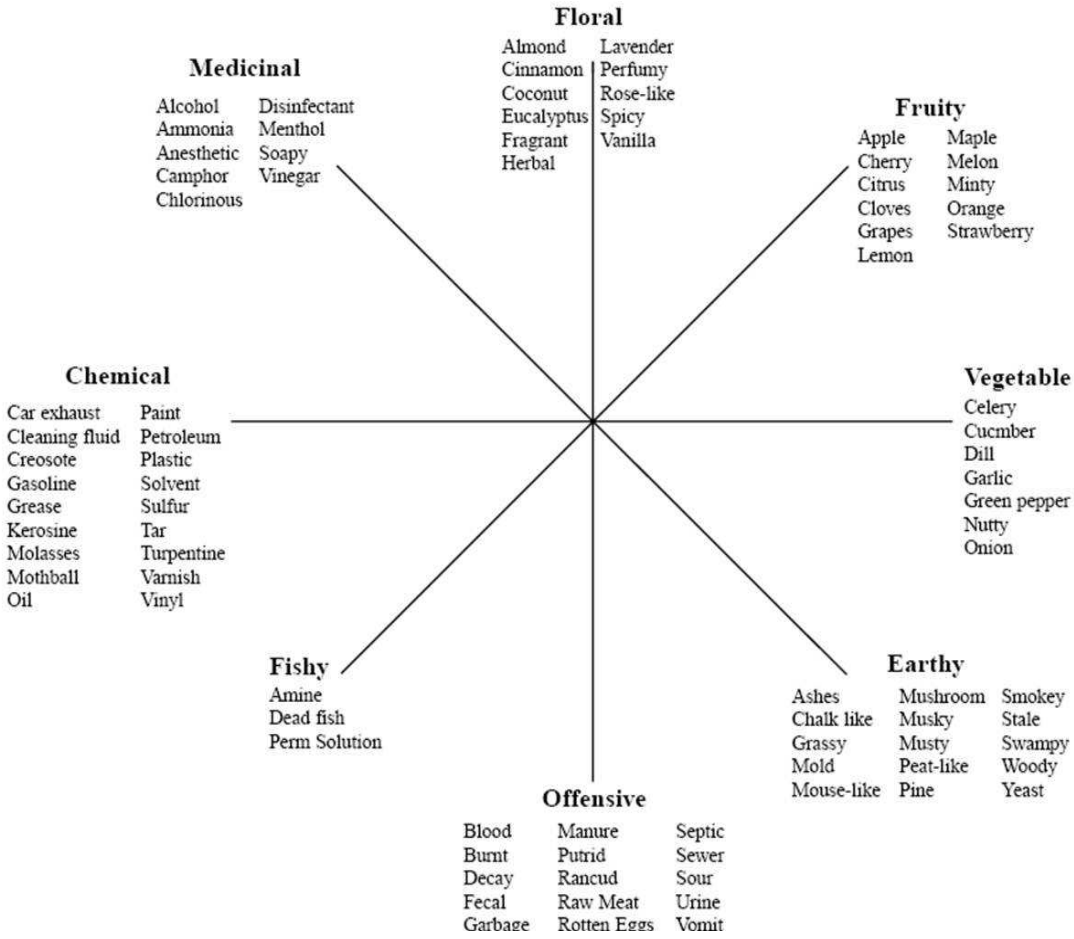

Figure B1. Odor descriptor wheel for environmental odors (McGinley et al. 2000). Permission to reprint figure obtained from St. Croix Sensory, Inc.