Polar Compounds in Alaska North Slope Oil and Other Oils: A Literature Survey and Synthesis

for

Prince William Sound Regional Citizens' Advisory Council (PWSRCAC)
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by

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Abstract

Polar compounds as found in oils are hydrocarbon compounds containing nitrogen, sulphur or oxygen. Measurement of the presence of these compounds in oils can be carried out using sophisticated analysis techniques, however quantification and separation of compounds is very difficult and will remain a problem for many years to come. Characterization of polar compounds in oils is at a state of infancy and little polar analysis for Alaska North Slope oil has been carried out to date.

In order to measure the toxicity of a specific compound or class of compounds, separation is needed. Separation is very difficult and in many cases, beyond the scope of today's technology. An alternative has been to synthesize the compound of concern and then test its toxicity. This approach ignores the matrix in which the compound is usually present and the compound of interest may be not bioavailable when present in the actual oil, due to its solubility in oil. Highly polar compounds are likely not present in produced oils such as ANS due to the polar compound's high water solubility. Compounds with moderate or less polarity are typically more soluble in oil than water. Similarly, highly polar compounds produced by biodegradation or photooxidation would be diluted in water during a spill.

The aquatic toxicity of polar compounds compared to aromatic compounds has been tested by using evaporative weathering. Aromatic compounds, particularly that of the 2 to 3 ring polyaromatic hydrocarbons (PAHs), are fairly well-established as the primary toxic component of oils. Polar compounds are soluble in water and thus may pose another source of toxicity. Evaporative weathering tests where photooxidation is not involved, in which some of the low molecular weight compounds and PAHs are lost from the oil, is thought to be one test of the comparison of polar compound toxicity compared to that of the PAHs. These tests show that polar compounds are generally less-aquatically-toxic than the 2 to 5-ring PAHs. Another test that has been performed is that of physical separation of oil components. In these type of tests, polar compounds have again been shown to have less aquatic toxicity that the PAHs in the same oil. Both tests have obvious limitations in that there are many compounds involved.

Naphthenic acids are polar compounds found in many oils and have been studied more extensively than other polar compounds. The lower-molecular weight naphthenic acids are more toxic than the high molecular weight acids, corresponding somewhat to the water solubility differences. Many low-molecular-weight naphthenic acids may not be in Alaskan and other oils that have been contacted with water as some of these compounds are more soluble in water and would largely be stripped out of the oil phase.

The data presented show that while there is little data for Alaskan oils, there is more data on other oils, which serve to scope out the problem of polar compounds in oils and serve as a model of what the compounds and their toxicity might be in Alaskan oils. Currently, it appears that polar compounds in Alaskan oils are generally of low toxicity.

Executive Summary

Polar compounds are hydrocarbon compounds containing nitrogen, sulphur or oxygen and are sometimes called NSO compounds, after the elements contained. Sometimes these are incorrectly called 'heterocycles', but only a few NSO compounds of interest, are heterocycles. These compounds are unique and are typically not found in environments not having come into contact with oil. Measurement of the presence of these compounds in oils can be carried out using sophisticated analysis techniques, however quantification and separation of compounds is very difficult and will remain a problem for many years to come. Characterization of individual polar compounds in oils is at a state of infancy. For Alaska North Slope oil, little polar compound analysis has been carried out to date.

This study shows that little is known about polar compounds in Alaska oils. Sulfur analysis shows that there should be some sulfur compounds (accounting for about 1% sulfur) and that the Dibenzothiophenes are about the same concentrations as in most oils. Aquatic toxicity studies show that for Alaska oils, that the 2 to 5 ringed PAHs account for toxicity observed as the oil is evaporatively weathered. Little photooxidation work has been carried out on Alaska oil, however, indications are that these compounds are somewhat toxic, but not more than the 2 to 5 ringed PAHs.

For several years, toxicologists have pursued studies looking for some hidden toxic compounds that may lie in oils. The tools used were evaporation, dissolution and photooxidation. While these are primitive tools, advances and separations still have not shown toxic and hidden compounds. The situation is similar in other studies of oils which have concluded that there are most probably no hidden toxic compounds in oils.

A list of about 200 polar compounds that might be in oils or had been separated from oils, was developed in this study. Some of these also had toxicity measurements – mostly aquatic. The compounds of interest include mostly oxygenated compounds including: low molecular weight carboxylic acids which typically have low toxicity and higher molecular weight carboxylic acids, which have very low toxicity (not very toxic). Also included are aromatic acids which typically are moderately toxic. Aromatic diones have been suggested and these have been found to be highly toxic. Compounds that may be in oils in low concentrations are the naphthenic acids, the lower molecular weight ones have moderate aquatic toxicities and the larger ones, low toxicity and are more soluble in whole oil.

The sulphur compounds in oil are many and varied. Many of these compounds have not been separated and studied. However, the benzothiophenes are typically studied along with the PAHs. The benzothiophenes have lower aquatic toxicity than most PAHs.

Nitrated compounds other than pyrene, pyridine and their derivatives have not been characterized in oil. Nitro-PAHs, compounds of toxicological concern, appear to only be formed by combustion processes such as in diesel emissions.

Highly polar compounds such as low-molecular weight acids largely are not present in pipelined oils as they are solubilized with water. Most production fields in Alaska have contact with water and thus the highly polar compounds have been solubilized, often millions of years ago. The moderately polar compounds in the oil will partition between the oil and the water after being spilled. Often the polarity is such that the compounds will partition into the oil because a

greater portion of the molecule is not polar. Such behaviour is true of benzothiophenes, for example. Thus, in summary, highly polar compounds are likely not present in pipelined oils such as ANS and compounds with moderate or less polarity are likely more soluble in oil than water.

Naphthenic acids have been well studied as a result of their presence in Alberta Oil Sands. Their presence, however, in Alaska oil has not been shown to date. Because of the water contact involved in most Alaskan oils and because of their different origin than bitumen, it is unlikely that there are high concentrations of these compounds.

Biodegradation of oils have been shown to produce oxygenated derivatives. Often these are more aquatically toxic than the starting compounds, however typically less than the PAHs in the oil. The biodegradation breakdown pathways are only understood for a few oil components. Further the speed of biodegradation is slow enough that high concentrations of oxygenated derivatives may not be produced faster than they are removed by water washing.

Photooxidation is known to produce oxygenated derivatives as well. Again these are sometimes more aquatically toxic than the originating compound. The photooxidation pathways are even less well known than those of biodegradation. The speed of photooxidation also may be slow enough to prevent the accumulation of toxic concentrations of breakdown products.

Information on polar compounds in oils can be achieved in many different ways. Of course, direct analysis is a prime way. Analysis of polars is complex, especially in an oil matrix, and thus this method has not been pursued as much as would typically be the case. As noted above, the toxicity has sometimes been pursued by following toxicity after evaporative weathering and because bulk analysis shows that there are more polars remaining, the result is typically that the toxicity is less. Other methods include separation of specific compounds and use of pure synthesized compounds that have been found in oils. There have been many studies of contaminated sites and emissions, but many of the compounds identified in these studies may not be present in oils. Other types of studies that have been performed are biodegradation and photooxidation studies. At this time many of the specific products of these processes have not been specifically identified and thus weakening the reliability of these results.

Analysis techniques are moving at a rapid pace. In the future, we may have the ability to determine more of the compounds in oils. At this time, petroleomics advances have given us a peek into what types of compounds may be present, but not enough to actually gauge specific structures or compositions. Specific advances have been created by using 2-D (two dimensional) chromatography which allows us to analyze the ‘hump’ or unresolved area in normal chromatography. This, however, offers little insight into most polar compounds. The development of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT ICR MS) has led to the indication of thousands of compounds in oils. At this time, the most successful studies that contribute to this present study have been the traditional and pain-staking separations and analysis.

List of Acronyms

ANS - Alaska North Slope - Usually referring to the crude oil mixture at the end of the pipeline
ARN - high molecular weight naphthenic acids
APPI - Atmospheric Pressure Photoionization
CALUX - Chemical Activated LUciferase gene eXpression – bioassay to detect effects of specific chemicals using a specific gene line
CEWAF - Chemically-Enhanced Water Accommodated Fraction - The sum total of oil in a water sample including chemically and physically dispersed and soluble oil
CYP1A - Cytochrome P450 1A -Liver enzymes that can be measured and indicators of stress in an organism, in cases of aquatic toxicity typically exposure to PAHs
Dalton - a unit of molecular weight
DART - Direct Analysis in Real Time
DBE - Double Bond Equivalent – a measure of the valence of a molecule
DESI - Desorption Electrospray Ionization
DMS - Differential Mobility Spectrometry
DNA - Deoxyribonucleic Acid
DWH - Deepwater Horizon, also known as the Macondo spill
EPA - U.S. Environmental Protection Agency
EROD - ethoxyresorufin-O-deethylase - an enzyme that is a good indicator of hydrocarbon exposure in an organism
ESI - Electrospray ionization
FID - Flame Ionization Detector - a gas chromatography detector
FT - Fourier Transform
GC - Gas chromatography - a separation technique that is very common
GC-MS - Gas Chromatography-Mass Spectrometry
GCxGC - two-dimensional chromatography
GPC - Gas Permeation Chromatography
Heterocycles – Carbon cyclic compounds containing an atom not being carbon, often NSO
HPLC - High Pressure Liquid Chromatography
IFO - Intermediate Fuel Oil - A mixture of Bunker C and diesel used for ship propulsion - eg. IFO 180 and 380 refer to the viscosity of the oil at about 38^{o}C
LC - Lethal Concentration
LC_{50} or LC_{50} - Lethal concentration to 50% of the test population
LDH - lactate dehydrogenase - an enzyme that is measured and an indicator of death of liver cells in an organism
LDI - Laser Desorption Ionization
LOEC - Lowest Observable Effect Concentration - the lowest concentration of a test substance that produces a response of exposed organisms that is statistically different from the response of control organisms
Microtox - A simplified toxicity measuring system using light-emitting bacteria
NA - Naphthenic acid
NESI - Negative Electrospray Ionization
NOEC - No-Effect Concentration - the highest concentration of a test substance that does not

produce a statistically-significant difference in response of exposed organisms compared to the response of control organisms

OSPW - Oil Sands Process Water
OPAHs – Oxygenated PAHs
Oxy - containing oxygen
PAH – Polycyclic Aromatic Hydrocarbon(s)
PCR - Polymerase Chain Reaction - a technique in genetics to amplify DNA
Petroleomics - Identification of the totality of the constituents of naturally-occurring petroleum and crude oil using high resolution mass spectrometry
PWSRCAC - Prince William Sound Regional Citizens' Advisory Council
QA – Quality Assurance
QC – Quality Control
QTOF - Quadrupole Time-of-Flight – a mass spectral detector
RNA - Ribonucleic Acid - part of genetic system
SFEF - Supercritical Fluid Extraction and Fractionation
SPE - Solid Phase Extraction – a material that absorbs analytes of interest which then can be extracted
Spiggin - is a natural adhesive used by sticklebacks to bind together filamentous algae to construct a nest
SRM - Standard Reference Material - a standard material such as an industrial effluent prepared for analysis comparison
∑PAH - the sum of PAHs in a given sample
TOF - Time of Flight
TPAH - Total Petroleum Aromatic Hydrocarbons – the sum of the concentrations PAHs in a sample
TPH - Total Petroleum Hydrocarbons - a measure of total hydrocarbons in a sample, usually by GC – FID - the sum of the concentrations of analyzed hydrocarbons in a sample
TSR - Thermochemical Sulfur Reduction
UPLC – Ultra-high Pressure Liquid Chromatography
UV - Ultra-violet light, a high frequency (past violet) portion of light spectrum
VOC - Volatile Organic Carbon - fraction of hydrocarbons which evaporate readily
WAF - Water-Accommodated Fraction - The sum total of oil in a water sample
WSF - Water Soluble Fraction

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1. Foreword

1.1 Objectives

The objectives of this review are to examine literature related to polar compounds and their toxicity in Alaska North Slope oil. As there was little information on Alaskan oils, the author extended the study to other oils that might provide insight into the polar compounds and their toxicity.

1.2 Scope

This review covers the literature from 2000 through to end of 2015. A wide-ranging literature search was carried out and included literature from all known sources. More than 125 pieces of literature were found. Of these, some were related to the issue of naphthenic acids as relevant to Alberta oil sands processing. This is relevant to Alaska North Slope oil as some of these compounds might also be found in ANS. A number of papers were found relating to sources of oxygenated or nitrated compounds such as emissions or industrial waste. It is noted in each case that the relevant compounds may not be found in petroleum oils nor formed by weathering processes of these oils when spilled. This version is a summary technical version and a separate detailed version with reviews of individual studies is also available.

1.3 Organization

Each section of the report begins with a summary and then provides a detailed review of the literature. The information contained in the review portions of the report is often quite detailed and technical. Results are often summarized in tables. This information is included to provide in-depth background to the summary statements.

2 Introduction

Polar compounds and their toxicity have been discussed for many years. There is an extensive body of literature on this topic, some of which relates to spilled petroleum oils. This paper reviews the known literature on the topic and presents this in the context of what is known and relates this information to Alaska North Slope oil.

3 Analysis of Alaska North Slope Oil

Hollebone (2012) analyzed a 2012 sample of ANS crude oil and the following is a table of results, Table 1. Of particular interest is the sulfur content, which is indicative of the amounts of some polar compounds in the oil.

Table 1 Analysis of Alaska North Slope Oil (Hollebone, 2012)

		%Evaporative Mass Loss			
		0.0%	12.08%	24.05%	36.05%
Sample #		2152.2	2152.1	2152.3	2152.4
Density (g/mL)	0ºC	0.8764	0.9087	0.9327	0.9558
	15ºC	0.8649	0.8978	0.9214	0.9437
API Gravity		31.14			
Dynamic Viscosity (mPa·s)	0ºC	22.8	91.7	538	9.30E+03
	15ºC	14.2	38.5	169.0	1.36E+03
Surface Tension (mN/m)	0ºC	27.4	29.2	31.3	NM
	15ºC	26.9	27.3	29.8	30.9
Interfacial Tension - Oil/Water (mN/m)	0ºC	23.9	25.9	27.6	NM
	15ºC	21.8	23.8	26.3	23.5
Interfacial Tension - Oil/ 33‰ Brine (mN/m)	0ºC	23.2	26.5	28.1	NM
	15ºC	20.6	22.6	27.0	22.4
Sulfur Content (%w/w)		0.93%	1.09%	1.25%	1.47%
Emulsion Formation	Visual Stability	Unstable	Unstable	Unstable	Unstable
	Complex Modulus (Pa)	NM	NM	NM	20.1
	Water Content (%w/w)	NM	NM	NM	23.7%

Of importance is the chemistry of ANS oil. Some work on this has been done in terms of BTEX and PAHs. Table 2 shows the BTEX content and Table 3 shows the PAH content (ESTD Data Base, 2015). Table 2 shows that the BTEX content is rapidly eliminated by evaporation by the time the oil is 30% evaporated. The other compounds lost are lower-molecular weight hydrocarbons.

Table 2 BTEX Content of Alaska North Slope Oil

	Concentration (µg/g oil) % Weathered	
Component	0%	30.50%
Benzene	2866	0
Toluene	5928	0
Ethylbenzene	1319	0
Xylenes†	6187	0
C3-Benzenes‡	5620	30
Total BTEX	16300	0
Total BTEX and C3-Benzenes‡	21920	30

†"Xylenes" include o-, m-, and p-xylene isomers.

‡"C3-Benzenes" include eight isomers.

Table 3 PAH Content of Alaska North Slope Oil (2002 sample)

	Concentration (µg/g oil) % Weathered			Concentration (µg/g oil) % Weathered	
Alkylated PAH					
Naphthalene	0%	30.50%		0%	30.50%
C0-N	261	167	Other PAHs		
C1-N	1015	1288	Biphenyl	135	177
C2-N	1800	2712	Acenaphthylene	12	18
C3-N	1702	2575	Acenaphthene	13	20
C4-N	815	1174	Anthracene	3	5
Sum	5594	7919	Fluoranthene	3	4
Phenanthrene			Pyrene	8	12
C0-P	209	295	Benz(a)anthracene	5	8
C1-P	666	932	Benzo(b)fluoranthene	5	7
C2-P	710	988	Benzo(k)fluoranthene	0.5	0.7

C3-P	486	707
C4-P	296	432
Sum	2368	3354
Dibenzothiophene		
C0-D	122	174
C1-D	225	319
C2-D	318	456
C3-D	265	362
Sum	931	1310
Fluorene		
C0-F	142	197
C1-F	328	449
C2-F	447	647
C3-F	379	525
Sum	1295	1819
Chrysene		
C0-C	48	68
C1-C	74	107
C2-C	99	141
C3-C	84	115
Sum	306	430
TOTAL	10493	14834

Benzo(e)pyrene	10	15
Benzo(a)pyrene	2	4
Perylene	3	4
Indeno(1,2,3cd)pyrene	0.1	0.3
Dibenz(a,h)anthracene	0.6	1
Benzo(ghi)perylene	3	5
TOTAL	204	281

Table 3 shows some interesting trends that should be borne in mind when considering oils and oil weathering. First, one will note that the larger PAHs are increased in concentration as the oil weathers, that is compared to the smaller PAHs. Second, naphthalene itself, is less in evaporatively-weathered oil than in fresh oil. This is important as naphthalene is considered to be important from a toxicological point of view. The loss of BTEX and naphthalene may account partially for the reduced toxicity of evaporatively-weathered ANS oil. It should also be noted that the dibenzothiophenes are polar compounds and are found in most oils. The distributions of these compounds are also unique to specific oils and have been used to fingerprint oils. Further, as shown later in this paper, they are relatively less toxic than their PAH counterparts.

4 Toxicity

4.1 Toxicity Generally

A standard toxicity test is to measure the acute lethal toxicity to a standard species such as the rainbow trout. The LC_{50} of a substance is the 'Lethal Concentration to 50% of a test population', usually given in mg/L, which is approximately equivalent to parts per million. The specification is also given with a time period, which is often 96 hours for larger test organisms such as fish. The smaller the LC_{50} number, the more toxic is the product. The toxicity of oils

themselves range from about 5 to 50 mg/L measured as an LC_{50} to the rainbow trout over 96 hours. Some components have toxicities greater than that.

Aquatic toxicity is a complex topic and as time progresses, more and more studies are added to the complex. More and more facets of toxicity are found to be relevant and important. The following summarizes just a few of the facets of toxicity:

End Points – either Lethal or Sub-lethal

Time – Acute or Chronic - acute usually is on a time frame of hours, time can vary up to months for chronic studies

Species – could be any and often includes local species. The most commonly target species are:

Rainbow trout, Fathead Minnow, Daphnia magna (water flea), varieties of shrimp, Zebrafish, and Vibrio fisheri (fluorescent bacteria used in Microtox). It is important to use at least some of these common indicator species so that comparisons can be made.

Types of test - Lethality – the oldest and most common
Sublethal includes the following:
Growth
Reproduction
Genotoxicity – effect on DNA or RNA
Developmental
Infection resistance
EROD - liver enzyme effects
Androgenic – effect on gender
Endocrine disruption – similar to above
Effects to target organs or tissue
and so on….

4.2 Toxicity of Weathered Oils

The question of which bulk component (usually aromatics versus polars) are more toxic in oils has been addressed by several researchers. One method has been to evaporatively weather the oil and then presumably the polar fraction rises and the aromatic fraction falls, leading to a simple difference between the measured toxicities. This is because many of the polar compounds are less volatile than many of the aromatic compounds. This comparison has led most researchers to conclude that aromatics are more toxic than the polar components. The matter is not that simple. It is now well known that the 2-5 ring aromatics are more toxic than the mono-aromatic and naphthalene type compounds on a short term basis, which are significantly evaporated during weathering experiments. In chronic tests, it has generally been shown that the 3 to 5 ring compounds are most toxic. Thus, weathering changes the toxicity profile of the aromatic compounds, while leaving the polar compounds relatively unchanged. There are data showing the concentrations of PAHs before and after evaporative weathering. For example, Table 3 shows the concentrations of key PAH components of Alaska North Slope oil (ESTD, 2015).

Table 3 shows that although most concentrations of PAHs go up with weathering. However, actual mass should be considered. Figure 1 shows the relative mass change of the various PAH groups and sulfur as weathering occurs for ANS oil.

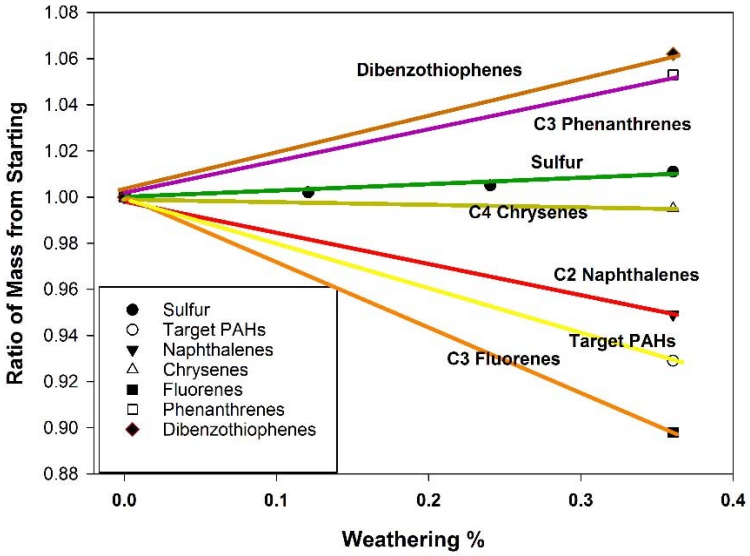

Figure 1 Illustration of the change in PAH and sulfur masses for ANS oil as it is weathered. These are mass-adjusted values from Table 3. It should be noted that the more toxic components, C2 Naphthalenes and Target PAHs including EPA priority PAHs, go down while the lesser toxic components such as Dibenzothiophenes and Phenanthrenes go up.

Figure 1 shows that some concentrations increase, while others decrease. What is important, however is the toxicity of these changes. As one can see from Figure 1, the compound classes with the increases include sulfur (as an element), Dibenzothiophenes and Phenanthrenes. These groups however have low aquatic toxicity. A more specific toxicity comparison can be done however. For some of the compounds listed in Table 3, aquatic toxicities are available (Ecotox, 2015; Vershueren, 2001). For this study, an aquatic toxicity comparison of the weathered compared to the starting oil was carried out. This consisted of locating the aquatic toxicities of as many compounds in Table 3 as possible and then interpolating those not available to complete a toxicity value for Rainbow Trout and Fathead Minnow for each of the compounds. Then the toxicities of the starting and weathered components were compared. The toxicities were taken from the literature (Verschueren, 2001, Ecotox, 2015). These are shown in Figures 2 and 3. As can be seen in these figures, the overall toxicities went down with weathering and can account for the decrease in toxicity with evaporative weathering as observed by several researchers as described below. The fact that the calculated aquatic toxicity decrease matches the empirical studies, does indicate that the loss of components, including some PAHs, accounts for this decrease.

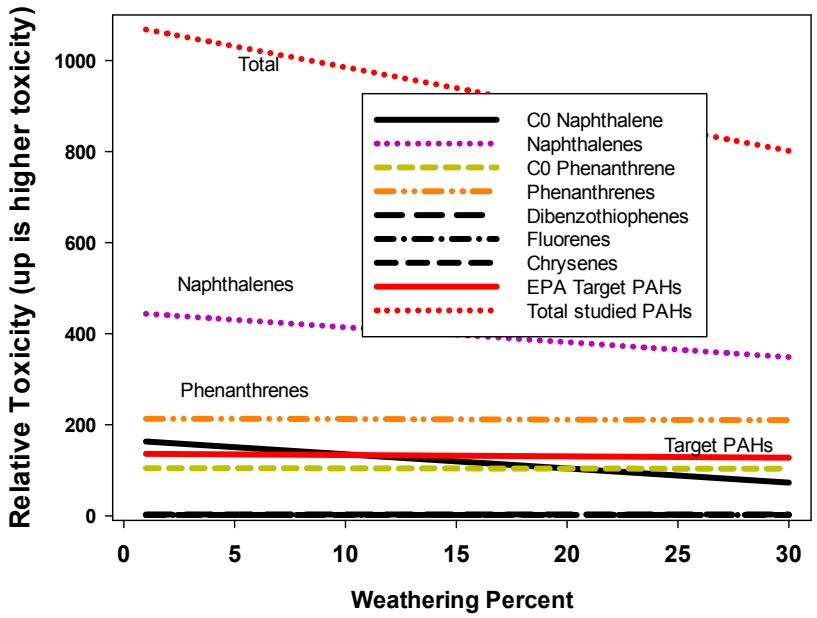

Figure 2 The change in aquatic toxicity to the Rainbow Trout with increased weathering of the oil and changing PAH concentrations.

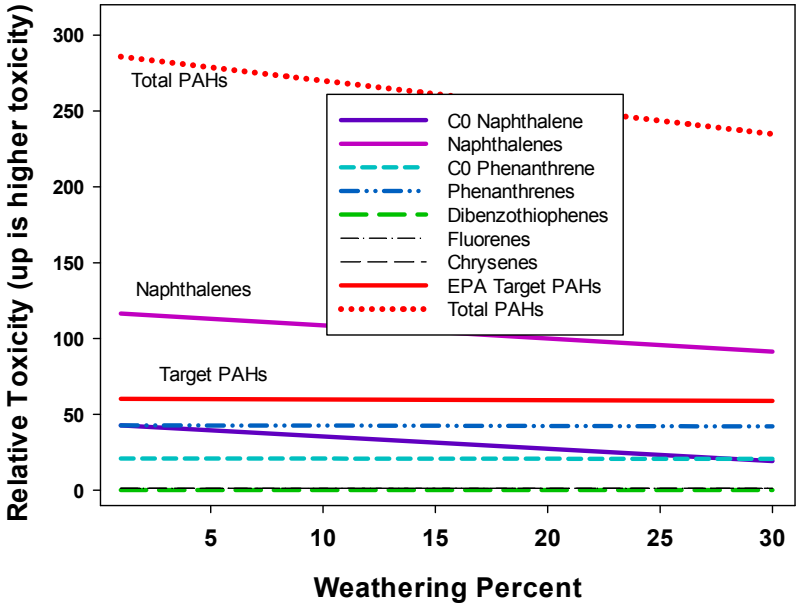

Figure 3 The change in aquatic toxicity to the Fathead Minnow with increased weathering of ANS oil and changing PAH concentrations.

Several authors have studied the toxicity of oils and have noted that the aromatics, particularly the PAHs are more toxic than the remaining components of the oil, based on evaporative weathering studies (VanScoy et al., 2012; Carls et al., 2008). This implies that the polar compounds, which are not generally counted with the aromatics, are less toxic than the aromatics. Radniecki et al. (2013) found similar results when exposing oils, weathered oils and dispersed oils to the ammonium oxidizing bacterium, *Nitrosomonas europaea*. In particular, they found that weathered oils showed less toxicity to Alaska North Slope oil, whether chemically dispersed or not. In fact, dispersed oil that was weathered before dispersion was less toxic than the dispersed unweathered oil. This again implies that the weathered oils (more polar compounds, but probably more importantly, less volatile aromatic compounds) are less toxic.

Others have noted the opposite trend (Barron et al., 1999). Barron et al. (1999) exposed mysid shrimp (*Mysidopsis bahia*) to water accommodated fractions of California spilled oils and found that the toxicity of the oils, both lethal and sublethal, went down with increasing PAHs and Alkylated benzenes. In addition, it also went down with alkane content. These trends are shown in Figure 4. This suggests that the remaining compounds of the water soluble fraction, were more toxic than the PAHs, possibly the polar soluble compounds. Other explanations of this are that the balance of PAHs was changed by the processes in the study and that the more toxic PAHs were left in the oil.

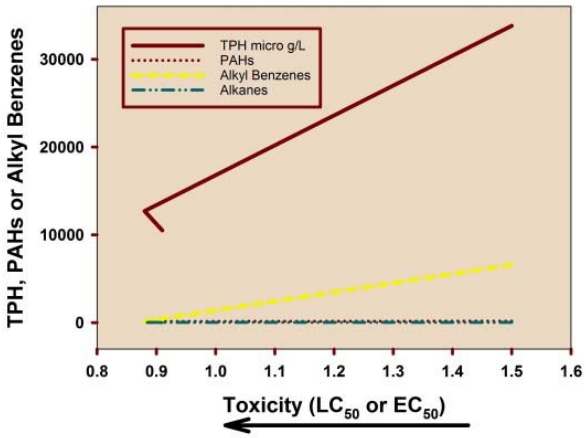

Figure 4 A study showing the toxicity increasing with increasing PAH and alkylated benzene content (After Barron, 1999). Note that the upward trend is decreasing toxicity here, opposite of the visualization.

A decrease in toxicity with weathering has been shown by a number of aquatic toxicologists. In moderately or lightly-weathered oil, it appears that the shifting PAH concentration can change toxicity (Jung et al., 2013). The effect here is mostly certainly that of weathering of the 2-ring aromatics leaving more of the 3-ring compounds. Jung et al. (2013) noted this and also noted in toxicity studies to the Zebrafish that cardiotoxicity was evident for different oils of different geological origins as well as by different exposures (soluble fractions plus high energy dispersed whole oil fractions). Jung et al. noted that the toxicity pattern changed as weathering progressed. This was also noted by others (Carls et al., 1999, 2000; Incardona et al.,

2013; Heintz et al., 1999; Dupuis and Ucan-Marine, 2015). These effects have some relevance to polar compound toxicity but this was not measured and the effects could be explained by PAH distributions.

4.3 Comparison between the Toxicity of Aromatics and Other Components

Weathering experiments are not the only way of assessing the difference in the toxicity of aromatic and polar compounds. Another way to approach the issue is to separate the compounds in oils using techniques such as high-pressure liquid chromatography (HPLC) and solvent extraction. When this was carried out, mixed outcomes result, with one study showing that the aliphatics were more toxic than the polars which were more toxic than the aromatics (Rial et al., 2013a). This study may not be representative as the fractions were extracted using DMSO, which itself is toxic and reactive. Another study showed that many of the polar fractions were not so toxic, but that the production of liver enzymes largely resulted from one of the heaviest polar fractions (Melbye et al., 2009). There are several issues with separation studies; first, the contents of the separated fractions are unknown; second, the fractions may contain aromatic compounds and third, any toxicity differences may be as a result of quantities of the toxicant rather than their specific toxicity. It is also important to note that some of the polar compounds are more soluble than other compounds; therefore, they may be more bioavailable. Traditional tests indicate that polar compounds are less toxic than the aromatic fraction and the toxicity of oil is highest in the 2-5 ring aromatic fraction (see the previous section). Further studies have shown that this doesn't change much through weathering processes such as photooxidation. Another consideration should be that highly soluble polar compounds are probably not remaining in the oil and have been concentrated by water contact and removed before shipment through pipelines.

Several researchers have shown that aromatics are more toxic than other components of the oil by carrying out weathering and/or fractionation studies. Incardona et al. attributed oil toxicity to Pacific herring to the 3 to 4 ring PAHs. Moles (2009) showed that weathering decreased the avoidance preference of pink salmon fry, implicating the aromatics that were removed by weathering. Radovic et al. (2014) tested fractions of North Sea Oil for aryl hydrocarbon receptor antagonist and found less with polar fractions. The only study to show contrary results was by Zemanek et al. (1997) in study of contaminated soils with oil and some with creosote. Tests with Microtox showed toxicity resided in polar fractions, the applicability to oil spills is unknown. These findings are elaborated further in the detailed studies below.

Zemanek et al. (1997) carried out Microtox and Ames bioassays to assess acute toxicity and mutagenicity of water soluble components of fractionated oils extracted from one creosote- and four petroleum-contaminated soils. Microtox results revealed that potential acute toxicity resides mainly in the polar class fractions at three sites and indicated potential synergistic and antagonistic effects between compounds in the total extracts at two sites. Ames *Salmonella* testing indicated that the polyaromatic fractions at two sites exhibit weak mutagenicity with enzymatic activation, while the polar fractions at two sites are weakly mutagenic without enzyme activation. These study results appear to contradict other studies in the field.

Incardona et al. (2009) in one study showed that weathered oil subjected Pacific herring to cardiac arrhythmia when exposed to oil. This was attributed to the 3 to 4 ring PAHs in the oil. This highlights the changes in toxicity when losing the 2-ring PAHs through weathering.

Rial et al. (2013a) fractionated Maya crude oil into aliphatics, aromatics and polars. Fractions were dissolved in dimethyl sulfoxide (DMSO) and subsequently toxicity of single fractions and binary mixtures was assessed using the sea urchin embryo test. The descriptive ability of concentration addition, independent action and modifications of both models for describing the joint toxicity of mixtures had also been evaluated. The hydrocarbon content extractable with dichloromethane of the fractions dissolved in DMSO was: 12.0 mg/L, 39.0 mg/L and 20.5 mg/L for aliphatics, aromatics and polars, respectively. The toxicity of the extracts in DMSO of the fractions as EC_{50} (μL/L) was: aliphatics (165.8 to 242.3) < polars (87.1-115.7) < aromatics (20.5-34.6). Rial et al. (2013b) weathered Angolan crude and a Heavy Fuel Oil by evaporation and photooxidation. The aliphatic, aromatic, polar and asphaltene fractions of the fresh and weathered oils were isolated. The toxicity of the water accommodated fraction or an oil/fraction dissolved in DMSO was assessed using the sea urchin embryo test. Photooxidation was observed to decrease the aromatic content and increase polar compounds. A slight reduction in the toxicity of Angolan crude was observed following weathering for the water-accommodated fraction and the extract in DMSO, but no effect was seen for the heavy fuel oil. For aliphatic compounds, the toxicity decreased in the order fresh > evaporated >photooxidized for both Angolan crude and heating fuel oil. Weathering slightly increased the toxicity of the aromatic and polar fractions of the oil. The aromatic fractions were responsible for most of the toxicity and the polar compounds were the second most important toxic components, despite having less or similar abundance than the aliphatic fraction. The toxic contribution of the aromatic compounds was found to be higher for the fuel oil than for the Angolan crude. A decrease in the toxicity of Angolan crude following weathering, correlated with a reduction in the aromatic fraction.

Melbye et al. (2009) studied the chemical and toxicological properties of the water-soluble fraction of an artificially weathered Norwegian Sea crude oil, by a combination of chemical analysis and toxicity testing in fish in vitro bioassays. The water-soluble fraction of the crude oil was separated into 14 increasingly-polar fractions by preparative high-pressure liquid chromatography. The in-vitro toxicity (7-ethoxyresorufin O-deethylase activity, estrogenicity, and metabolic inhibition) of these fractions was characterized in a primary culture of liver cells (hepatocytes) from rainbow trout (*Oncorhynchus mykiss*). The main contributor to liver enzyme production was one of the most polar fractions, accounting gravimetrically for more than 70% of the organic material in the water-soluble fraction and dominated by an unresolved complex mixture. Chemical analysis by gas chromatography-mass spectrometry and comprehensive two-dimensional gas chromatography-time of flight-mass spectrometry identified a large number of cyclic and aromatic sulfoxide compounds and low amounts of benzothiophenes (<0.1% of total mass). The commonly-monitored, toxic components of crude oil (e.g., naphthalenes, polycyclic aromatic hydrocarbons, and alkylated phenols) eluted in less polar fractions, characterized by somewhat lower toxicity. Normalization of in-vitro responses to the mass in each fraction demonstrated a more even distribution of toxicity, indicating that toxicity in the individual fractions was related to the amount of material present. Although polar and nonpolar compounds contribute additively to crude oil toxicity, the water-soluble fraction was dominated by polar compounds because of their high aqueous solubility and the high oil-water loading. Under these conditions, the polar unresolved-complex mixture-rich fraction might account for a large portion of crude oil EROD activity because of its high abundance in the water-soluble fraction.

Moles (2009) studied preference tests using outmigrant pink salmon fry, *Oncorhynchus gorbuscha*. Fish were allowed to choose between various concentrations of oil/seawater solutions and uncontaminated seawater at a temperature of 7°C. Test oils used to prepare the water accommodated fractions were fresh oil, artificially weathered oil, and dispersed oil. Concentrations of 48 aromatic and 30 alkane hydrocarbons were measured. Twenty trials were run at each tested concentration. The avoidance threshold was the lowest concentration where the number of minutes spent on the uncontaminated side was significantly greater than in the reference period prior to toxicant delivery. Fry avoided water-accommodated mixtures of Alaska North Slope crude oil in seawater at concentrations of 960 mg/L total aromatic hydrocarbons fresh oil and 873 mg/L total aromatic hydrocarbons dispersed oil. These concentrations correspond to 75% and 68% of the reported median lethal concentration. Fry did not avoid weathered oil, indicating that monoaromatic hydrocarbons may be the chemical component of oil being detected. The avoidance of oil suggests a potential for oil pollution to alter pink salmon migration behavior when leaving their native streams, but only at nearly lethal concentrations. This also shows that the polar compounds, which increased in the weathered fraction, were not a factor in avoidance.

Radovic et al. (2014) tested aliphatic, aromatic, and polar oil fractions of North Sea crude oil for the presence of aryl hydrocarbon receptor agonist and androgen receptor antagonist, the AR antagonist effects in the aromatic and, to a lesser extent, polar fractions. Data from two-dimensional gas chromatography-time-of-flight mass spectrometry (GC × GC-TOFMS) data set to the bioassay data obtained from normal-phase LC fractions was shown. The predicted AhR binding effects in the fresh and artificially weathered aromatic oil fractions facilitated the identification of alkyl-substituted three- and four-ring aromatic systems in the active fractions through the weighting of their contributions to the observed effects.

In summary, most tests conclude that weathered oils show less acute and chronic toxicity, indicating that the polar compounds are either not bioavailable or less toxic.

4.4 Toxicity of Specific Polar Compounds

A few studies specifically focused on studying polar compound toxicity, appear in the literature. Carlsson et al. (2014) studied water produced effluents from the North Sea and found that the particulate fraction was 10 times less toxic than the soluble fraction (mostly aromatic), indicating that the polar fractions may be less toxic to the zebrafish. Elie et al. (2015) showed that metabolic profiles of compounds did not show many differences between oxygenated and unoxygenated species. Fallahtafti et al. (2012) showed that hydroxylated products including metabolic products of PAHs were more toxic than the parent PAHs, however, the applicability to oil spills was not shown.

In summary, the toxicity of some specific polar compounds are toxic to fish, however others in weathered oils appear to be less toxic.

4.5 Toxicity of Photo-oxidized Oils

While several researchers studied photo-oxidized oils, only one studied this facet separately. Duesterloh et al. (2002) exposed copepods to photooxidized and base PAHs and found that the photooxidized PAHs were as much as 10 times more toxic. Maki et al. (2001) studied the

effects of biodegraded and sunlight-exposed oil on the bioavailabilty and found it increased by the sunlight exposure. These studies indicate that while photooxidized oils appear to be more toxic in the laboratory, the oxidized compounds may be carried away by water solubility at sea.

In summary, photooxidized oils are generally more toxic to aquatic life than oils that were not exposed to light, however, the question of whether this photooxidation process is rapid enough to cause concern is another issue. In fact, in nature these photooxidation products may be diluted faster than they are created (Maki et al., 2001). This may be true as similar oxidized products in the oil sands are diluted with the process water.

4.6 Photo-enhanced Toxicity

One finding in recent times was that some species, typically transparent or with translucent life stages, were subject to enhanced toxicity to oil when exposed to sun. This is quite different than the formation of photooxidation products. Barron and Ka'aihue (2001) studied the potential for photo-enhanced toxicity in Prince William Sound and Gulf of Alaska waters. Photo-enhanced toxicity is the increase in the toxicity of a chemical in the presence of ultraviolet light (UV) compared to a standard laboratory test conducted with fluorescent lighting (minimal UV). Oil products, weathered oil, and specific polycyclic aromatic compounds present in oil are 2 to greater than 1000 times more toxic in the presence of UV. The photo-enhanced toxicity of oil to fish and aquatic invertebrates appears to occur through a process of photosensitization, rather than photo-modification of the aqueous phase oil. In photosensitization, the bioaccumulated chemical transfers light energy to other molecules causing toxicity through tissue damage. The available evidence indicates that phototoxic components of oil are specific 3-5 ring polycyclic aromatic hydrocarbons (PAHs) and heterocycles. Determinants of photo-enhanced toxicity include the extent of oil bioaccumulation in aquatic organisms and the spectra and intensity of UV exposure. This was noted earlier by a number of authors (Pelletier et al., 1999; Boese et al., 1999) This photo-enhanced toxicity is explained further in Barron et al. (2005, 2008).

The results of these studies, while interesting may not have relevance to the toxicity of polar compounds in and created from oils as photo-enhanced toxicity relates only to oil that is already in a transparent organism.

4.7 Toxicity of Naphthenic Acids (NAs)

Naphthenic acids are a large family of compounds that contain both a ring and an acid function. Most work has been carried out on the characterization and toxicity of naphthenic acids, especially those related to the oil sands of Alberta (Nero et al., 2006). This is because the naphthenic acids are largely separated from the bitumen during processing and discharged along with the process water. Most petroleum, including probably Alaska North Slope, contains some naphthenic acids which is an oxidation product of the petroleum. Low-molecular-weight naphthenic acid content may be low in most pipeline oils as they would be removed by solubility in water and removal before pipeline injection, often in the producing formation. This is especially true for low-molecular weight naphthenic acids. However, naphthenic acids should be considered as models, as they do represent polar compounds and may provide information about polar compound toxicity, even if they are not in high concentrations in Alaskan oils.

Naphthenic acids are a wide group of compounds that can be characterized by the general equation:

$$C_n H_{2n+z} O_z \quad (1)$$

Where: n is the number of carbons

Z is the number of hydrogen atoms removed for a cycloalkane structure (typically 0 to -12). The number of rings in the structure is given by Z/2.

The toxicity of naphthenic acids is summarized in Table 4.

Naphthenic acids can be found in most heavy oils, but typically in low concentrations. An example of this is the Hebei Spirit spill in Korea in 2007 (Wan et al., 2014). Wan et al. developed a sensitive method for analysis of naphthenic acids, together with oxy-NAs in sediment samples then the method was applied to determine the NA mixtures in crude oil, weathered oil, and sediments from the spilled sites after the Hebei Spirit oil spill, South Korea. Concentrations of naphthenic acids, O_3-NAs, and O_4-NAs were found to be 7.8 to130, 3.6 to 44, and 0.8 to 20 mg kg^{-1} in sediments from the spill area, respectively, which were much greater than those measured in the reference sites. Concentrations of naphthenic acids were 50-100 times greater than those (0.077-2.5 mg kg^{-1}) of PAHs in the same sediment samples. The sedimentary profiles of oil-derived naphthenic acids centered around compounds with 21-35 and 12-21 carbons, respectively, indicating that the crude-derived naphthenic acids mixtures originating from the 2007 oil spill, were persistent. Acyclic naphthenic acids of n = 5 to 20 were easily degraded compared to cyclic naphthenic acids n = 21 to 41 during the oil weathering processes, and the ratio of oxy-naphthenic acids n = 21 to 41, relative to naphthenic acids n = 21 to 41 could be an index to estimate the degree of oil weathering in sediments. Altogether, the persistent oil-derived naphthenic acids n = 21 to 41 could be used as a potential indicator for oil-specific contamination, as such compounds would not be much affected by the properties of coastal sediments possibly due to the high sorption of the naphthenic acids in sediment. This study indicates that the NA content could even be used as a tracer of environmental exposure.

Extensive toxicology has been performed on various naphthenic acids. Table 4 summarizes the results. In summary, low-molecular-weight naphthenic acids are found to be acutely toxic and higher-molecular-weight naphthenic acids are less so. The following is a brief textual summary of the results.

Algae growth suppression tests show that some NA compounds suppressed growth and others did not (Goff et al., 2012; Woodsworth et al., 2015; Sweigert et al., 2015). Leishman et al. (2013) studied plant growth suppression with NA concentrations. When the NAs were in process water, growth was not suppressed, however some neat NAs did show plant suppression. The growth and pupation of *Chironomus dilutus* (water midge) was studied in response to NA addition (Anderson et al., 2012; Wiseman et al., 2013b). Generally, little effect was shown at low concentrations but effects were seen at higher concentrations. NA acute toxicity tests with *Daphnia magna* showed that LC_{50} values of about 10 mg/L were less than some whole oils (Sweigert et al., 2015). Mckee et al. (2014) exposed rats to oral doses of NAs and found that effects were observed, however, at relatively high levels. Zhang et al. (2011) exposed E. Coli to various concentrations of NAs in a genetic study. It was found that at environmental concentrations, some genetic effects could be observed. Numerous studies on fathead minnow were carried out (Kavanagh et al., 2011, 2012, 2013; He et al., 2012; Sweigert et al., 2015;

Wiseman et al., 2013a). Generally, it was found that environmentally-relevant concentrations showed, little effect, however elevated levels of NAs showed acutely toxic effects, and lesser levels showed reproductive effects. Some genetic effects were also observed at moderate doses.

NA toxicity tests on frogs and tadpoles showed toxic effects only at high levels (Melvin and Trudeau, 2012, Melvin et al., 2013; Smits et al., 2012; Sweigert et al., 2015). Chronic goldfish exposure to NA showed that this caused less resistance to infection, however, short term exposure increased resistance to the same infection (Hagen et al., 2012). Peters et al. (2007) exposed eggs and larvae of yellow perch and Japanese medaka to oil sands process water containing NAs. Deformities resulted and hatchability was affected, especially at higher concentrations. Tests on mouse NA oral exposure to NAs changed the expression of anti-inflammatory genes, either up or down depending on time after exposure. Numerus Microtox tests were carried out showing both the acute toxicity of NA mixtures and individual compounds, results are given in Table 4 and the Appendix (Frank et al., 2008; Jones et al., 2011, Sweigert et al., 2015). In summary, acute toxicities of NAs were greatest for low-molecular-weight or aromatic-containing compounds.

Collier et al. (2013) reviewed the toxicity of PAHs and Naphthenic acids and the effects of naphthenic acids on fish. NA chronic and acute exposures to rainbow trout were carried out showing some effects at low concentrations and more at higher concentrations (LeClair et al., 2013; MacDonald et al., 2013; McNeill et al., 2012; Peters et al., 2007; Tollefson et al. 2012). Knag et al. (2013) carried out chronic tests on the three-spined stickleback showing at the concentrations tested that NAs did not have estrogenic effects. Acute exposures of yellow perch to NAs showed some gill effects (Nero et al., 2006). Zebrafish larvae when exposed to varying levels of NAs showed acute toxicity however an aromatic NA showed more toxicity (Scarlett et al., 2012, 2013; Reinardy et al., 2013). Sansom et al. (2013) correlated fish cell lines for cellular integrity and these values correlated with NA exposure.

In summary, the following observations can be made about naphthenic acid toxicity. First and foremost, is that NA toxicities vary widely from relatively non-toxic to moderately toxic. The low-molecular weight compounds are more toxic than the higher-molecular weight compounds. Similarly, the aromatic NAs are more toxic than the aliphatic ones. The levels of NAs in oil sands process water doesn't appear to be acutely toxic. Further, mixtures of acids appear to be less toxic than many of the separated compounds. This may be due simply to the presence or absence of particularly toxic compounds. No single mechanism of aquatic toxicity is evident for NAs, however, the effects on juveniles of any species is more pronounced than on adults – as is the case with most aquatic toxicants.

Studies on naphthenic acid shows that the lower molecular species exhibit some toxicity. The fact remains that many of the naphthenic acid compounds studied were separated by water processing of oil sands bitumen. It is highly unlikely that a large portion of these same low-molecular-weight compounds would remain in any oil as almost all oils produced would come into contact with water and thus would remove a large portion of such water-soluble compounds.

Table 4 Toxicity of Naphthenic Acids

Name	Details	Separation	Info	Characterization - Separation		Toxicity	Species	Value	Exposure time	Test	Reference
Extracted from pond at Ft. Mc.				different pond types		pupation	*Chironomus dilutus*	8 - 13 % emergence			Anderson et al., 2012
Extracted from pond at Ft. Mc.			separated by distillation			EC_{50}	*Vibrio fisheri* - Microtox				Frank et al.,2008
Extracted from pond at Ft. Mc.			separated by distillation		lowest MW	EC_{50}	Microtox	10 mM	15 min.	lowest MW cmpd.	Frank et al.,2008
			Distill temp	Mean Molecular Wt.							Frank et al.,2008
			130	223		EC_{50}	Microtox	42 mg/L	15 min.		Frank et al.,2008
			160	243		EC_{50}	Microtox	58 mg/L	15 min.		Frank et al.,2008
			190	261		EC_{50}	Microtox	43 mg/L	15 min.		Frank et al.,2008
			220	287		EC_{50}	Microtox	55 mg/L	15 min.		Frank et al.,2008
			residue >220	346		EC_{50}	Microtox	65 mg/L	15 min.		Frank et al.,2008
			stock			EC_{50}	Microtox	53 mg/L	15 min.		Frank et al.,2008
					Highest MW	EC_{50}	Microtox	0.6 mM	15 min.	highest MW cmpd.	Frank et al.,2008
Extracted from pond at Ft. Mc.			compared to commercial mixture				mice	50 or 100 mg/kg	8 wk	expression of pro-inflammator	Garcia-Garcia et al., 2012
										genes was down in animals ex	Garcia-Garcia et al., 2012
										but little effect on extracts fro	Garcia-Garcia et al., 2012
Isolated from waste water							Green Algae	up to 100 mg/L	24 hr	relatively tolerant	Goff et al., 2012
Commercial Mixture			not characterized				Goldfish	20 mg/L	7 d	> resistant to infection	Hagen et al., 2012
							Goldfish	20 mg/L	12 wk	< resistant to infection	Hagen et al., 2012
Extracted from pond at Ft. Mc.				ozonated or not - content 19.7 mg/L			fathead minnow	eggs hatched	7 d	many developmental	He et al., 2012
							larvae	in full exp. Water		effects noted, ozone-treated w	He et al., 2012
1-adamantane carboxylic acid				pure compound		EC_{50}	Microtox	0.667 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
1-adamantane ethanoic acid				pure compound		EC_{50}	Microtox	0.784 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
2,6,10-trimethyl undecanoic acid				pure compound		EC_{50}	Microtox	0.015 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
2,6-dimethyl heptanoic acid				pure compound		EC_{50}	Microtox	0.240 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
3,5,dimethyl adamantane carboxylic acid				pure compound		EC_{50}	Microtox	0.565 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
3,5,dimethyl adamantane ethanoic acid				pure compound		EC_{50}	Microtox	0.337 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
3,7-dimethyl octanoic acid				pure compound		EC_{50}	Microtox	0.060 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
3-cyclohexyl propanoic acid				pure compound		EC_{50}	Microtox	0.410 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
3-decalin-1-yl propanoic acid				pure compound		EC_{50}	Microtox	0.004 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-cyclohexyl butanoic acid				pure compound		EC_{50}	Microtox	0.110 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-ethyl cyclohexyl ethanoic acid				pure compound		EC_{50}	Microtox	0.340 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-methyl dodecanoic acid				pure compound		EC_{50}	Microtox	0.012 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-methyl nonanoic acid				pure compound		EC_{50}	Microtox	0.20 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-methyl octanoic acid				pure compound		EC_{50}	Microtox	0.480 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-butyl cyclohexyl ethanoic acid				pure compound		EC_{50}	Microtox	0.095 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-butyl phenyl ethanoic acid				pure compound		EC_{50}	Microtox	0.250 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-hexyl cyclohexyl ethanoic acid				pure compound		EC_{50}	Microtox	0.012 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-hexyl phenyl ethanoic acid				pure compound		EC_{50}	Microtox	0.023 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-pentyl cyclohexyl ethanoic acid				pure compound		EC_{50}	Microtox	0.030 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-pentyl phenyl ethanoic acid				pure compound		EC_{50}	Microtox	0.044 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-propyl cyclohexyl ethanoic acid				pure compound		EC_{50}	Microtox	0.200 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
4-n-propyl phenyl ethanoic acid				pure compound		EC_{50}	Microtox	0.394 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
5-cyclohexyl pentanoic acid				pure compound		EC_{50}	Microtox	0.050 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011

Table 4 Toxicity of Naphthenic Acids

Name	Details	Separation	Info	Characterization - Separation	Toxicity	Species	Value	Exposure time	Test	Reference
6-cyclohexyl hexanoic acid				pure compound	EC_{50}	Microtox	0.040 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
7-methyl dodecanoic acid				pure compound	EC_{50}	Microtox	0.080 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Decalin-2-carboxylic acid				pure compound	EC_{50}	Microtox	0.218 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Decalin-2-ethanoic acid				pure compound	EC_{50}	Microtox	0.027 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Decanoic Acid (DA)				pure compound	EC_{50}	Microtox	0.12 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Dodecanoic Acid				pure compound	EC_{50}	Microtox	0.019 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Hexanoic Acid				pure compound	EC_{50}	Microtox	0.7 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Nonanoic Acid				pure compound	EC_{50}	Microtox	0.36 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Octanoic Acid				pure compound	EC_{50}	Microtox	0.38 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Synthesized or commercial				individual compounds	EC_{50}	Microtox	0.004 to 0.7 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Undecanoic Acid				pure compound	EC_{50}	Microtox	0.042 mM	15 min.	*Vibrio fischeri*	Jones et al., 2011
Extracted from demo pond at Ft. M			not characterized		immunity to gill diseases	Fathead minnow	10 mg/L	nr	immunity	Kavanagh et al., 2011
Extracted from pond at Ft. Mc.			not characterized		Reproduction suppressed	Fathead minnow	~ 25 mg/L	21 d	reproduction	Kavanagh et al., 2011
Extracted from pond at Ft. Mc.			not characterized		Reproduction suppressed	Fathead minnow	~ 5-10 mg/L	21 d	reproduction	Kavanagh et al., 2011
Two commercial mixtures						three-spined stickleback		21 d		Knag et al., 2013
						did not show estrogenic nor anti-androgenic properties				Knag et al., 2013
Purified extracts from oil sands process water - not further characterized						Rainbow trout		7 d		LeClair et al., 2013
							decrease in A. salmonicida antibodies			LeClair et al., 2013
Extracted acids from process water							extracted did not inhibit germination			Leishman et al., 2013
Surrogate naphthenic acids			not characterized			Arabidopsis - plant	surrogate inhibited		seed germination	Leishman et al., 2013
Extracted from pond at Ft. Mc.				not characterized		Rainbow trout	0, 10 or 100	5 or 21 d	low effect on EROD	MacDonald et al., 2013
						intraperitoneal	mg/kg		low effect on immunity to A. s	MacDonald et al., 2013
Commercial Mixture			90% were C10 to C16		no effect level	target organs -rats	100 mg/kg/d			McKee et al., 2014
			39% had one ring, 31% two rings		no effect level	developmental effects -rats	100 mg/kg/d			McKee, 2014
			5 % had three rings, 1% four rings and 24% no rings							McKee, 2014
Process water in pond			three different water systems			Rainbow Trout		21 d	high liver enz./gill erosion	McNeil et al., 2012
Commercial mixture			not characterized		LC_{50}	Tadpole Larvae	4.8 mg/L			Melvin & Trudeau et al., 2012
					LC_{50}	Tadpoles	3 mg/L			Melvin et al., 2012
Commercial mixture			48	40 12 84 16	LC_{100}	yellow perch	3.6 mg/L	21 d	low salt	Nero et al., 2006
Extracted from Mildred Lake			28%	55% 17% 61% 39%	LC_{100}	yellow perch	6.8 mg/L	21-d	low salt	Nero et al., 2006
			C5-13	C14-21 C22-33 Z9 to -4 Z-6 to -12					high salt reduced toxicity	Nero et al., 2006
									high salt reduced toxicity	Nero et al., 2006
Commercial mixture			not characterized		Deformity	yellow perch		24 h	larval	Peters et al., 2007
Extracted from Mildred Lake			not characterized		LC_{50}	Rainbow trout	3-5 mg/L		calculated	Peters et al., 2007
					Deformity	yellow perch	> 1.6% dilution	24 hr		Peters et al., 2007
					Deformity	Japanese Medaka	> 1.6% dilution	24 hr		Peters et al., 2007
					Deformity	Japanese Medaka		24 h	larval	Peters et al., 2007
Extract from oil sands			fractions using argentation column			Zebrafish larvae	>20 to 2000 µg/L	96 hr	no estrogenic effects	Reinardy et al.,2013
Extracted from pond at Ft. Mc.			unseparated		LC_{50}	Zebrafish larvae	8.4 mg/L	96 hr		Scarlett et al., 2013
			acid extract		LC_{50}	Zebrafish larvae	5.4 mg/L	96 hr		Scarlett et al., 2013
			alicyclic		LC_{50}	Zebrafish larvae	13.1 mg/L	96 hr		Scarlett et al., 2013
			aromatic		LC_{50}	Zebrafish larvae	8.1 mg/L	96 hr		Scarlett et al., 2013

Table 4 Toxicity of Naphthenic Acids

Name	Details	Separation	Info	Characterization - Separation	Toxicity	Species	Value	Exposure time	Test	Reference
				dehydroabietic acid	LC_{50}	Zebrafish larvae	1.2 mg/L	96 hr		Scarlett et al., 2013
Commercial mixture				not characterized		northern leopard frog	0, 20, or 40 mg/L	28 d	Nas deposited in muscle tissue with little negative effec	Smits et al., 2012 Smits et al., 2012
Commercial Mixture			1-ring >2-ring > acyclic >3-ring acids used fractionated sample containing n-acids from C10 to C14		LL_{50}	Fathead minnow	9.0 mg/L	96 hr		Sweigert et al., 2015
					LC_{50}	Fathead minnow	5.6 mg/L	96 hr		Sweigert et al., 2015
					LL_{50}	Daphnia magna	24 mg/L	48 hr		Sweigert et al., 2015
					LC_{50}	Daphnia magna	20 mg/L	48 hr		Sweigert et al., 2015
					LL_{50}	Algae - subcapitata	43 mg/L	96 hr		Sweigert et al., 2015
					LC_{50}	Algae - subcapitata	40 mg/L	96 hr		Sweigert et al., 2015
					EC_{50}	Microtox	46 mg/L	15 min.		Sweigert et al., 2015
Synthetic compounds					EC_{50}	Rainbow trout	24-89 mg/L	96 hr	metabolic effects	Tollefson et al., 2012
					EC_{50}	Rainbow trout	43-148 mg/L	96 hr		Tollefson et al., 2012
Extracted from pond at Ft. Mc.			Treated with ozone and untreated			Fathead minnow		7 d	transcriptions varied	Wiseman et al., 2013a
Extracted from pond at Ft. Mc.			Fresh and aged process water (ospw)			*Chironomus dilutus*		4 & 7 d	growth less in fresh ospw	Wiseman et al., 2013b
Extracted from pond at Ft. Mc.				not characterized		algae - 6 species	1000 mg/L	14 d	lowest observed effect	Woodsworth et al., 2015
						algae - 4 speciies	300 mg/L		on growth	Woodsworth et al., 2015
Commercial mixture				not characterized		E Coli	0, 10 or 100 mg/L	3 hr	transcriptional reporters altered some up some down	Zhang et al., 2011 Zhang et al., 2011

4.8 Toxicity of Polar Compounds Generally

A number of studies have been carried out on oxygenated PAHs (OPAHs), many of which cannot be related directly to degradation products of petroleum. Many of these studies are related to degradation of PAHs derived from industrial sources other than petroleum sources and degradation processes involving combustion or reactions in industrial wastes. They may serve to provide information on petroleum oxygenates, however it should be noted in most cases that the compounds noted have not been identified at spill scenes. Knecht et al. (2013) for example studied oxygenated PAHs and found that the most toxic were those where the ketone compounds were on adjacent carbons.

Hazard-level screening of heavy oils from a human toxicity point of view was carried out by EPA (EPA, 2012). This shows that heavy oils are less toxic (both acute and chronic) than lighter oils in tests using models that relate to humans, e.g. rats. This may be significant here, in that the heavy oils contain a larger proportion of polar compounds than do light oils.

4.9 Photooxidation Products and Pathways

One important line of studies is to determine the products and pathways of petroleum photooxidation. To summarize these studies, Bobinger et al. (2009) studied the photooxidation of benzothiophenes. Lampi et al. (2006, 2007) studied the toxicities of parent PAHs and oxygenated PAHs to *Daphnia Magna* and to duckweed. Many of the PAH photoproducts were much more toxic than the parent PAHs. This appears to be generally the case, that the photoproducts are more toxic than the parent compounds. One question that remains, however is if the products would actually be bioavailable. Further, the rate at which photooxidation occurs, over days to achieve a few percent, implies that a toxic concentration may not be reached at sea. Photoproducts are often much more water soluble than the starting compounds and thus may be solubilized in water and diluted at sea (Maki et al., 2001). This may be true as similar oxidized products in the oil sands are diluted with the process water. So in summary, photooxidation is known to produce oxygenated derivatives. These are sometimes more aquatically toxic than the originating compound. The speed of photooxidation also may be slow enough to prevent the accumulation of toxic concentrations of breakdown products. Soluble photo-products would be diluted at sea before causing a high toxicity.

4. 10 Toxicity Studies and Their Implications

An important issue when discussing oil and oil components is certainly toxicity, both of the oil itself and of any separated components. As noted in the text and also in the Appendix, many polar compounds have not been separated from oil and tested for toxicity. In general, one must bear in mind that highly polar compounds (especially those of lower molecular weight) are not in transported oils and will long have leached or solubilized in water. Most oils come into contact with water in the producing formations and sometimes thereafter. Further, many polar compounds still are highly soluble in the oil and are often separated with the PAHs.

Aquatic toxicity is a complex topic and as time progresses, more and more studies are added to the complex. More and more facets of toxicity are found to be relevant and important.

The most commonly target species are: Rainbow trout, Fathead Minnow, *Daphnia magna* (water flea), varieties of shrimp, Zebrafish, and *Vibrio fisheri* (fluorescent bacteria used in Microtox). It is important to use at least some of these common indicator species so that comparisons can be made.

The tests and species used for studying polar compounds in this particular study are summarized in Table 5 below.

Table 5 Toxicity End Points and Species Used

Lethality	Toxicity	Species	Exposure time	Reference
Sub-Lethal	pupation	*Chironomus dilutus*		Anderson et al., 2012
Lethal	LC_{50} and EC_{20}	Mysid shrimp	various	Barronet et al., 1999
Lethal	EC_{50}	Pacific Herring eggs/larvae	96 hr	Barron et al., 2003
Sub-Lethal	Reproduction	Pacific Herring eggs/larvae	14 d	Carls et al., 2000, 2008
Lethal	LC_{50}	Pacific Herring eggs/larvae	96 hr	Carls et al., 2008
Sub-Lethal	developmental toxicity	Zebrafish	various	Carlsson et al., 2014
Sub-Lethal	various - swimming etc.	Copepods	24 hr	Duesterloh et al., 2002
Lethal	EC_{50}	*Vibrio fisheri* - Microtox		Frank et al.,2008
Sub-Lethal	Genetic effects	mice	8 wk	Garcia-Garcia et al., 2012
Sub-Lethal	relatively tolerant	Green Algae	24 hr	Goff et al., 2012
Sub-Lethal	resistant to infection	Goldfish	7 d	Hagen et al., 2012
Sub-Lethal	resistant to infection	Goldfish	12 wk	Hagen et al., 2012
Sub-Lethal	Reproduction	fathead minnow	7 d	He et al., 2012
Sub-Lethal	edema	Herring embryos	various	Incardona et al., 2009
Sub-Lethal	embryotoxicity	Zebrafish larvae	various	Incardona et al., 2013
Lethal	EC_{50}	*Vibrio fischeri*	15 min.	Jones et al., 2011
Sub-Lethal	Organal effects	Zebrafish larvae	various	Jung et al., 2013
Sub-Lethal	Reproduction suppressed	Fathead minnow	21 d	Kavanagh et al., 2011

Sub-Lethal	immunity to gill diseases	Fathead minnow	nr	Kavanagh et al., 2012

Table 5 Toxicity End Points and Species Used

Lethality	Toxicity	Species	Exposure time	Reference
Sub-Lethal	estrogenic effects	three-spined stickleback	21 d	Knag et al., 2013
Sub-Lethal	decrease in A. salmonicida antibodies	Rainbow trout	7 d	LeClair et al. 2013
Sub-Lethal	surrogate inhibited	Arabidopsis - plant		Leishman et al., 2013
Sub-Lethal	EROD effects	Rainbow trout	5 or 21 d	MacDonald et al., 2013
Sub-Lethal	target organ effects	target organs -rats		McKee et al., 2014
Sub-Lethal	high liver enz. /gill erosion	Rainbow Trout	21 d	McNeil et al., 2012
Sub-Lethal	EROD, Estrogenicity	Liver cells from Rainbow Trout		Melbye et al., 2009
Lethal	LC_{50}	Tadpole Larvae		Melvin & Trudeau et al., 2012
Lethal	LC_{50}	Tadpoles		Melvin et al., 2012
Sub-Lethal	preference tests	Pink Salmon fry		Moles et al., 2009
Lethal	LC_{100}	yellow perch	21-d	Nero et al., 2006
Lethal	LC_{50}	Rainbow trout		Peters et al., 2007
Sub-Lethal	Deformity	yellow perch	24 hr	Peters et al., 2007
Sub-Lethal	Deformity	Japanese Medaka	24 hr	Peters et al., 2007
Lethal	EC_{50}	Nitrogen oxidizing bacteria		Radniecki et al., 2013
Sub-Lethal	androgen reception	chemical reactivity		Radovic et al., 2014
Sub-Lethal	no estrogenic effects	Zebrafish larvae	96 hr	Reinardy et al.,2013
Sub-Lethal	EC_{50}	Sea Urchin embryos	various	Rial et al., 2013a

Lethal	LC_{50}	Zebrafish larvae	96 hr	Scarlett et al., 2013
Sub-Lethal	Genetic effects	northern leopard frog	28 d	Smits et al., 2012
Lethal	LL_{50}	Fathead minnow	96 hr	Sweigert et al., 2015
Lethal	LL_{50}	Daphnia magna	48 hr	Sweigert et al., 2015

Table 5 Toxicity End Points and Species Used

Lethality	Toxicity	Species	Exposure time	Reference
Lethal	LL_{50}	Algae - subcapitata	96 hr	Sweigert et al., 2015
Lethal	EC_{50}	Microtox	15 min.	Sweigert et al., 2015
Sub-Lethal	EC_{50}	Rainbow trout	96 hr	Tollefson et al., 2012
Lethal	LC_{50}	Top Smelt and Embryos	96 hr	VanScoy et al., 2012
Sub-Lethal	Transcription	Fathead minnow	7 d	Wiseman et al., 2013a
Sub-Lethal	Growth	*Chironomus dilutus*	4 & 7 d	Wiseman et al., 2013b
Sub-Lethal	Lowest observed effect	algae - 6 species	14 d	Woodsworth et al., 2015
Lethal	LC_{50}	*Vibrio fischeri*	various	Zemanek et al., 1997
Sub-Lethal	EC_{50}	Ames test	various	Zemanek et al., 1997
Sub-Lethal	Genetic effects	E Coli	3 hr	Zhang et al., 2011

This shows how varied and complex the aquatic testing situation can be. In summary:

1. The separation of polar compounds is detailed in chapter 5 and is far more complex and sophisticated than even the toxicity testing. It will be many years before many compounds are separated such that toxicity testing on them can be performed. Further one must always question whether a separated compound is truly representative of the toxicity as its mutual solubility in the oil matrix may render it non-toxic when not separated. Both the toxicity and separation are noted in the Appendix.
2. Minimum tests should include key indicator species and key tests for both chronic and acute tests. It is estimated that a minimum of 6 different tests would be needed before one had an idea of the relative toxicity of a new substance or compound and that about 20 different tests would give a more complete picture.
3. A full toxicity picture is only obtained when several tests are conducted and on several species.

4. Local species, in this case Alaskan species, may provide useful information but it is more important to first test key species to provide comparison points. AOSRT (2014) notes some of the species that might be used for the Arctic.

5 Separation and Analysis

Oil analysis is a complex topic and entire encyclopaedias could be devoted to the topic – which indeed there are. The standard method for oil analysis is to use a gas chromatograph (GC). A small sample of the oil extract, often in hexane or dichloromethane (DCM), and a carrier gas, usually helium or hydrogen, are passed through a capillary column. The sample is injected into a heated chamber from where its vapors pass into the silica column. The silica column is coated with absorbing materials and, because the various components of the oil have varying rates of adhesion, the oil separates because these components are absorbed at different rates onto the column walls. The gases then pass through a sensitive detector. The injector, column and detector are often maintained at constant temperatures to ensure repeatability. The system is calibrated by passing known amounts of standard materials through the unit. The amount of many individual components in the oil is thereby measured. The components that pass through the detector can also be totaled and a TPH value determined. It is important to note that only the vapors pass into the column initially. Heavier contaminants can foul the injector and first part of the column. It is therefore important the sample is subjected to a cleanup procedure before it is injected into the column. Cleanup procedures can be complex and involve several steps.

While a GC measurement is highly accurate, this standard measurement does not include resins, asphaltenes, and some other components of the oil with higher molecular weight which do not vaporize and pass through the column. These heavier components can be determined separately using open column chromatography or precipitation techniques. Many methods have been used to analyze oils in the past and many new methods are being developed. For polar compounds there have been several developments in recent times. These are summarized very briefly in Figure 5, and will be described briefly below.

	sample prep	extraction	Injection	Column	Detector	Results
Standard	cleanup	DCM	On-column	Std.	MS	few polars
GC X GC	cleanup	DCM	On-column	Std.	MS	hump cmpds.
TOF	cleanup	specialized	On-column	Std.	MS	some polars
ESI - ICR	little cleanup	specialized	ESI		ICR	many polars
LCMS	cleanup	special	Liquid		MS	some polars
Specialized	cleanup	special	On-column		MS	selected polars

Figure 5 An approximation of the six most common methods to have been used to analyze oils for various purposes as well as to identify polar compounds. Sample 'prep' is sample preparation. This is often a defining step as in traditional chromatography it removes many of the polar compounds.

Standard gas chromatography has been summarized above and is very limited in polar compound analysis as many of the polars are removed before chromatography can proceed.

GC X GC or comprehensive two-dimensional gas chromatography, in GC × GC two columns are connected sequentially, typically the first dimension is a conventional column and the second dimension is a short fast GC type, with a modulator positioned between them. The function of the modulator is to continuously collect small fractions of the effluent from 1D, ensuring that the separation is maintained in this dimension and to quickly transfer the 2D fraction collected and focused as a narrow pulse. The result is to remove the traditional chromatography 'hump' and analyze it. This is very good for analyzing isomers of hydrocarbons which tend to group in the 'hump' but less so at highly polar compounds. Compounds of low polarity such as alcohols may be separated by this method.

Time-of-flight mass spectrometry has been used to analyze oils for polar compounds. The success relies not so much in the mass spectrometry, but in the sample preparation and chromatography which is more flexible than in standard chromatography. Success in measuring some polar compounds has been record.

ESI or Electrospray Ionization is a technique used in mass spectrometry to produce ions using an electrospray in which a high voltage is applied to a liquid to create an aerosol. It is especially useful in producing ions from macromolecules such as larger polar compounds because it overcomes the propensity of these molecules to fragment when ionized. ESI is different from other atmospheric pressure ionization processes since it may produce multiply charged ions, effectively extending the mass range of the analyser to accommodate the larger orders of magnitude observed in resins and asphaltenes. ESI may be used on a variety of mass spectrometers.

ICR or Ion Cyclotron Resonance refers to the selective movement of ions in a magnetic field. This selectivity can be used for measuring the masses of an ionized analyte in mass spectrometry, particularly with Fourier transform ion cyclotron resonance mass spectrometers. When combined with the ESI, it can yield the elemental formulae of hundreds and thousands of molecules.

LCMS or Liquid Chromatography Mass Spectrometry can be used for polar analysis of oils. In HPLC, the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase generally composed of particles chosen or derivatized to accomplish particular types of separations. The sample is then injected into a mass spectrometer. The application has yield good information about some types of polar compounds.

Specialized methods have been developed to shed light on specific families of polar compounds (Strausz and Lown, 2003). These methods often involve some of the above injections, separations and mass spectrometric types, however, very specific methods are developed for each sub-family of compounds. To analyze a number of compound families can take a life time.

5.1 Polar Compounds in Weathered Oils

Weathered oils contain a significant amount of polar compounds and their content increases as the weathering progresses. This is especially true of oxygenated compounds. Ruddy et al. (2014) studied weathered Macondo oil samples taken from a Pensacola beach by both ESI FT ICR and APPI in GC X GC. They found complex compound distribution in the weathered oil consisting of ketones, hydroxyl and carboxylic compounds and mixed compounds of these. In fact, weathering processes in the Gulf of Mexico resulted in more than a doubling of the

compound complexity of the crude oil. Most of the transformations in the Gulf were found to be oxygenated aromatic hydrocarbons between C_{20} to C_{85}. Table 6 shows some of the major species found in the fresh and weathered oil. Ruddy et al. (2014) also noted that aerobic alkane biodegradation proceeds by terminal or sub-terminal oxidation however in this study these products would be suppressed in the analytical method. Ruddy et al. (2014) also performed GC X GC/TOF MS analysis of fractionated and unfractionated extracts. The extracts largely yielded ketones and carboxylic acids. The first fraction yielded largely single or double ketones; the second fraction, O_1 to O_5 compounds: the third fraction, O_1 to O_7 and the fourth fraction yielded polyfunctional ketone and carboxylic compounds O_1 to O_8.

Table 6 Significant Polar Species in Macondo Oil and Weathered Oil (Ruddy et al., 2014)

Species in Fresh DWH Oil	Species in highly weathered DWH oil
N	N
NO	NO
NO_2	NO_2
NS	NS
OS	OS
	NO_3
	O
	O_2
	O_3

Islam et al. (2013, 2015) compared two sets of oil samples, one obtained from different weathering stages of the M/V Hebei Spirit oil spill site and the other prepared by a photo-degradation experiment. Samples were compared by atmospheric pressure photo-ionization coupled with Fourier transform ion cyclotron resonance mass spectrometry. The oil samples were separated into saturate, aromatic, resin, and asphaltene fractions before analysis. Gravimetric analysis of the SARA fractions revealed a decreased weight percentage of the aromatic fraction and an increased resin fraction in both sets of samples. Molecular-level investigations of the SARA fractions showed a significant reduction in the S_1 class in the saturate fraction and increase of S_1O_1 class compounds with high DBE values in resin fraction. Levels of N_1 and N_1O_1 class compounds increased after degradation compared to compounds generating molecular ions. This study revealed changes occurring in heteroatom polar species of crude oils such as sulfur- and nitrogen-containing compounds that would not be detected with conventional GC based techniques. This study presented evidence demonstrating that secondary and tertiary amines were more vulnerable to photo-degradation than compounds containing pyridine, and hence, preferential degradation depending on chemical structures must be considered in the study of hazardous or toxic components.

5.2 Separation and Analysis Techniques

Analytical techniques are an issue and many of the polar compounds of interest may not be detected by standard petroleum analysis methods (Drozdova et al., 2013). Further, there exist no simple methods by which the polar compounds can be separated or analyzed. Analytical techniques that have been applied are summarized in Table 7.

Table 7 Separation and Analytical Techniques

Number	Type	Technique	Reference
1	GC	Standard extraction and GC	
2	LC ISI MS	Liquid Chromatography Electrospray Ionization Mass Spectrometry	Porter et al., 2004
3	FT ICR MS	Fourier Transform Ion Cyclotron Resonance Mass Spectrometry	Wang et al., 2011
4	GC X GC	Two Dimensional Gas Chromatography	Radovic et al., 2014
5	DMS	Differential Mobility Spectrometry	Noesthedon et al., 2014
6	nano-DESI	Nanospray Desorption Electrospray Ionization	Eckert et al., 2012
7	NIOMS	Negative Ion Orbitrap Mass Spectrometry	Headley et al., 2013
8	APPI-FTICR-MS	Atmospheric Pressure Photoionization Source - Fourier Transform Ion Cyclotron F	Walters et al., 2015
9	NESI-FTICR-MS	Negative Electrospray Ionization	Walters et al., 2015
10	PESI-FTICR-MS	Positive Electrospray Ionization	Walters et al., 2015
11	AG - SPE	argentation solid phase extraction - separate Aromatic Naphthenic acids	Jones et al., 2012
12	UPLC-ESI-QTOF-MS	MAX column followed by Ultrapressure Liquid Chromatography....	Wang et al., 2013
13	LDI-FTICR-MS	Laser Desorption Ionization - Fourier Transform Ion Cyclotron Resonance	Cho et al., 2012
14	Resin Column	Separation of nitrogen-containing compounds	Oliveira et al., 2004
15	KOH extraction	Removed acidic compounds	Fafet et al., 2008
16	Cyclodextrin Adsorption	Adsorbed species of oxygenated compound including naphthenic acids	Headley et al., 2014
17	DART - FTICR MS	Direct Analysis in Real Time - Fourier Transform Ion Cyclotron Resonance ...	Lobodin et al., 2015
18	NOM - ESI -ICR	negative-ion electrospray ionization - ElectroSpray Ionization -	Mapolelo et al., 2011
19	UPLC-ESI-QTOF-MS	Ultrahigh Pressure -ElectroSpray Ionization	Wan et al., 2014
20	Distillation then ESO	distillation then negative-ion ESI FT-ICR MS	Wang et al., 2011a,b
21	µ SPE then GC MS	Micro Solid Phase Extraction, then GC MS	Yang et al., 2013
22	alumina then GC MS	polar 1 separation technique	Strausz & Lown et al., 2003
23	GPC then GC MS	GPC Biobeads SX-1 - polar 1 separation technique	Strausz & Lown et al., 2003
24	Sil. Gel then GC MS	Silica Gel - polar 2 separation technique	Strausz & Lown et al., 2003
25	GPC then GC MS	GPC Biobeads SX-1 - polar 2 separation technique	Strausz & Lown et al., 2003
26	Sil. Gel then GC MS	Silica Gel - sulfoxide separation technique	Strausz & Lown et al., 2003
27	Sil. Gel then GC MS	Silica Gel - thiophene separation technique	Strausz & Lown et al., 2003
28	Alumina then GC MS	Alumina - basic nitrogen separation technique	Strausz & Lown et al., 2003
29	$TiCL_4$ & $CuCl_2$	Complexation to yield basic nitrogen compounds	Strausz & Lown et al., 2003
30	Pyrolysis	Pyrolysis of non-distillable fractions, then separation	Strausz & Lown et al., 2003

McKenna et al. (2013) analyzed Macondo residual oil using Fourier Transform - Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). The analytical window for different methods was discussed, noting that only a small percentage of compounds in oils, especially weathered oils, are accessible by standard GC MS techniques. They characterized more than 30,000 acidic, basic and nonpolar compounds from the Macondo spill to create a baseline for future analysis. The analytical situation is illustrated in the following figures:

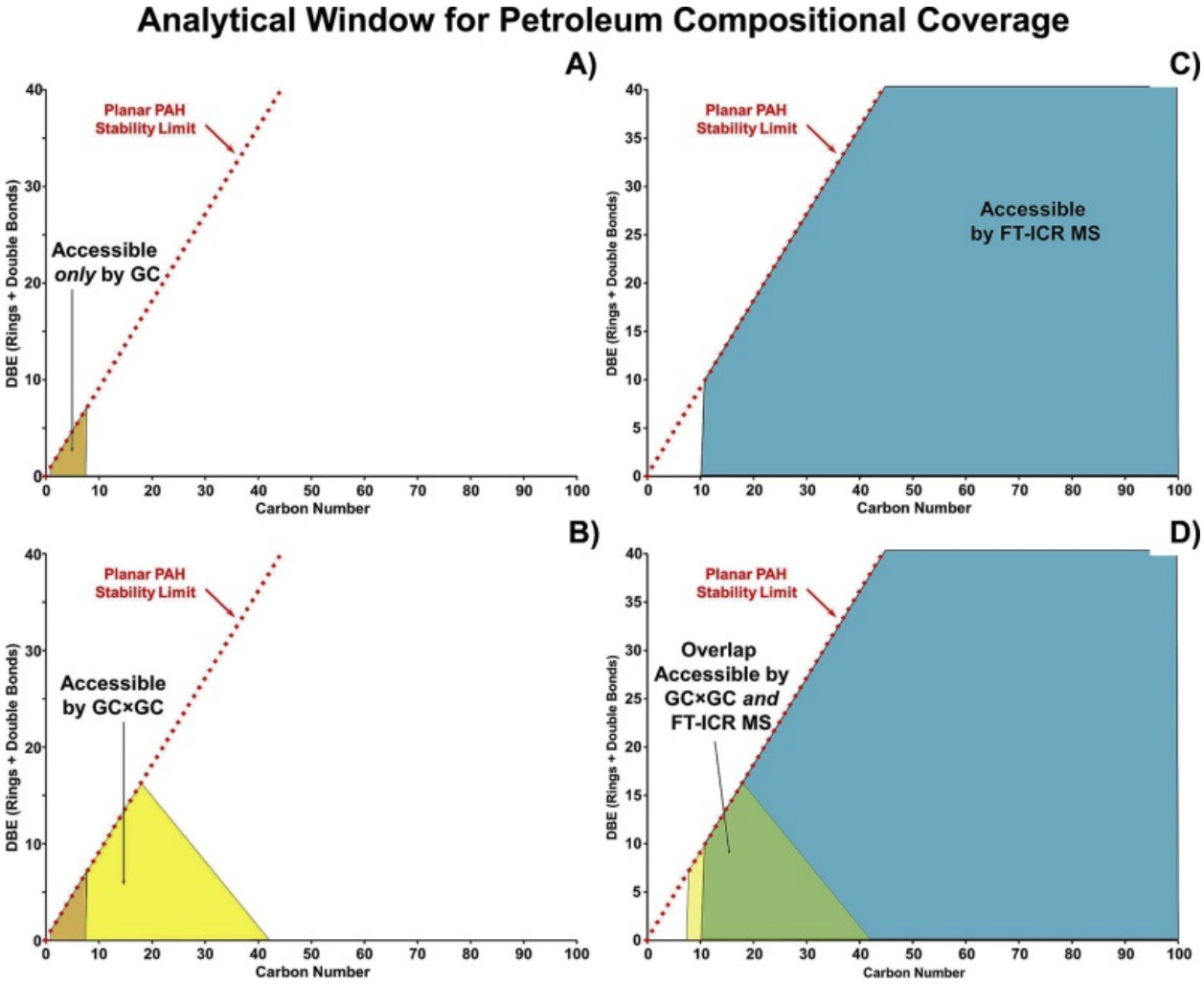

Figure 6 (from McKenna et al., 2013) Schematic diagram of the compositional continuum of petroleum reported as double bond equivalents (DBE, number of rings plus double bonds) versus carbon number. The analytical window accessible only by conventional gas chromatography (GC) is displayed in part A. The extension to hybrid analytical techniques (GC×GC) is shown in Figure 6B. Part C shows the extension to larger petroleum molecules (up to C_{100}) that have been observed in Macondo petroleum. Part D combines all three techniques to highlight the need for advanced analytical techniques beyond gas chromatography for oil spill studies.

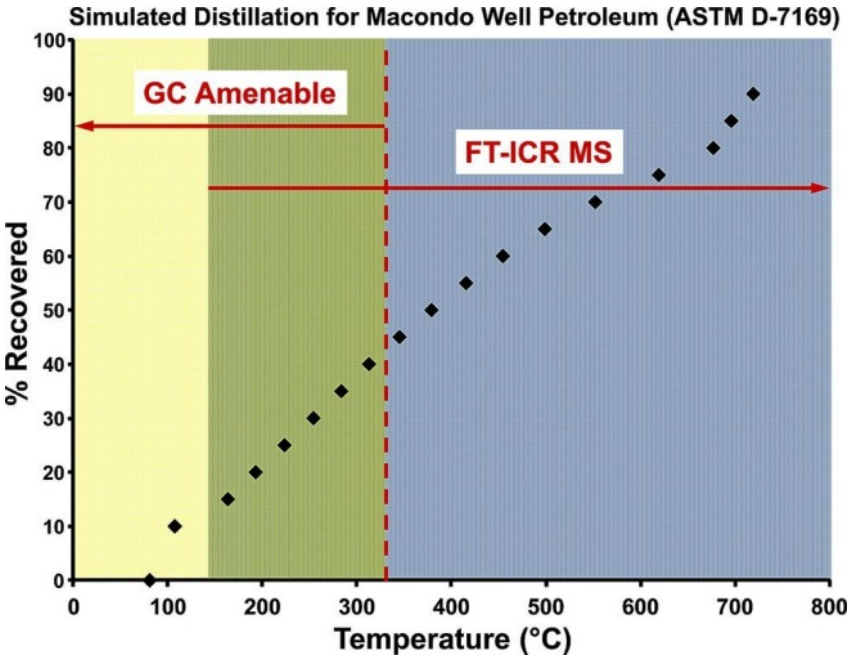

Figure 7 (from McKenna et al., 2013) Simulated distillation for Macondo well petroleum showing percentage removed as a function of temperature. The boiling range for Macondo petroleum obtained through gas chromatography (GC) distillation, reveals that more than 40% of the Macondo compounds have boiling points above the upper limit for GC-based techniques.

Standard chromatography techniques can be used with some adaptation. Li et al. (2012) used modified techniques to reconfirm retention indices for dibenzothiophenes. Oliviera et al. (2004) used liquid chromatography and conventional chromatography techniques to measure nitrogen compounds in oil. Ortiz et al. (2014) and Rowland et al. (2011a) used variants of conventional methods to separate naphthenic acids into individual compounds and classes. Yang et al. (2013) used variants of conventional techniques and micro-extraction to separate dibenzothiophenes and reclassify their retention times. Zhang et al. (2010) used similar techniques plus elemental analysis to characterize naphthenic acids. Conventional GC with Air Pressure Photoionization (APPI) has been used by a number of researchers to study oils and hydrocarbons (Strausz and Lown, 2003; Lundstedt et al., 2006; Headley et al., 2014). APPI is well suited to study of hydrocarbons as it results in less fragmentation, permitting study of more isomers. Eckert et al. (2012) used electrospray ionization, a technique which is very much used in present day study of hydrocarbons. This technique also results in less fragmentation, permitting more study of hydrocarbon isomers. Time-of-flight (TOF) mass spectrometry separates compounds by the arrival time after passage through a flight tube. This technique has more separation than conventional mass spectrometry and has been used by some workers to separate oil compounds (Fathalla et al., 2011; Wang et al., 2013).

Two-dimensional chromatography (2-D or GC x GC chromatography) injects part of the compounds under analysis into a second column, which is chosen to separate those compounds not separated in the first column. This results in analysis of the typical ‘hump’ found in

conventional chromatography. Some mildly polar compounds, sulphur, alcohols, etc. reside in this fraction and thus may be resolved. Several workers have use 2-D chromatography to study polar compounds such as naphthenic acids (Jones et al., 2012; Rowland et al.; 2011b, c, d; Shepherd et al., 2010; Wilde et al., 2015). Aeppli et al. (2012) studied weathering of oil and found that oil residues were often 50% oxygenated compounds. Most of these would not have been resolved by conventional chromatography. Manzano et al. (2013) use 2-D chromatography to delineate polar compounds in NIST standards.

The most commonly-used method for accessing compounds not typically analyzed by conventional chromatography is to use ICR or Ion Cyclotron Resonance. The selectivity of ions moving about in a high magnetic field, can be used for measuring the masses of an ionized analyte, particularly with Fourier transform ion cyclotron resonance mass spectrometers. When combined with the ESI, it can yield the elemental formulae of hundreds and thousands of molecules. The disadvantage of this expensive technique is that quantities of ions or the analyte can only be estimated. Walters et al. (2015) processed oils and estimated the abundance of NSO compounds as shown in Table 8. This data is more useful than simple appearance of compound tables. Several workers analyzed oils and identified polar compounds generically in these oils (Hegazi et al., 2012; Hegazi et al., 2014; Hu et al., 2011; Lu et al., 2013; Wang and Tu, 2011a, b; Cho et al., 2012; Mapolelo et al., 2010; Zhang et al., 2010). These studies have pushed back the frontiers of knowledge of oil polar compounds, however there are few quantitative data. Any quantitative data obtained is listed in the appendix to this study. Similarly, several authors have used ICR to separate naphthenic acids (Da Campo et al., 2009; Headley et al., 2011a, b, 2013). Quantitative results are in the appendix. Some researches used ICR to identify polar compounds in wastes and contaminated soils (Lobodin et al., 2015; Wang et al., 2013).

Liquid separation techniques have been used by several researchers to separate oil compounds before analysis (Porter et al., 2004; Fafet et al., 2008; Meyer et al., 1999; Noesthenden et al., 2014). These studies have generally resulted in some quantitative information which is listed in the appendix. Specific details on these studies follow.

Aeppli et al. (2012) studied the effect of weathering on surface slicks, oil-soaked sands, and oil-covered rocks and boulders over an exposure period of 18 months. With time, oxygen content increased in the hydrocarbon residues. Furthermore, a weathering-dependent increase of an oxygenated fraction relative to the saturated and aromatic fractions was observed. This oxygenated fraction made up >50% of the mass of weathered samples, and had an average carbon oxidation state of -1.0, and an average molecular formula of $(C_5H_7O)_n$. The oxygenated hydrocarbon residues were determined to be from a fossil source. The incorporation of oxygen into the oil's hydrocarbons was confirmed from the detection of hydroxyl and carbonyl functional groups and the identification of long chain (C_{10}-C_{32}) carboxylic acids as well as alcohols. The authors conclude that biodegradation and photooxidation were responsible for the accumulation of oxygen in the oil residues. These results show that molecular-level transformations of petroleum hydrocarbons lead to increasing amounts of oxyhydrocarbons that dominate the solvent-extractable material from oiled samples. Furthermore, use of conventional extraction and GC methodology would only account for about 25% of the material. Aeppli et al. (2014) also noted that the basis of comparison, the biomarkers, may not be present to provide comparison.

Table 8 - Compound Classes by Walters et al., 2015

Compound class	Relative Amount*	Compound class	Relative Amount*
S_1	9	S_1O_2	2
S_2	8	S_1O_3	9
S_3	7	S_1O_4	8
S_4	2	S_1O_5	5
N_1	3	S_1O_6	2
O_1	9	S_1O_7	2
O_2	8	S_2O_2	2
O_3	3	S_3O_2	4
O_4	8	S_4O_2	3
O_5	1	N_2	2
O_6	3	N_1O_1	6
O_7	1	N_1O_2	7
O_8	1	N_1O_3	6
S_1N_1	9	N_1O_4	3
S_2N_1	8	S_1N_1	3
S_3N_1	7	S_4N_1	1
S_1O_1	6	$S_1N_1O_2$	1
N_2O_1	3	$S_4N_1O_1$	1

*rated 1 to 10 - ten is the highest of the oils tested in a given group

- these are estimates only based on GCMS data

Strausz and Lown (2003) studied Alberta bitumens and published a book showing extensive separation techniques and results of the analysis of many classes of compounds. For polar compounds, they separated and classified the following groups:

- Alkyl carbazoles
- Benzyl carbazoles
- Sulfoxides
- Alkyl fluoren-9-ones
- Fluorenones
- Naphthalenic carboxylic acids
- Hexacyclic acids
- Cyclic sulfoxides
- Monocyclic terpenoid sulfides
- Monocyclic terpenoid sulfoxides
- Bicyclic terpenoid sulfides

- Bicyclic terpenoid sulfoxides
- Tricyclic terpenoid sulfides
- Tricyclic terpenoid sulfoxides
- Tetracyclic terpenoid sulfides
- Tetracyclic terpenoid sulfoxides
- Pentacyclic terpenoid sulfides
- Pentacyclic terpenoid sulfoxides
- Hexacyclic terpenoid sulfides
- Hexacyclic terpenoid sulfoxides
- Terpenoid sulfides and sulfoxides
- Carboxylic acids and esters
- Tricyclic acids and esters
- Tricyclanes
- Pentacyclic acids and esters
- Acyclic acids and esters
- Ketones
- Alcohols
- Fluorenols
- Carbazoles
- Azaarenes
- Pyridines
- Quinolines
- Porphyrins
- Thiophenes
- Indenes

In summary many polar compounds and classes of polar compounds have been found in oils, almost none in Alaskan oils. Only Dibenzothiophenes as noted in Table 3 have been found in Alaskan oils. This section noted many analytical methods, results of these are included in the Appendix along with any toxicity data found.

Analytical chemistry is moving along swiftly and along many different lines. No doubt, within a few years, one will be able to see a lot more compounds in oils. Hopefully one will be able to separate them or synthesize them to know what the toxicity of these compounds are. Currently however, the ability to do any of this is limited and very expensive.

5.3 The Deepwater Horizon Studies

The Deepwater Horizon spill engendered many polar studies as noted above (Aeppli et al., 2012, 2014; and others). Some studies have already been covered above such as in section 5.2. Several studies that were also carried out will be summarized below. Tidwell et al. (2015) used passive sampling devices measure air vapor and water dissolved phase concentrations of 33 polycyclic aromatic hydrocarbons

(PAHs) and 22 oxygenated PAHs (OPAHs) at four Gulf of Mexico coastal sites prior to, during, and after shoreline oiling from the Deepwater Horizon oil spill. Measurements were taken at each site over a 13-month period, and flux across the water-air boundary was determined. Vapor phase sum PAH and OPAH concentrations ranged between 1 and 24 ng/m^3 and 0.3 and 27 ng/m^2, respectively. PAH and OPAH concentrations in air exhibited different spatial and temporal trends than in water, and air-water flux of 13 individual PAHs were strongly associated with the DWH incident. The largest PAH volatilizations occurred at the sites in Alabama and Mississippi in the summer, each nominally 10 000 ng/m^2/day. Acenaphthene was the PAH with the highest observed volatilization rate of 6800 ng/m^2/day in September 2010. The concentration of the OPAHs was not relatable to the PAH concentrations, nor were the individual OPAHs classified. Volatilization would reduce the amount of PAHs in the oil exposing the remainder to organisms. It is questionable whether the toxicity of the remainder would be the same.

King et al. (2014) studied the photochemical behavior of Deepwater Horizon oil collected from the surface of the Gulf of Mexico. Thin oil films on water were subjected to simulated sunlight, and the resulting chemical and optical changes were observed. Polycyclic aromatic hydrocarbons (PAHs) showed substantial photodegradation, with larger PAHs being more rapidly decomposed. About 60% of the fluorescence at the excitation and emission maxima was observed with 12 hr of simulated solar irradiation equivalent to approximately 3 days of sunlight. Synchronous scan fluorescence measurements showed 80-90% loss of larger PAHs with 12 hr of simulated solar irradiation. Specific PAHs or degradation products were not identified. Alkanes showed no significant photochemical losses. After irradiation, the toxicity of water in contact with the oil significantly increased, presumably due to the release of water soluble photoproducts that were toxic.

5.4 Oxygenated Compounds

Oxygenated compounds are often the products of biodegradation and photooxidation and have been cited as being a source of toxicity (Fingas, 2014). Oxygenated compounds at industrial sites and at oil spills are often different compounds and may have no relationship. Forsberg et al. (2014) investigated coupling passive sampling technologies with ultraviolet irradiation experiments to study polycyclic aromatic hydrocarbon (PAH) and oxygenated PAH transformation processes in bioavailable mixtures. Passive sampling device (PSD) extracts were obtained from coastal waters impacted by the Deepwater Horizon oil spill and Superfund sites in Portland, Oregon, USA. Oxygenated PAHs were found in the contaminated waters with PSDs. All mixtures were subsequently exposed to a mild dose of ultraviolet B (UVB). A reduction in PAH levels and simultaneous formation of several oxygenated PAHs were measured. Site-specific differences were observed with UVB-exposed PSD mixtures.

Lundstedt et al. (2007) studied oxygenated polycyclic aromatic hydrocarbons (oxy-PAHs) and demonstrated that oxy-PAHs are abundant at contaminated sites. The oxy-PAHs show relatively high persistency and because they are formed through transformation of PAHs, their concentrations in the environment may even increase as the sites are remediated by methods that promote PAH degradation. They show that oxy-PAHs are toxic to both humans and the environment, although the toxicity seems to be manifested through other effects than those known to be important for polycyclic aromatic compounds in general, that is, mutagenicity and carcinogenicity. Finally, they presented data that support the hypothesis that oxy-PAHs are more mobile in the environment than PAHs, due to their polarity, and thus have a

higher tendency to spread from contaminated sites via surface water and groundwater. It is not known whether any of these PAHs or oxy-PAHs would be present from oil exposure – but probably not as the processes that form them are different from the processes that oils undergo.

Layshock et al. (2010) studied polycyclic aromatic hydrocarbons substituted with a ketone or quinone functionality in various substrates. An optimized method was developed to quantify five ketone and four quinone OPAHs from matrices ranging from biological tissue to diesel particulates. Five National Institute of Standards and Technology Standard Reference Materials were analyzed. This is the first report of OPAH quantitation in SRM 2977 (mussle tissue), SRM 1944 (waterway sediment), SRM 1975 (diesel extract), and SRM 1650b (diesel particulate matter) and among the few to report concentrations from SRM 1649 (urban dust). Furthermore, this is one of the first reports of OPAHs in biological tissue. Σ 9OPAHs were 374 mg/kg (mussle tissue), 5.4 mg/kg (sediment), 16.9 mg/kg (urban dust), 33.4 mg/kg (diesel extract), and 150 mg/kg (diesel particulate matter). In all SRMs, the levels of OPAHs were similar to or exceeded levels of PAHs. Of the OPAHs tested, the most frequently occurring in the environmental matrices were 9-fluorenone, 9,10-anthraquinone, benzofluorenone, and 7,12-benz[a]anthracenequinone. It is not known whether these compounds appear in oil or photooxidized oil, however to date they have not been identified in oils or oil residues.

5.5 Nitrated Compounds

Nitro-PAHs have been considered to be a toxic component of oil but have to date only been found in burn samples such as diesel exhaust. Kawanaka et al., 2007 developed a method for determination of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs; mono-nitro-PAHs and dinitropyrenes) in diesel exhaust particles by gas chromatography-negative ion chemical ionisation tandem mass spectrometry (GC/NCI/MS/MS). They used two types of column in GC/NCI/MS/MS analysis. A polar column was used for determination of mono-nitro-PAHs, and a non-polar column was used for determination of dinitropyrenes and mono-nitro-PAHs except nitrofluoranthenes. Similarly, Jariyasopit et al. (2013) studied the atmospheric reactions which produce nitrated PAHs.

5.6 Degradation Compounds and their Measurement

Oil that has undergone forms of oxidation such as biodegradation or photooxidation, contains oxygenated compounds (Fingas, 2014). The end products of biodegradation and oxidation such as photooxidation are similar or the same compounds and include acids, esters, ketones and aldehydes. Some of these compounds cannot be analyzed by standard extraction and gas chromatographic methods. Conventional methods do not analyze for polar compounds and would not count them in the analytical results. Studies have shown that highly oxidized oil, including that undergoing biodegradation and photooxidation, is not properly analyzed by conventional techniques. Conventional analytical techniques may miss as much as 75% of the oil mass. Similarly, for biodegradation analysis, conventional techniques may overstate biodegradation by as much as four times.

Five possible analytical solutions to this problem are suggested and include; thin layer chromatography, derivitization and then GC analysis, FTIR, NMR, HPLC and LTPS. The latter three techniques are typically applied to only the separated polar fractions of the oil. These techniques are in their infancy and much more work needs to be carried out.

The biodegradation pathways of oil compounds are largely unknown. Biodegradation steps are known only for a few of the thousands of compounds in oils. Analytical methods for many of the biodegradation products are also absent or need development. The situation is very complex and only extensive research over dozens of years will improve the knowledge. The first step will be a generalized analysis step that can include many oxygenated compounds**.**

5.7 Petroleomics

Petroleomics is the identification of the constituents of naturally-occurring petroleum and crude oil using high resolution mass spectrometry (Lobodin et al., 2015; Song et al., 2015; Cho et al., 2015, Ruddy et al., 2014; Islam et al., 2012). In addition to mass determination, petroleomic analysis sorts the chemical compounds into heteroatom class (nitrogen, oxygen and sulfur) and type (degree of unsaturation, and carbon number). Petroleomics is now a fast-moving science and is resulting in many papers and much information. Table 6 (Ruddy et al., 2014) shows the typical output of a petroleomics study.

Rodgers et al. (2011) studied the weathering of Macondo oil. They noted that weathering of the hydrocarbon matrix increases the polarity of components of the oil spill, further hindering GC characterization. Characterization of the water-soluble, acidic, basic, and neutral species by FT-ICR MS was carried out, along with characterization of the polar and heavy oil fractions outside the analytical window of most techniques. The most volatile components of an oil spill are lost quickly due to evaporation, but the residuals are what remain. Rodgers et al. (2011) present the detailed chemical composition of the reservoir crude oil before the spill, as well as surface and subsurface oil samples and tar balls from the Mississippi Canyon 252 Deepwater Horizon oil spill, as well as chromatographic fractions.

While these studies are extremely useful in pushing the boundaries of understanding of petroleum composition, they do not answer some of the prime questions in this study. Petroleomics has not yet been able to identify individual compounds of interest and show their structure. The specifics of a compound are what is really needed. In a sense they are adding another ‘alphabet soup’ to the problem of petroleum analysis.

6 Conclusions

Polar Compounds in Alaska Oils

This study shows that little is known about polar compounds in Alaska oils. Sulfur analysis shows that there should be some sulfur compounds (accounting for about 1% sulfur) and that the Dibenzothiophenes are about the same concentrations as in most oils. Toxicity studies show that for Alaska oils, that the 2 to 5 ringed PAHs account for toxicity observed as the oil is evaporatively weathered. Little photooxidation work has been carried out on Alaska oil, however, indications are that these compounds are somewhat toxic, but not more than the 2 to 3 ringed PAHs.

Hidden Toxic Compounds

For several years toxicologists have pursued studies looking for some hidden toxic compounds that may lie in oils. The tools used were evaporation, dissolution and photooxidation.

While these are primitive tools, advances and separations still have not shown more toxic and hidden compounds. The situation is similar in a National Academy of Sciences study of heavy oils which stated that 'The complexities of the mixtures of compounds in the resin and asphaltene fractions are so great that it's possible that some exotic, diluted-bitumen-specific needle is hiding in the molecular haystack, but the overall structures of the mixtures, as portrayed by the most recent analyses, do not suggest that it's likely' (NAS, 2015).

Solubility of Polar Compounds

An important consideration throughout this report is the solubility of polar compounds in oil versus that in water. This consideration is behind many of the studies summarized in this report, however, is seldom dealt quantitatively. A first consideration is that all polar compounds are more soluble in water than most non-polar compounds; however, the size and substituents on the polar compounds makes a large difference. A concept of this chemistry is shown in Figure 8.

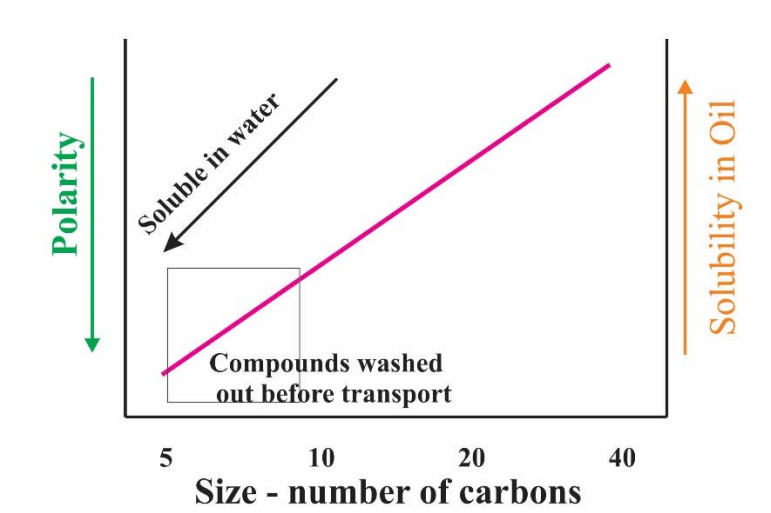

Figure 8 A concept drawing of the relationships among polarity, solubility and molecular size. This shows that solubility increases with increasing polarity and decreasing molecular size. Further, it shows that many of the smaller polar compounds may be solubilized out of oil before transport because contact with water would have removed them. This is found on a routine basis with oil sands oil, where the process water contains most of the more polar compounds and only the larger and/or less polar compounds remain in the oil.

The chemical concept is variable with the type of polar compound and the type of hydrocarbon that it is bonded with. For example, an acid is much more polar and soluble in water than is an alcohol. Similarly, a polar constituent on a long chain aliphatic is much less soluble than the same constituent on an aromatic molecule. Notwithstanding the variabilities noted, small and polar compounds are generally quite soluble in water and at sea would largely be diluted before much time passes.

A further and important concept is that of time. Processes such as biodegradation and photo-oxidation are much slower than dissolution and thus many of the highly polar and soluble

compounds are diluted or dissolved at sea, before these polar-forming processes reach terminal extents. This was shown by the studies performed during the Deepwater Horizon, many of which were summarized in this report. Although the processes of biodegradation and photo-oxidation were noted, the soluble and polar products of these processes were not present at the time of the measurement. In fact, biodegradation takes months to years to reach significant extent, while dissolution is very rapid and is on the order of seconds.

The end result of this is that highly polar compounds resulting from processes such as biodegradation and photo-oxidation are diluted and dissolved at sea, leaving the less polar or larger compounds in the oil. This also has significant impact on the toxicity of the highly polar products as they would be largely diluted before impacting biota.

Types of Polar Compounds

A list of about 200 polar compounds that might be in oils or had been separated from them was developed. Some of these also had toxicity measurements – mostly aquatic. The compounds of interest include mostly oxygenated compounds including: low molecular weight carboxylic acids which typically have low toxicity and higher molecular weight carboxylic acids, which have very low toxicity (not very toxic). Also included are aromatic acids which typically are moderately toxic. Aromatic diones have been suggested and these have been found to be highly toxic. Compounds that may be in oils in low concentrations are the naphthenic acids, the lower molecular weight ones have moderate aquatic toxicities and the larger ones, low toxicity.

The sulphur compounds in oil are many and varied. Many of these compounds have not been separated and studied. However, the benzothiophenes are typically studied along with the PAHs. The benzothiophenes have lower aquatic toxicity than most PAHs.

Nitrated compounds other than pyrene, pyridine and their derivatives have not been characterized in oil. Nitro-PAHs, compounds of toxicological concern, appear to only be formed in combustion processes such as diesel emissions.

Presence of Polar Compounds

Highly polar compounds such as low-molecular weight acids largely are not present in pipelined oils as they are solubilized out by contact with water. Most production fields in Alaska have contact with water and thus the highly polar compounds have been solubilized out, often millions of years ago. The moderately polar compounds in the oil will partition between the oil and the water after being spilled. Often the polarity is such that the compounds will partition into the oil because a greater portion of the molecule is not polar. Such behaviour is true of benzothiophenes, for example. Thus, in summary, highly polar compounds are likely not present in pipelined oils such as ANS and compounds with moderate or less polarity are likely more soluble in oil than water. Further, highly polar compounds created by biodegradation and photooxidation would be diluted during spills on water at rates that are faster than could cause toxicity.

Naphthenic Acids

Naphthenic acids have been well studied as a result of their presence in Alberta Oil Sands. Their presence, however, in Alaska oil has not been shown to date. Because of the water contact

involved in most Alaskan oils and because of their different origin than bitumen, it is unlikely that there are high concentrations of these compounds.

Biodegradation

Biodegradation of oils have been shown to produce oxygenated derivatives. Often these are more aquatically toxic than the starting compounds, however typically less than the PAHs in the oil. The biodegradation breakdown pathways are only understood for a few oil components. Further the speed of biodegradation is slow enough that high concentrations of oxygenated derivatives may not be produced faster than they are removed by water washing.

Photooxidation

Photooxidation is known to produce oxygenated derivatives as well. Again these are sometimes more aquatically toxic than the originating compound. The photooxidation pathways are even less well known than those of biodegradation. The speed of photooxidation also may be slow enough to prevent the accumulation of toxic concentrations of breakdown products.

Studies of Polar Compounds

It is prudent to note that information on polar compounds in oils can be achieved in many different ways. Of course, direct analysis is a prime way. Analysis of polars is complex, especially in an oil matrix, and thus this method has not been pursued as much as would typically be the case. As noted above, the toxicity has sometimes been pursued by following toxicity after evaporative weathering and because bulk analysis shows that there are more polars remaining, the result is typically that the toxicity is less. Other methods include separation of specific compounds and use of pure synthesized compounds that have been found in oils. There have been many studies of contaminated sites and emissions, but many of the compounds identified in these studies may not be present in oil. Other types of studies that have been performed are biodegradation and photooxidation studies. At this time many of the specific products of these processes have not been specifically identified and thus weakening the reliability of these results.

Analysis

Analysis techniques are moving at a rapid pace. In the future, we may have the ability to determine more of the compounds in oils. At this time, petroleomics advances have given us a peak into what types of compounds may be present, but not enough to actually gauge specific structures or compositions. Specific advances have been created by using 2-D (two dimensional) chromatography which allows us to analyze the ‘hump’ or unresolved area in normal chromatography. This, however, offers little insight into most polar compounds. The development of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT ICR MS) has led to the indication of thousands of compounds in oils. At this time, the most successful studies that contribute to this present study have been the traditional and pain-staking separations and analysis such as described by Strausz and Lown (2003).

7 Recommendations

The recommendations come in two parts; that related to aquatic toxicity studies and that related to separation. It should be noted that both types of studies are quite complex and require extensive funding to make even an incremental improvement. Analytical approaches are extremely complex and expensive. Even smaller incremental improvements would be achieved in conducting analytical studies.

This study shows how varied and complex the aquatic testing situation can be. In summary:

1. A full toxicity picture is only obtained when several tests are conducted and on several species.
2. Minimum tests should include key indicator species and key tests for both chronic and acute tests. It is estimated that a minimum of 6 different tests would be needed before one had an idea of the relative toxicity of a new chemical substance and that about 20 different tests would give a more complete picture. To date many of these have been completed for Alaskan oils, however it should be noted that these oils are constantly changing with production changes.
3. Local species, in this case Alaskan species, may provide useful information but it is more important to first test key species to provide comparison points.
4. The separation of polar compounds is detailed in chapter 5 and is far more complex and sophisticated than even the toxicity testing. It will be many years before many compounds are separated such that toxicity testing on them can be performed. Further one must always question whether a separated compound is truly representative of the toxicity as its mutual solubility in the oil matrix may render it non-toxic when not separated or just part of the total oil toxicity matrix. The question of bioavailability should be addressed in any study. Both the toxicity and separation are noted in the Appendix.
5. At this stage, it is felt that it would be best to fill any picture of the effects of oil evaporative weathering on Alaskan or indicator species, employing some of the experts already working on these specific species.
6. One suggested experiment which would open a lot of questions is to perform an 8-way study involving forms of weathering as well as the water under the weathered oil. The test would involve performing a standard(s) toxicity test using eight challenges:

Unweathered oil	weathered oil	Photooxidized oil	Biodegraded oil
water from under	water from under	water from under	water from under

The test would require good design to ensure that the items are compared fairly, however it would give hints as to the relative toxicities of the 8 items above. This is important as the toxicity of degradation products of photooxidized and biodegraded oils have not been studied extensively to date.

In terms of the analytical and separation efforts, little can be recommended at this stage other than to carry out good and extensive chemical analysis on the target oils used in any studies carried out as a result of recommendation 6 above.

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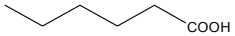
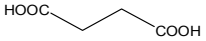
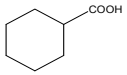
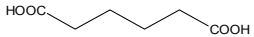
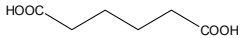
Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
$C_6H_{12}O_2$	Hexanoic Acid		116	142-62-1	HA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	2200 mg/L		Frank et al., 2009
$C_6H_{12}O_2$	Hexanoic Acid		116	142-62-1	HA	none	chem suppl.	LC_{50}	*Daphnia Magna*	1170 mg/L		Frank et al., 2009
$C_6H_{12}O_2$	Hexanoic Acid		116	142-62-1	HA	synthesized or pure		EC_{50}	*Vibrio fischeri*	0.7 mM		Jones et al., 2011
$C_4H_6O_4$	Succinic Acid		118	110-15-6	SA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	74000 mg/L		Frank et al., 2009
$C_4H_6O_4$	Succinic Acid		118	110-15-6	SA	none	chem suppl.	LC_{50}	*Daphnia Magna*	3200 mg/L		Frank et al., 2009
$C_7H_{12}O_2$	Cyclohexanecarboxylic acid		128	98-89-5	CHCA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	1200 mg/L		Frank et al., 2009
$C_7H_{12}O_2$	Cyclohexanecarboxylic acid		128	98-89-5	CHCA	none	chem suppl.	LC_{50}	*Daphnia Magna*	860 mg/L		Frank et al., 2009
$C_9H_{10}O$	2,3-dihydro-1H-inden-4-ol		134.2		PhOH_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ GGT, +	predicted	Scarlett et al., 2004
$C_9H_{12}O$	2-isopropylphenol		136.2		PhOH_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ GGT, LDH, +	predicted	Scarlett et al., 2004
$C_9H_{12}O$	4-isopropylphenol		136.2		PhOH_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ GGT, LDH, +	predicted	Scarlett et al., 2004
$C_9H_{12}O$	3,4,5-trimethylphenol		136.2		PhOH_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ GGT, +	predicted	Scarlett et al., 2004
$C_6H_{10}O_4$	Adipic Acid		146	124-04-9	AA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	68000 mg/L		Frank et al., 2009
$C_6H_{10}O_4$	Adipic Acid		146	124-04-9	AA	none	chem suppl.	LC_{50}	*Daphnia Magna*	3000 mg/L		Frank et al., 2009
$C_9H_{10}O_2$	4-methylphenylethanoic		150.2		M-aro_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_9H_{10}O_2$	3,5-dimethylbenzoic		150.2		M-aro_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_9H_{10}O_2$	3-phenylpropanoic		150.2		M-aro_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, +	predicted	Scarlett et al., 2004
$C_9H_{10}O_2$	p-ethylbenzoic		150.2		M-aro_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, +	predicted	Scarlett et al., 2004
$C_{10}H_{14}O$	3,5-diethylphenol		150.2		PhOH_5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ GGT, +	predicted	Scarlett et al., 2004

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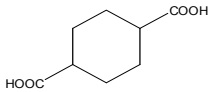
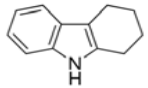
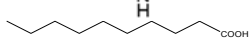
Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
$C_{14}H_{14}N$	9 Ethyl Carbazole		196	86-28-2		LC MS MS	resins					Porter et al., 2004
$C_9H_{16}O_2$	3-cyclohexylpropanoic		156.2		cyc_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_9H_{16}O_2$	4-methylcyclohexylethanoic		156.2		cyc_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_9H_{18}O_2$	2,6-dimethylheptanoic		158.2		br-ali_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{10}H_{12}O_2$	4-ethylphenylethanoic		164.2		M-aro_5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{10}H_{12}O_2$	3,4,5-trimethylbenzoic		164.2		M-aro_6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT +	predicted	Scarlett et al., 2004
$C_{10}H_{12}O_2$	4-isopropylbenzoic		164.2		M-aro_7	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{10}H_{16}O_2$	Bicyclo[4.3.0]nonane-2-carboxylic		168.2		Bi_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP, +	predicted	Scarlett et al., 2004
$C_{10}H_{16}O_2$	3-methylbicyclo[3.3.0]octane-1- carboxylic		168.2		Bi_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{10}H_{18}O_2$	4-cyclohexylbutanoic		170.3		cyc_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{10}H_{18}O_2$	4-ethylcyclohexylethanoic		170.3		cyc_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_8H_{12}O_4$	1,4-Cyclohexanedicarboxylic Acid		172	1076-97-7	CHDCA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	80000 mg/L		Frank et al., 2009
$C_8H_{12}O_4$	1,4-Cyclohexanedicarboxylic Acid		172	1076-97-7	CHDCA	none	chem suppl.	LC_{50}	*Daphnia Magna*	2600 mg/L		Frank et al., 2009
$C_{12}H_{13}N$	1,2,3,4-Tetrahydro Carbazole		172	942-01-8		LC MS MS	resins					Porter et al., 2004
$C_{10}H_{20}O_2$	Decanoic Acid (DA)		172.3	334-48-5	DA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	57 mg/L		Frank et al., 2009
$C_{10}H_{20}O_2$	Decanoic Acid (DA)		172.3	334-48-5	DA			LC_{50}	*Daphnia Magna*	220 mg/L		Frank et al., 2009

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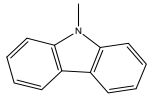
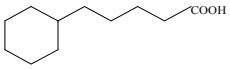
Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
$C_{10}H_{20}O_2$	Decanoic Acid (DA)		172.3	334-48-5	DA	synthesized or pure		EC_{50}	*Vibrio fischeri*	0.12 mM		Jones et al., 2011
$C_{10}H_{20}O_2$	n-decanoic		172.3		n-ali-1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{10}H_{20}O_2$	3,7-dimethyloctanoic		172.3		br-ali_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{12}H_{16}O$	4-cyclopentylphenol		176.3		PhOH_6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ GGT, +	predicted	Scarlett et al., 2004
$C_{11}H_{14}O_2$	4-i-propylphenylethanoic		178.2		M-aro_8	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{11}H_{14}O_2$	4-phenylpentanoic		178.2		M-aro_9	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{11}H_{16}O_2$	Adamantane-1-carboxylic		180.3		tri_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{13}H_{11}N$	9 methyl Carbazole		182	1484-12-4		LC MS MS	resins					Porter et al., 2004
$C_{11}H_{18}O_2$	Bicyclo[4.3.0.]nonane-2-ethanoic		182.3		Bi_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{11}H_{18}O_2$	Decahydronaphthalene-2-carboxylic		182.3		Bi_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{11}H_{20}O_2$	CyclohexanePentanoic acid		184	5962-88-9	CHPA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	13 mg/L		Frank et al., 2009
$C_{11}H_{20}O_2$	CyclohexanePentanoic acid		184	5962-88-9	CHPA			LC_{50}	*Daphnia Magna*	110 mg/L		Frank et al., 2009
$C_{11}H_{20}O_2$	5-cyclohexylpentanoic		184.3		cyc_5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{11}H_{20}O_2$	4-n-propylcyclohexylethanoic		184.3		cyc_6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{11}H_{22}O_2$	n-undecanoic		186.3		n-ali-2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004

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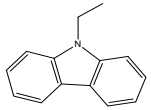
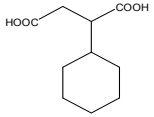
Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
$C_{11}H_{22}O_2$	7-methyldecanoic		186.3		br-ali_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{12}H_{16}O_2$	3-methyl-5-phenylhexanoic		192.3		M-aro_10	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{12}H_{16}O_2$	4-(3,5-dimethylphenyl)butanoic		192.3		M-aro_11	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{12}H_{18}O_2$	Adamantane-1-ethanoic		194.3		tri_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{14}H_{14}N$	9 Ethyl Carbazole		196	86-28-2		LC MS MS	resins					Porter, 2004
$C_{12}H_{20}O_2$	Decahydronaphthalen-2-ylacetic		196.3		Bi_5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{10}H_{16}O_4$	Cyclohexylsuccinic Acid		200	1489-63-0	CHSA	none	chem suppl.	EC_{50}	*Vibrio fischeri*	5200 mg/L		Frank et al., 2009
$C_{10}H_{16}O_4$	Cyclohexylsuccinic Acid		200	1489-63-0	CHSA			LC_{50}	*Daphnia Magna*	1300 mg/L		Frank et al., 2009
$C_{12}H_{24}O_2$	n-dodecanoic acid		200.3		n-ali-3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{13}H_{18}O_2$	4-(3,5-dimethylphenyl)pentanoic		206.3		M-aro_12	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{13}H_{20}O_2$	3-methyl-adamantane-1-ethanoic		208.3		tri_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, +	predicted	Scarlett et al., 2004
$C_{13}H_{20}O_2$	3,5-dimethyladamantane-1-carboxylic		208.3		tri_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{13}H_{20}O_2$	3-ethyladamantane-1-carboxylic		208.3		tri_5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{13}H_{22}O_2$	3-(bicyclo[4.4.0]non-2′-yl)propanoic		210.3		Bi_6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{13}H_{26}O_2$	n-tridecanoic		214.4		n-ali-4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{13}H_{26}O_2$	4-methyldodecanoic		214.4		br-ali_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004

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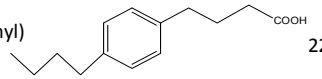
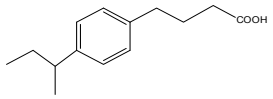
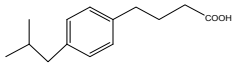
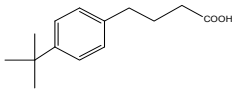
Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
$C_{14}H_{24}O_2$	4-(4'-n-butylphenyl) butanoic acid		220		4-n-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	74 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-n-butylphenyl) butanoic acid		220		4-n-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	87 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-n-butylphenyl) butanoic acid		220		4-n-BPBA	none	synthesized	EC_{50}	*Vibrio fischeri*	85 mg/L	Microtox	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-iso-butylphenyl) butanoic acid		220		4-i-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	89 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-iso-butylphenyl) butanoic acid		220		4-i-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	102 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-iso-butylphenyl) butanoic acid		220		4-i-BPBA	none	synthesized	EC_{50}	*Vibrio fischeri*	67 mg/L	Microtox	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-sec-butylphenyl) butanoic acid		220		4-s-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	51 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-sec-butylphenyl) butanoic acid		220		4-s-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	82 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-sec-butylphenyl) butanoic acid		220		4-s-BPBA	none	synthesized	EC_{50}	*Vibrio fischeri*	18 mg/L	Microtox	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-tert-butylphenyl) butanoic acid		220		4-t-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	67 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-tert-butylphenyl) butanoic acid		220		4-t-BPBA	none	synthesized	EC_{50}	*Rainbow trout*	82 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{14}H_{24}O_2$	4-(4'-tert-butylphenyl) butanoic acid		220		4-t-BPBA	none	synthesized	EC_{50}	*Vibrio fischeri*	43 mg/L	Microtox	Tollefson et al., 2012, 2012
$C_{14}H_{20}O_2$	Tetracyclo[7.3.1.06,11]tridecane-3-carboxylic		220.3		te_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{14}H_{22}O_2$	3,7-dimethyl-1-adamantane ethanoic		222.3		tri_6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, +	predicted	Scarlett et al., 2004

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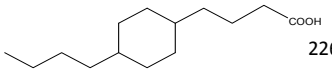
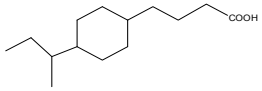
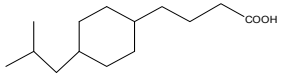
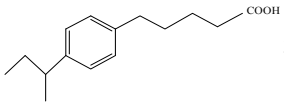
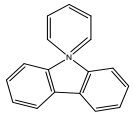
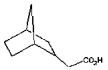
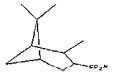
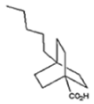
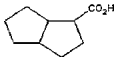
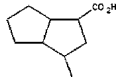
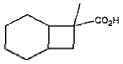
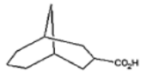
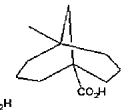
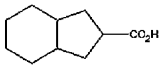
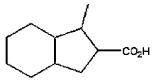
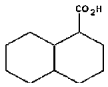
$C_{14}H_{26}O_2$	4-(4'-n-butylcyclohexyl) butanoic acid		226		4-n-BCHBA	none	synthesized	EC_{50}	*Rainbow trout*	24 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-n-butylcyclohexyl) butanoic acid		226					EC_{50}	*Rainbow trout*	43 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-n-butylcyclohexyl) butanoic acid		226					EC_{50}	*Vibrio fischeri*	293 mg/L	Microtox	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-iso-butylcyclohexyl) butanoic acid		226		4-i-BCHBA	none	synthesized	EC_{50}	*Rainbow trout*	88 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-iso-butylcyclohexyl) butanoic acid		226		4-i-BCHBA	none	synthesized	EC_{50}	*Rainbow trout*	148 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-iso-butylcyclohexyl) butanoic acid		226		4-i-BCHBA	none	synthesized	EC_{50}	*Vibrio fischeri*	35 mg/L	Microtox	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-sec-butylcyclohexyl) butanoic acid		226		4-s-BCHBA	none	synthesized	EC_{50}	*Rainbow trout*	31 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-sec-butylcyclohexyl) butanoic acid		226		4-s-BCHBA	none	synthesized	EC_{50}	*Rainbow trout*	52 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{14}H_{26}O_2$	4-(4'-sec-butylcyclohexyl) butanoic acid		226		4-s-BCHBA	none	synthesized	EC_{50}	*Vibrio fischeri*	33 mg/L	Microtox	Tollefson et al., 2012, 2012
$C_{14}H_{28}O_2$	n-tetradecanoic		228.4		n-ali-5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{14}H_{28}O_2$	2,6,10-trimethylundecanoic		228.4		br-ali_5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{15}H_{20}O_2$	Diamantane-1-carboxylic		232.3		pe_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004
$C_{15}H_{20}O_2$	Diamantane-3-carboxylic		232.3		pe_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP, +	predicted	Scarlett et al., 2004
$C_{15}H_{20}O_2$	Diamantane-4-carboxylic		232.3		pe_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP	predicted	Scarlett et al., 2004

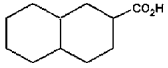
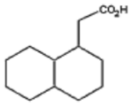
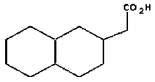
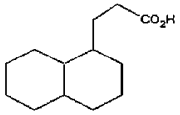
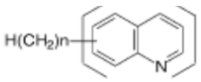
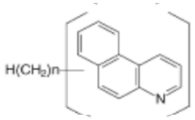
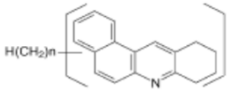
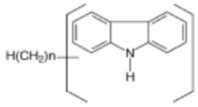
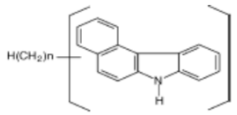
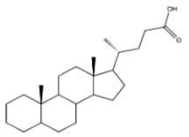
Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
$C_{15}H_{22}O_2$	4-(2'+ 3' + 4'-iso-butylphenyl) butanoic acid		234		4-i-BPPA	none	synthesized	EC_{50}	*Rainbow trout*	73 mg/L	Metabolic Inhibition	Tollefson et al., 2012, 2012
$C_{15}H_{22}O_2$	4-(2'+ 3' + 4'-iso-butylphenyl) butanoic acid		234		4-i-BPPA	none	synthesized	EC_{50}	*Rainbow trout*	133 mg/L	Membrane Integrity	Tollefson et al., 2012, 2012
$C_{15}H_{22}O_2$	4-(2'+ 3' + 4'-iso-butylphenyl) butanoic acid		234		4-i-BPPA	none	synthesized	EC_{50}	*Vibrio fischeri*	nd	Microtox	Tollefson et al., 2012, 2012
$C_{15}H_{22}O_2$	3-methyltetracyclo[7.3.1.06,11] tridecane-3-carboxylic		234.3		te_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP, +	predicted	Scarlett et al., 2004
$C_{15}H_{30}O_2$	13-methyltetradecanoic		242.4		br-ali_6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT	predicted	Scarlett et al., 2004
$C_{18}H_{13}N$	9 Phenyl Carbazole		244	1150-62-5		LC MS MS	resins					Porter et al., 2004
$C_{17}H_{24}O_2$	3,4-dimethyl-diamantane-3-carboxylic		260.3		pe_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, ALP, +	predicted	Scarlett et al., 2004
$C_{18}H_{36}O_2$	n-octadecanoic		284.5		n-ali-6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ LDH, SCOT, REP	predicted	Scarlett et al., 2004
$C_{19}H_{26}O_2$	Polycyclic monoaromatic 4		286.4		PMaro_4	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{19}H_{24}O_3$	Polycyclic monoaromatic 2		300.4		PMaro_2	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT +	predicted	Scarlett et al., 2004
$C_{20}H_{28}O_2$	Polycyclic monoaromatic 6		300.4		PMaro_6	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT +	predicted	Scarlett et al., 2004
$C_{20}H_{26}O_3$	Polycyclic monoaromatic 1		314.4		PMaro_1	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT +	predicted	Scarlett et al., 2004
$C_{20}H_{28}O_3$	Polycyclic monoaromatic 3		316.4		PMaro_3	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT	predicted	Scarlett et al., 2004
$C_{21}H_{28}O_3$	Polycyclic monoaromatic 5		328.4		PMaro_5	none	Theoretical	Pred. Effect	*human liver, Endocrine*	+ SCOT +	predicted	Scarlett et al., 2004

Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
Organization by Author												
	Bicyclo[2.2.l]heptane-l-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[2.2.l]heptane-2-ethanoic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	2,6,6-trimethylbicyclo[3.l.l]heptane-3- carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[2.2.2]octane-l-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	4-pentylbiyclo[2.2.2]octane-2-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[3.2.l]octane-6-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[3.3.0]octane-2-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	4-methylbicyclo[3.3.0]octane-2- carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	7-methylbicyclo[4.2.0]octane-7- carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[3.3.l]nonane-l-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[3.3.l]nonane-3-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	5-methylbicyclo[3.3.l]nonane-l-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[4.3.0]octane-3-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	2-methylbicyclo[4.3.O]octane-3-carboxyllic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[4.4.0]decane-2-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015

Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
	Bicyclo[4.4.0]decane-3-carboxylic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[4.4.0]decane-2-ethanoic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[4.4.0]decane-3-ethanoic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Bicyclo[4.4.0]decane-2-propanoic acid					synthesized		separation only but located in oil sands water				Wilde et al., 2015
	Alkyl quinolines		129 (base)			Separated		separation only from Brazilian oil residue				Oliveira et al., 2004
	Alkyl benzoquinolines		179 (base)			Separated		separation only from Brazilian oil residue				Oliveira et al., 2004
	Alkyl tetrahydrodibenzoquinolines		233 (base)			Separated		separation only from Brazilian oil residue				Oliveira et al., 2004
	Alkyl carbazoles		167 (base)			Separated		separation only from Brazilian oil residue				Oliveira et al., 2004
	Alkyl benzocarbazoles		217 (base)			Separated		separation only - from Brazilian oil residue				Oliveira et al., 2004
	Stearic acid				in a commercial mixture							Da Campo et al., 2009
	β-Cholanic Acid				in a commercial mixture							Da Campo et al., 2009

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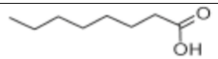
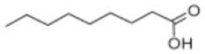
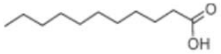
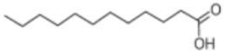
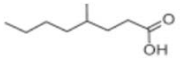
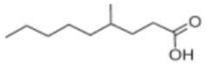
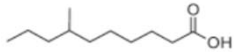
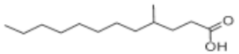
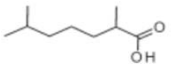
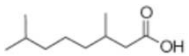
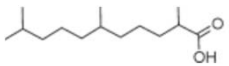
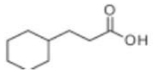
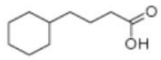
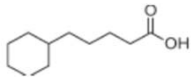
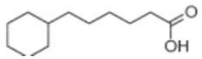
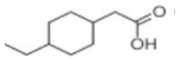
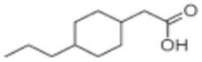
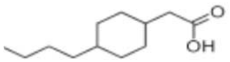
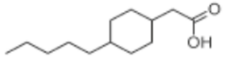
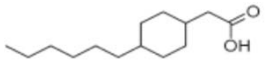
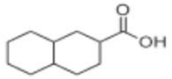
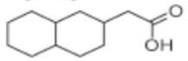
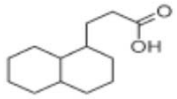
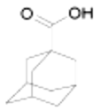
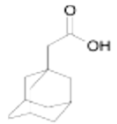
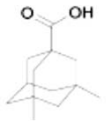
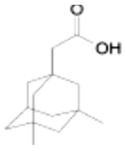
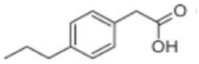
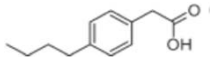
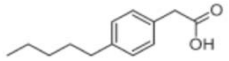
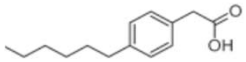
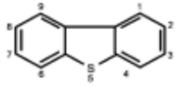
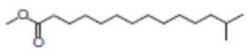
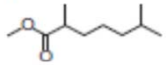
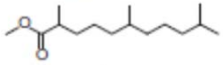
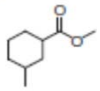
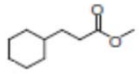
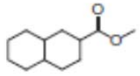
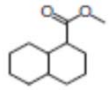
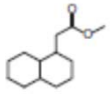
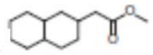
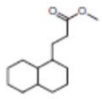
Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
	Octanoic Acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.38 mM		Jones et al., 2011
	Nonanoic Acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.36 mM		Jones et al., 2011
	Undecanoic Acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.042 mM		Jones et al., 2011
	Undecanoic Acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Dodecanoic Acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.019 mM		Jones et al., 2011
	4-methyl octanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.480 mM		Jones et al., 2011
	4-methyl nonanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.20 mM		Jones et al., 2011
	7-methyl dodecanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.080 mM		Jones et al., 2011
	4-methyl dodecanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.012 mM		Jones et al., 2011
	2,6-dimethyl heptanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.240 mM		Jones et al., 2011
	3,7-dimethyl octanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.060 mM		Jones et al., 2011
	2,6,10-trimethyl undecanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.015 mM		Jones et al., 2011
	3-cyclohexyl propanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.410 mM		Jones et al., 2011
	4-cyclohexyl butanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.110 mM		Jones et al., 2011
	5-cyclohexyl pentanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.050 mM		Jones et al., 2011
	6-cyclohexyl hexanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.040 mM		Jones et al., 2011

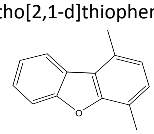
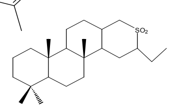
Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
	4-ethyl cyclohexyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.340 mM		Jones et al., 2011
	4-n-propyl cyclohexyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.200 mM		Jones et al., 2011
	4-n-butyl cyclohexyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.095 mM		Jones et al., 2011
	4-n-pentyl cyclohexyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.030 mM		Jones et al., 2011
	4-n-hexyl cyclohexyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.012 mM		Jones et al., 2011
	Decalin-2-carboxylic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.218 mM		Jones et al., 2011
	Decalin-2-ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.027 mM		Jones et al., 2011
	3-decalin-1-yl propanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.004 mM		Jones et al., 2011
	1-adamantane ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.784 mM		Jones et al., 2011
	1-adamantane carboxylic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.667 mM		Jones et al., 2011
	3,5,dimethyl adamantane carboxylic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.565 mM		Jones et al., 2011
	3,5,dimethyl adamantane ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.337 mM		Jones et al., 2011
	4-n-propyl phenyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.394 mM		Jones et al., 2011

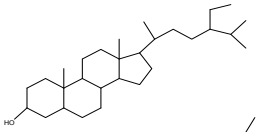
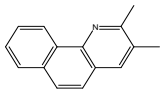
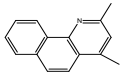
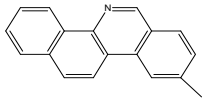
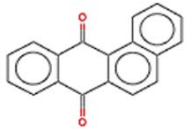
Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
	4-n-butyl phenyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.250 mM		Jones et al., 2011
	4-n-pentyl phenyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.044 mM		Jones et al., 2011
	4-n-hexyl phenyl ethanoic acid					synthesized or pure		EC_{50}	*Vibrio fischeri*	0.023 mM		Jones et al., 2011
	Dibenzothiophenes				identified the retention indices of 45 DBT isomers and noted these for coal, oil and sediment							Li et al., 2012
	13-methyl undecanoic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	2,6-dimethyl heptanoic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	2,6,10-trimethyl undecanoic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	3-methylcyclohexyl carboxylic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Cyclohexyl-3-propanic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Decalin-2-carboxylic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Decalin-1-carboxylic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Decalin-1-ethanoic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Decalin-2-ethanoic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Decalin-1-proprionic acid					synthesized or pure	identified by EI MS					Rowland et al., 2011c
	Benzothiophene				BT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2-Methylbenzothiophene				2-MBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	5-Methylbenzothiophene				5-MBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Ethylbenzothiophene				EBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Dimethylbenzothiophene				DMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Dimethylbenzothiophene				DMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013

Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
	2,7-Dimethylbenzothiophene				2,7-DMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	3,5-Dimethylbenzothiophene				3,5-DMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2,3-Dimethylbenzothiophene				2,3-DMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Ethylmethylbenzothiophene				EMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Ethylmethylbenzothiophene				EMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2,3,4-Trimethylbenzothiophene				2,3,4-TBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Trimethylbenzothiophene				TBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2,3,5-Trimethylbenzothiophene				2,3,5-TBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Ethyldimethylbenzothiophene				EDMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Ethyldimethylbenzothiophene				EDMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Ethyldimethylbenzothiophene				EDMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Ethyldimethylbenzothiophene				EDMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Tetramethybenzothiophene				TMBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Dibenzothiophene				DBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	4-Methyldibenzothiophene				4-MDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2-/3-Methyldibenzothiophenes				2/3-MDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1-Methyldibenzothiophene				1-M DBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	4-Ethyldibenzothiophene				4-EDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	4,6-Dimethyldibenzothiophene				4,6-DMDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2,4-Dimethyldibenzothiophene				2,4-DM DBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	3,6-Dimethyldibenzothiophene				3,6-DM DBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2,7-/2,8-/3,7-Dimethyldibenzothiophenes				2,7/2,8/3,7-DM D	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1,4-/1,6-/1,8-Dimethyldibenzothiophenes				1,4/1,6/1,8-DM D	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1,3-/3,4-Dimethyldibenzothiophenes				1,3/3,4-DM DBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1,7-Dimethyldibenzothiophene				1,7-DM DBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2,4,6-Trimethyldibenzothiophene				2,4,6-TMDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	2,4,7-/2,4,8-Trimethyldibenzothiophenes				2,4,7/2,4,8-TMDB	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1,4,8-Trimethyldibenzothiophene				1,4,8-TMDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1,4,7-Trimethyldibenzothiophene				1,4,7-TMDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1,3,7-Trimethyldibenzothiophene				1,3,7-TMDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	3,4,7-Trimethyldibenzothiophene				3,4,7-TMDBT	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Benzo[b]naphtho[2,1-d]thiophene				BN[2,1-d]T	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Methylbenzo[b]naphtho[2,1-d]thiophene				MBN[2,1-d]T	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Methylbenzo[b]naphtho[2,1-d]thiophene				MBN[2,1-d]T	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Methylbenzo[b]naphtho[2,1-d]thiophene				MBN[2,1-d]T	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Methylbenzo[b]naphtho[2,1-d]thiophene				MBN[2,1-d]T	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	Methylbenzo[b]naphtho[2,1-d]thiophene				MBN[2,1-d]T	Extracted from Bohai oil using micro solid phase extraction						Yang et al., 2013
	1,4-dimethylfluoren-9-one					extracted from bitumen in subfraction 8						Strausz & Lown, 2003
	tetracyclic sulfone					extracted from bitumen and reduced from sulfoxide						Strausz & Lown, 2003

Appendix - Specific Polar Compounds and Data *(arranged by molecular weight if known and then by author)*

Molecular Formula	Name	Structure	Molecular Weight	CAS #	Abbreviation	Separation	Source	Toxicity	Species	Value	Test	Reference
C_{29}	β-sitosterol					extracted from bitumen and saponified from asphaltene						Strausz & Lown, 2003
	2,3-dimethylbenzo-[h]quinoline					extracted from bitumen and separated						Strausz & Lown, 2003
	2,4-dimethylbenzo-[h]quinoline					extracted from bitumen and separated						Strausz & Lown, 2003
	6-methylbenzo-[c]phenanthridine					extracted from bitumen and separated						Strausz & Lown, 2003
$C_{18}H_{10}O$	Benz(a)anthracene-7,12-dione		258.3	2498-66-0	BAQ	purchased			*Zebra fish*	Development five days	4 µm	Eli et al., 2015

* ALP, GGT, LDH, SCOT, SGPT are liver enzymes, Rep = endocrine reproduction, + after means there are reproductive effects