NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 121
BROAD SPECTRUM ANALYSIS OF MUNICIPAL AND INDUSTRIAL EFFLUENTS DISCHARGED INTO THE PEACE, ATHABASCA AND SLAVE RIVER BASINS: CHARACTERIZATION OF EFFLUENT SAMPLES, 1994 - VOLUME 1 of 2
Prepared for the
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by

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PREFACE:

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

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May 29/96

(Robert McLeod, Co-chair)  
May 31/96
BROAD SPECTRUM ANALYSIS OF MUNICIPAL AND INDUSTRIAL EFFLUENTS
DISCHARGED INTO THE PEACE, ATHABASCA AND SLAVE RIVER BASINS:
CHARACTERIZATION OF EFFLUENT SAMPLES, 1994

STUDY PERSPECTIVE

Under the Northern River Basins Study (NRBS), water, effluent, sediment and biota have been sampled extensively and analyzed for specific contaminants known to be associated with developments within the study area, or known to be transported by aerial transport. To date, only “target compound” contaminant analyses have been conducted on these samples, and the results show generally low levels of these compounds. However, these types of specific analyses do not include other potential contaminants that are not currently known to be associated with man-made developments within the basins, or aerial transport, or for which there is little understanding of their environmental effects. Target compound analyses have been done with selected ion monitoring gas chromatography or mass spectrometry (GC/MS) with specific detectors. However, this method gives no indication of the other non-target compounds present, nor does it provide an “archive” record of chromatograms. An alternative experimental approach to characterizing the major effluents and receiving waters of the Athabasca and Peace river systems is by broad spectrum analysis.

The project conducted broad spectrum analyses of water and effluent samples upstream and downstream of major effluent sources on the Athabasca, Peace and Wapiti-Smoky River systems. Analytical methods to classify organic constituents in effluents were based on full scan coupled GC/MS, and all significant compounds were characterized with respect to mass spectra and GC retention indices. The task was accomplished in three stages: (1) summary of results and review of raw GC/MS data from previous effluent analyses conducted between 1989 and 1994, (2) collection and analysis of current effluents, and (3) collection and analysis of receiving water samples.

Routine priority pollutant data for the analyses of municipal and industrial effluents, produced between 1989 and 1994, were reevaluated. Searchable mass spectral libraries were prepared for the organic components that were characterized. During that time period, improvements in effluent quality were observed, particularly for conventional bleached kraft mills. Generally, only low concentrations of contaminants were observed in sewage treatment plant (STP) effluent. Under the second task, 260 compounds were characterized from 1994 effluent samples, and a comparison of results revealed that the improvement in pulp mill effluent quality has continued. The third task determined that none of the contaminants observed in the discharged effluents were observed in surface waters in significant concentrations. Some of the compounds observed are ubiquitous in nature, and their presence cannot be attributed solely to industrial and municipal effluents.

Based on these results, it was concluded that the scope of future investigations should be narrowed to lipophilic classes of compounds in effluents and receiving waters, eliminating the compromises necessary to include hydrophilic compounds in the analysis. These analytical results will provide a permanent record of GC/MS data, allowing researchers to revisit the data in future years if other compounds become of interest.

Related Study Questions

4a) What are the contents and nature of the contaminants entering the system and what is their distribution and toxicity in the aquatic ecosystem with particular reference to water, sediments and biota?

8b) Recognizing that people drink water and eat fish from these river systems, what is the current concentration of contaminants in water and edible fish tissue and how are these levels changing through time and by location?

13b) What are the cumulative effects of man-made discharges on the water and aquatic environment?
This report provides information on the collection and analysis of current effluents (Task 2). A summary of the results and review of raw GC/MC data from previous effluent analysis conducted between 1989 and 1994 (Task 1) is provided in Northern River Basins Study Project Report No. 111. Northern River Basins Study Project Report No. 138 provides analytical results arising from the collection and analysis of receiving water samples in 1994 (Task 3).
REPORT SUMMARY

Samples of effluents discharging into the Alberta northern river basins were collected, extracted by solid phase extraction and fractionated before analysis by coupled gas chromatography-mass spectroscopy. Observed organic contaminants were characterized and quantified. The results are compared to results of previous analyses of these effluents. Chromatograms characteristic of the contaminants present are also presented.
ACKNOWLEDGMENTS

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1.0 INTRODUCTION

Under the Northern River Basins Study Board, water, effluent, sediment, fish and benthic invertebrates have been sampled extensively and analyzed for a wide variety of specific contaminants known to be associated with the developments within the Northern Basins. To date only target compound analysis for specific contaminants has been undertaken. These analyses are for specific contaminants and yield no information regarding other contaminants which may be present. To observe these other compounds full scan coupled gas chromatography-mass spectroscopy (GC-MS) analysis of samples, followed by interpretation of the generated mass spectra is required.

This report is the second in a series of three reports describing the results of broad characterization of effluents discharged in the Alberta northern river basins and the analysis of surface waters for effluent components and other contaminants. This report describes the characterization of effluents discharged into the basin in 1994. In this work effluent samples were extracted by solid phase extraction (XAD-2 resin), separated into high and low molecular weight fractions by gel permeation chromatography, and fractionated by solvent partitioning. The extraction/fractionation process is shown in Scheme 1. Fractions of the effluent extracts were characterized by GC-MS and evaluated for acute toxicity with Microtox®.

2.0 MATERIALS AND METHODS

2.1 Solvents, Reagents and Equipment

All solvents were distilled in glass reagent grade purchased from BDH Inc. (Omnisolv grade). Tetrahydrofuran was purchased with butylated hydroxy toluene (0.25%) present as preservative and was redistilled in glass and preserved with ethanol (0.25%) prior to use. Acetic anhydride was freshly distilled prior to use. Amberlite XAD-2 resin was purchased from Axys Environmental Systems Ltd. and used without modification or was purchased from the Aldrich Scientific Company and soxhlet extracted with methyl-<i>t</i>-butyl ether (4 hr) followed by soxhlet extraction with methanol (4 hr) prior to use. Glass fibre filters used in the extraction apparatus were Gelman Type A/E 142 mm glass fiber filters prepared following AEC Environmental Chemistry SOP SB16.0, “Preparation of Gelman type A/E filters for Infiltrex II sampler”.
Extractions were done with an Infiltrex II sampler purchased from Axys Environmental Systems Ltd.

2.2 **Sample Collection, Transport, and Storage**

Grab samples of effluents were collected, without preservation, in methyl-\(\tau\)-butyl ether rinsed 4L amber glass bottles fitted with PFTE lined screw caps. Samples were shipped by overnight courier to the Alberta Environmental Centre in Vegreville Alberta where they were stored at 4° C until analyzed.

2.3 **Sample Extraction**

Solid phase, XAD-2 extraction of acidified (pH 2, HCl) samples was conducted using an Infiltrex automatic sampler with a Gelman Type A/E glass fiber filter and XAD-2 extraction cartridge. A 4.0 L aliquot of acidified sample was pumped through the sampler at a rate of 40 mL/min. The glass fiber filter was removed from the filter assembly and extracted with 300 mL of freshly distilled tetrahydrofuran in a soxhlet extractor for 4 hr. The extraction cartridge was removed and excess water was expelled with a gentle stream of UHP grade nitrogen gas. The extraction column was then eluted with 150 mL of tetrahydrofuran which was collected and combined with the filter extract. The column was then eluted with nitrogen-purged methanol and stored for further use. The sample bottle was rinsed with 100 mL of tetrahydrofuran, and the rinse which was combined with the previous tetrahydrofuran extracts and concentrated by rotary evaporator and made up to 10 mL in tetrahydrofuran. The extract was then dried by passing through 1 g of granular anhydrous sodium sulphate packed in a 6” Pasteur pipette.

2.4 **Gel Permeation Chromatography Separation of Effluent Extracts**

Extracts were separated into high and low molecular weight fractions by nonaqueous gel permeation chromatography. The chromatography system involved a Spectra Physics 8770 isocratic HPLC pump, two 300 x 10 mm Biobeads SX-3 chromatography columns, prepared following AEC Environmental Chemistry SOP SB24.0 “Preparation of gel permeation chromatography columns for lipid cleanups”, connected in series and a Rheodyne HPLC injector with a 2.0 mL sample loop. The fraction collection times were determined following AEC
Environmental Chemistry SOP SB25.0 “Calibration of gel chromatography cleanup columns”, based on corn oil used as a lipid standard. One half of each extract was fractionated using repeated 1.0 mL injections and the remaining half was stored at -20° C for future reference. The low molecular weight cuts were then pooled, concentrated and then diluted to 10 mL with tetrahydrofuran. A 2.0 mL aliquot was set aside and stored at -20° C for Microtox® evaluation.

### 2.5 Fractionation of Low Molecular Weight Fraction of Effluent Extracts

Extracts were fractionated repeatedly in 2 mL portions. A 2 mL portion of the low molecular weight fraction of extract was combined with 40 mL of distilled deionized water and 1.0 mL of aqueous potassium carbonate (75%) in a 50 mL Mixxor liquid/liquid extractor. The aqueous phase was extracted with 10 mL of pentane which was then dried by elution through a 1 g column of granular anhydrous sodium sulphate (fraction A).

Freshly distilled acetic anhydride, 0.3 mL, was then added to the aqueous solution which was then extracted with 10 mL pentane. The pentane was then dried by elution through a 1 g column of granular anhydrous sodium sulphate (fraction B).

The aqueous solution was then extracted with 10 mL of methyl-\(-\)butyl ether, which was then dried by elution through a 1 g column of granular anhydrous sodium sulphate (fraction C). The remaining aqueous solution was acidified by dropwise addition of 30% sulphuric acid to adjust the pH to below 2 and then extracted with methyl-\(-\)butyl ether. The extract was also dried by elution through a 1 g column of granular anhydrous sodium sulphate (fraction D).

Dried fractions from the low molecular weight fraction were pooled, concentrated and then diluted to 10 mL with methyl-\(-\)butyl ether. A 2.0 ml portion of each fraction was set aside for Microtox® evaluation. Fractions A and B were then concentrated to 1 mL under a stream of nitrogen and stored at -20°C for analysis by coupled gas chromatography- mass spectroscopy. Fraction C was divided in two equal portions and one portion was methylated with diazomethane generated from Diazald® following AEC Environmental Chemistry SOP SB22.0, “Methylation of organic acids with diazomethane generated from Diazald®”. Both portions were then concentrated to 1 mL under a stream of nitrogen and stored at -20°C for analysis by coupled gas chromatography-mass spectroscopy. Fraction D was evaporated under a stream of nitrogen in a 15 mL culture tube and dissolved in 1 mL of methanol and 0.4 mL of methanolic boron.
trifluoride (10%). A PFTE line screw cap was fitted and the solution was heated to 60° for 1 hr.
before the addition of 10 mL of deionized distilled water. A 1 mL portion of hexane and 10 μL
of internal standard solution were then added and the tube shaken for 20 min. The tube was the
centrifuged and the hexane layer transferred with a Pasteur pipette to a 2 mL autosampler vial
and stored at -20°C for analysis by coupled gas chromatography-mass spectroscopy.

2.6 Coupled Gas Chromatography-Mass Spectroscopy Analysis

Effluent extract fractions to which d10-phenanthrene had been added as the internal
standard (2.4 μg/mL) were analyzed using a Hewlett Packard 5890 gas chromatograph coupled
to a Hewlett Packard 5970 mass selective detector. The gas chromatograph was equipped with
an HP 7673A autosampler, a split/splitless injector run in the splitless mode, and a fused silica
capillary column (30m x 0.20 mm i.d.) coated with DB-1 methylsilicone stationary phase (film
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multiplier to increase sensitivity. The mass spectrometer was tuned using perfluorotributylamine
as calibrant, to give a 502 ion 25% of the 69 ion and a 219 ion 150% of the 69 ion. The injector
was maintained at 290°C for 1.0 μL sample injections. The initial column oven temperature was
50°C, which was maintained for 2 minutes before being increased to 300°C at a rate of 5°C/min.
and then maintained for 5 minutes at 300°C. The GC-MS interface was maintained at 280°C.
GC-MS information was recorded and the analyzed on an Everdata 486 computer using Hewlett
Packard G1045c MS Chemstation software.

2.7 Analysis of GC-MS Results

GC-MS data was analyzed using Hewlett Packard G1045c MS Chemstation software on
an Everdata 486 computer. The Wiley mass spectral library and broad spectrum analysis mass
spectral libraries constructed in the first phase of this project were used as the reference library
for probability bases matching (PBM) library searches. All spectra presented were “background
subtracted”. Background spectra for subtraction were obtained as the average of spectra over a
0.05 to 0.10 minute interval in a region with no peak, near the peak under consideration.

Spectra of coeluting compounds were obtained by first identifying ions representing of
each coeluting compounds (unique to the compound if possible, and as abundant as possible);
next obtaining background subtracted spectra from the apex of peaks in the extracted ion chromatograms (EIC) for the ions representative of the compounds; and then, for each compound, subtracting the component of the spectra from the other coeluting compounds (the amount determined from abundances in the EICs).

Kovats and PAH retention indices were calculated by linear interpolation of a compound’s retention time between that of the standards eluting immediately before and after the compound. The retention times of n-alkanes (C₉ to C₃₄) were used to calculate Kovats indices and the retention times of naphthalene, phenanthrene, chrysene and dibenzo[a,h]anthracene (2 to 5 ring PAHs) were used to calculate PAH retention indices (dibenzo[a,h]anthracene was used as the 5 ring PAH standard in place of picene which was not available).

Method blanks were evaluated to determine the contribution of the extraction and chromatographic materials to those observed in the final extract fractions. A mass spectral library of compounds observed in blanks was constructed for use in the evaluation of compounds observed in effluent extract fractions. Compounds observed in extract fractions which matched retention indices and spectra with those observed in blanks were not considered unless present in extracts in concentrations double those of the blank. The only compounds observed in the blanks and considered in this report are phthalate esters and linear carboxylic and dicarboxylic acids and esters.

2.8 Mass Spectral Evaluation and Library Compilation

Mass spectra were compared to the reference library using PBM software and evaluated using mass spectral interpretation techniques described by McLafferty (1980). Discussion of mass spectral interpretation is not presented in this report. Mass spectra were stored in another PBM searchable reference library using Hewlett-Packard MS Chemstation software.

Effluent GC-MS chromatograms were divided into three groups, i) bleached kraft pulp mill effluents (BKME), ii) high yield pulp mill effluents which comprised both thermomechanical and chemi-thermomechanical pulp mill effluents (CTMP), and iii) municipal sewage treatment plant effluents (STP). The Suncor effluent was considered separately. Separate searchable libraries were created for each group. Within each group, compounds were further divided by the fraction in which they are most concentrated. Within these subgroups the
compounds were numbered by order of elution. For example, BKME A 10, would be the 10th peak considered in the chromatograms of the A fraction of BKME.

2.9 Quantitation of Compounds

Compounds were quantified using d10-phenanthrene as internal standard. No standards for the compounds reported were run in the course of the analysis so compound concentrations were estimated assuming constant TIC response factors for compounds and internal standards. Concentrations in effluents were calculated by applying a concentration factor to the sum of concentrations observed in extract fraction concentrates.

2.10 Microtox® Evaluation of Effluent Extracts and Fraction Concentrates

Fractions set aside for Microtox® testing (1 mL) were evaporated to dryness under a stream of nitrogen and immediately redissolved in 2 mL of glass-distilled methanol. These solutions, and dilutions made into methanol, were used to measure Microtox® activity. The assay procedure described in AEC Microbiological Method Manual, based on the original Beckman Instruments procedure was employed to measure Microtox® activity except all dilutions and controls were done with methanol. Aliquots of 0.10 mL of methanol or the test substance serially diluted in methanol were added to the test media.

2.11 Construction of Characteristic Traces

The characteristic trace of mono- and dicarboxylic acids is the extracted ion chromatograms of m/e 74 and m/e 87 of the D fraction from 10 to 50 minutes added together. The extracted ion chromatogram of m/e 149 of the A fraction from 28 to 54 minutes is the characteristic trace of phthalate esters. The diterpene characteristic trace is the sum of the extracted ion chromatograms of m/e 272, m/e 270, m/e 257, m/e 255 and m/e 137 of the A fraction from 30 to 35 minutes. The characteristic trace of triterpenoids is the sum of the extracted ion chromatograms of m/e 380, m/e 382, m/e 384, m/e 394, m/e 396 and m/e 398 of the A fraction from 46 to 54 minutes merged with the sum of the extracted ion chromatograms of m/e 410 and m/e 412 of the A fraction from 50 to 56 minutes. The characteristic trace of nonylphenols is the sum of the extracted ion chromatograms of m/e 121, m/e 135, m/e 107 and
m/e 149 of the A fraction from 24 to 29 minutes. The characteristic trace of the unidentified acids in the municipal STP effluents is the sum of the extracted ion chromatograms of m/e 117, m/e 251 and m/e 265 of the D fraction from 36 to 46 minutes.

3.0 RESULTS AND DISCUSSION

Compounds found in effluents are tabulated in Tables 1 (bleached kraft mill effluents), 2 (chemi-thermomechanical pulp mill effluents) and 3 (municipal sewage treatment plant effluents). The Suncor effluent is discussed separately. These compounds are characterized and described by i) tentative identification based on interpretation of mass spectra, mass spectral library search results, elution order (evaluated using published retention indices) when ever possible, ii) class/ type of compound, i.e. chlorinated terpene, alkylated benzene, alkylated thiophene etc., and iii) substructure such as carboxylic acid or incorporation of special elements such as chlorine or sulphur as determined from the molecular ion cluster, characteristic losses or characteristic ions. The mass spectra of these compounds are attached to this report in Appendices 1, 2, and 3. They are also available in Probability Based Searchable digital format. Care must be used when using these spectra since they were obtained from complex chromatograms which required manipulation.

Kovats and PAH retention indices were calculated using data from GC-MS analysis of n-alkane and PAH standards shown in Figures 1 and 2. Kovats indices have been shown to be directly (linearly) related to the appearance temperatures, and hence retention times, of compounds eluting in temperature programmed gas chromatography (Watts and Kekwick 1974). This is obvious in Figure 1. That PAH retention indices are linearly related to retention times is apparent in Figure 2. Both Kovats and PAH retention indices of observed compounds are included in Tables 1, 2, and 3.

3.1 Bleached Kraft Mill Effluents (BKME)

The concentrations of compounds in BKMEs are reported by mill, in Appendix 4. The character of these effluents has changed considerably from those of previous years reported in the Phase I report. No monoterpenes, sesquiterpenes, alkylated sulphides or polysulphides, thiophenes, or chlorinated compounds were observed in any of the current effluents.
Nonylphenols, which were previously observed in Weldwood-Hinton mill effluent, were not in the current effluent but large numbers of phthalate esters were, perhaps replacing nonylphenols in the process. Characteristic traces of phthalate esters in the mill effluents are shown in Figure 3. Phthalate esters in the other mill effluents were not nearly as numerous or concentrated but do have a significant presence.

Both mono- and dicarboxylic acids were present in all effluents but in much lower amounts in the AlPac-Grasslands effluent. These compounds were observed as the free acid in the A fraction and as methyl esters in the D fraction. They were also present in blanks making accurate quantitation impossible. The characteristic chromatograms of these compounds in the D fraction, as methyl esters, are shown in Figure 4. The differences between amounts and pattern between the effluents and blank suggests a real contribution from the effluents.

Although no monoterpenes and sesquiterpenes were observed, diterpenes and triterpenoids (C27 to C30) were observed in some effluents. Figure 5 shows the characteristic traces of diterpenes, which were observed in the Weyerhaeuser-Grande Prairie effluent and Figure 6 shows the characteristic traces of triterpenoids for these effluents. Both classes of compounds are most prevalent in the Weyerhaeuser-Grande Prairie effluent in which 3 diterpene acids were also observed.

3.2 Chemi-thermomechanical Pulp Mill (CTMP) Effluents

The concentrations of compounds in CTMP effluents are reported, by mill, in Appendix 5. Phthalate esters, dioctyl hexanedioic acid, and mono- and dicarboxylic acids were observed in these effluents at significant concentrations. Mono-, sesqui-, and diterpenes were not observed although triterpenoids were observed in the Slave Lake Pulp-Slave Lake effluent. Contaminants previously observed in high concentrations (>50 µg/L) in the Slave Lake Pulp-Slave Lake effluent, discussed in the Phase I report (Johnson 1996), were not observed in this survey.

Characteristic traces for phthalates, acids as methyl esters, and triterpenoids are presented in Figures 7, 8, and 9 respectively.
3.3 Municipal Sewage Treatment Plant (STP) Effluents

The concentrations of compounds in STP effluents were reported, by municipality, in Appendix 6. Concentrations of contaminants observed in these effluents are generally higher than those observed in the pulp mill effluents. As with the pulp mill effluents, both phthalates and carboxylic acids were present in the STP effluents. Characteristic traces of these classes of compounds for these effluents are shown in Figures 10 and 11. In the carboxylic acid characteristic traces it can be seen that the Grande Prairie effluent differs from the others, with the fatty acid homologue series extending up to octacosanoic acid (C28:0). This homologue series is also extended in the Fort McMurray effluent, but only to pentacosanoic acid (C25:0). The source and variability of these compounds in the effluents are unknown, but they may prove useful as tracers to determine distribution and impact of these effluents.

2-Butoxyethanol phosphate (3:1) was observed in all effluents in significant concentrations (4.1 to 7.6 µg/L). Triphenyl phosphate was also observed, but in concentrations which ranged from 128 µg/L in the Whitecourt effluent to 0.4 µg/L in the Fort McMurray effluent. Caffeine, which was observed in previous work, was only observed in the Town of Athabasca effluent, at a low concentration (1.1 µg/L). Another class of compounds generally associated with sewage effluents were the sterol derived, sterols, sterones, stanols and stanones (triterpenoids). These were easily observed in the Grande Prairie effluent but were present in all STP effluents as is shown in characteristic traces, based on the molecular ions of these compounds, in Figure 12. Another group of hydrocarbons was observed in the acid fractions of the STP effluent extracts. These compounds have very similar mass spectra but have yet to be identified. The mass spectra all include abundant ions of m/e 117, 251 and 265 suggesting similar structures. Characteristic traces for these compounds are presented in Figure 13. These compounds were observed principally in the Fort McMurray STP effluent although trace amounts were present in other effluents. Nonylphenols, a group of anthropogenic hydrocarbons observed previously in STP effluents, were observed in all STP effluents surveyed. Characteristic traces of this group of compounds in STP effluents are shown in Figure 14.

Dialkyl polysulphides and substituted thiophenes, previously only observed in kraft mill effluents, were observed in the Grande Prairie effluent but not in BKMEs analyzed in this work.
3.4 Suncor Process Effluent

The organic component of the Suncor effluent consisted primarily of naphthenic acids, a very complex mixture of alicyclic carboxylic acids. Despite the carboxylic acid functionality these compounds appeared in fraction A, the base/neutral nonpolar fraction. This is shown in Figure 15, chromatograms of the A and B fractions of the Suncor effluent extract. Naphthenic acids, a very complex mixture, not resolvable by simple gas chromatography, appear as an unresolved "hump" between 20 and 55 minutes. The presence of these organic acids masks the presence of polycyclic aromatic hydrocarbons (PAHs) which are also present in the A fraction. The presence of these compounds is demonstrated in Figure 16, merged extracted ion chromatograms of the molecular ions of PAHs. Although identifications were confirmed with the retention times of authentic standards or comparison with reported retention indices (Vassilaros et. al. 1982), accurate quantitation was not possible with this data. The concentrations of these compounds range from 0.1 to 1 µg/L.

Alkylated PAHs were also observed by extracted ion chromatograms although identification was not confirmed with authentic standards. Figure 17 shows merged extracted ion chromatograms of molecular ions of alkylated PAHs. Accurate quantitation was not possible with this data but concentrations of these compounds range from 0.1 to 1 µg/L. Figure 18 shows the extracted ion chromatograms of the molecular ions of alkylated dibenzothiophenes. The identification of these peaks was not confirmed with authentic standards.

These chromatograms provide evidence of alkylated PAHs and heterocyclic PAHs in the process effluent indicating the need for further work to characterize and quantify these compounds in a further fractionated base/neutral fraction of the effluent.

3.5 Microtox® Evaluation of Effluent Extracts and Fractions

Effluent extracts and fractions were evaluated for toxicity using Microtox® as the toxicity measuring system. Extracts and fractions were solvent exchanged into methanol for the toxicity testing. The results of the testing, in percentage of solution required to attenuate light to...
50% after 15 min., multiplied by the concentration factor, are presented in Table 4. The cumulative toxicity of the fractions was calculated as the inverse of the sum of inverses of the toxicity of individual fractions. The value of the cumulative toxicity will be lower than the individual toxicities indicating higher toxicity. This summation procedure assumes a linear dose response for toxicants and that there are no antagonistic or synergistic interactions among toxicants and between toxicants and other contaminants. The results in Table 4 indicate extreme toxicity in all effluents analyzed which is known not to be the case. The observed toxicity is likely due to contaminants introduced by the use of tetrahydrofuran in the extraction and fractionation steps. This phenomena has been reported by others (Warner et. al. 1986). Although the toxicity observed is unlikely due to constituents of the effluents it is interesting to note that the cumulative toxicities of the fractions approximate that observed in the low molecular weight fraction of 8 of the 12 effluents.

4.0 SUMMARY OF FINDINGS

The improvement in the quality of pulp mill effluents since 1989 described in report 1 of the series (Johnson, 1996) has continued. In BKME, none of the chlorinated or sulphonated compounds observed previously were present. In all pulp mill effluents, mono- and sesquiterpenes were not present. Diterpenes and triterpenoids were observed but at lower concentrations than previously reported. Ubiquitous compounds like mono- and dicarboxylic acids and phthalate esters were present in these effluents. Heterocyclic compounds previously reported in high concentrations in the Slave Lake Pulp-Slave Lake effluent were not observed in this survey.

The improvement in the pulp mill effluents was not mirrored by improvements in the municipal STP effluents. High concentrations of organic phosphate were present in all effluents. Elongated fatty acids (>C20) were observed in the Grande Prairie and Fort McMurray effluents. Dialkyl polysulphides and alkylated thiophenes were also observed in the Grande Prairie STP.

The Suncor process effluent contained a mixture of PAHs and alkylated PAHs in addition to naphthenic acids. This effluent requires further study to fully characterize it and its environmental effects. Naphthenic acids are toxic but as soaps may also effect the distribution
and fate of the nonpolar PAHs in the environment. These effects must be better understood in order to assess the effect of the naphthenic acids on the environment.
5.0 REFERENCES


Table 1. Compounds present in bleached kraft pulp mill effluents

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Table 1 continued. Compounds present in bleached kraft pulp mill effluents.

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Table 2 continued. Compounds present in chemi-thermomechanical pulp mill effluents.

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Table 3. Compounds present in municipal sewage treatment plant effluents.

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<th>Retention Time (min.)</th>
<th>Kovats Index</th>
<th>PAH Index</th>
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Table 3 continued. Compounds present in municipal sewage treatment plant effluents.

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<th>PAH Index</th>
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Table 3 continued. Compounds present in municipal sewage treatment plant effluents.

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### Table 4. Microtox® toxicity of effluent extracts and fractions.

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1. Low molecular weight fraction.
2. The inverse of the sum of inverses of the toxicity of individual fractions.
3. No toxicity.
4. Not analyzed.
Scheme 1. Extraction/fractionation flowchart for broad spectrum analysis.
Figure 1. A plot of \( n \)-alkane (C10-C35) retention times by carbon number for calculation of Kovats indices.
Figure 2. A plot of PAH (naphthalene, phenanthrene, chrysene and dibenzol [a,h]anthracene) retention times by ring number for calculation of PAH retention indices.
Figure 3. Characteristic traces of phthalate ester in the A fraction of BKMEs from A. AlPac-Grasslands, B. Diashowa-Peace River, C. Weldwood-Hinton, and D. Weyerhaeuser-Grande Prairie.
Figure 4. Characteristic traces of mono- and dicarboxylic acids as methyl esters in the D fraction of BKMEs from A. AlPac-Grasslands, B. Diashowa-Peace River, C. Weldwood-Hinton, and D. Weyerhaeuser-Grande Prairie.
Figure 5. Characteristic traces of diterpenes in the A fraction of BKMEs from A. AIPac-Grasslands, B. Diashowa-Peace River, C. Weldwood-Hinton, and D. Weyerhaeuser-Grande Prairie.
Figure 6. Characteristic traces of triterpenoids in the A fraction of BKMEs from A. AIPac-Grasslands, B. Diashowa-Peace River, C. Weldwood-Hinton, and D. Weyerhaeuser-GrandePrairie.
Figure 7. Characteristic traces of phthalate esters in a fraction of CTMP effluents from A. Alberta Newsprint Company-Whitecourt, B. Millar Western-Whitecourt, and C. Slave Lake Pulp-Slave Lake.
Figure 8. Characteristic traces of mono- and dicarboxylic acids as methyl esters in the D fraction of CTMP effluents from A. Alberta Newsprint Company-Whitecourt, B. Millar Western-Whitecourt, and C. Slave Lake Pulp-Slave Lake.
Figure 9. Characteristic traces of triterpenoids in the A fraction of CTMP effluents from A. Alberta Newsprint Company-Whitecourt, B. Millar Western-Whitecourt, and C. Slave Lake Pulp-Slave Lake.
Figure 10. Characteristic traces of phthalate esters in the A fraction of municipal STP effluents from A. Athabasca, B. Fort McMurray, C. Grande Prairie and D. Whitecourt.
Figure 11. Characteristic traces of mono- and dicarboxylic acids as methyl esters in the D fraction of municipal STP effluents from A. Athabasca, B. Fort McMurray, C. Grande Prairie and D. Whitecourt.
Figure 12. Characteristic traces of triterpenoids in the A fraction of municipal STP effluents from A. Athabasca, B. Fort McMurray, C. Grande Prairie and D. Whitecourt.
Figure 13. Characteristic traces of unidentified acids as methyl esters in the D fraction of municipal STP effluents from A. Athabasca, B. Fort McMurray, C. Grande Prairie and D. Whitecourt.
Figure 14. Characteristic traces of nonylphenols in the A fraction of municipal STP effluents from A. Athabasca, B. Fort McMurray, C. Grande Prairie and D. Whitecourt.
Figure 15. A The total ion chromatograms of A fraction of the Suncor process effluent with blank contributions removed B. The total ion chromatograms of B fraction of the Suncor process effluent with blank contributions removed.
Figure 16. Merged extracted ion chromatograms of the A fraction of the Suncor process effluent showing 1. fluoranthene, 2. pyrene, 3. benzo[a]anthracene, 4. chrysene, 5. benzo[b]fluoranthene, 6. benzo[k]fluoranthene, 7. benzo[e]pyrene, 8. dibenzo[a,h]anthracene and 9. picene.
Figure 17. Merged extracted ion chromatograms of the molecular ion of alkylated PAHs in the A fraction of the Suncor process effluent: A. alkylated phenanthrene and anthracene, B. alkylated fluoranthene and pyrene, C. alkylated benzoanthracenes and chrysenes, and D. alkylated benzo[fluoranthenes and benzopyrenes.
Figure 18. Extracted ion chromatograms of the molecular ions of alkylated dibenzothiophenes in the A fraction of the Suncor process effluent.
APPENDIX A:  BROAD SPECTRUM ANALYSIS OF MUNICIPAL AND INDUSTRIAL EFFlUENT DISCHARGED INTO THE PEACE, ATHABASCA AND SLAVE RIVER BASINS - DATABASE FILES

This report was split into three separate project reports; namely, Northern River Basins Study (NRBS) Project Report No’s 138, 121 and 111. An electronic copy of these three reports and their appendices (where electronic copies exist) are contained on the three disks provided in NRBS Project Report No. 138. This information is being provided to facilitate use by researchers. Users are encouraged to contact the authors of these reports for additional background information.

There is no warranty expressed or implied for the use of this database; the Northern River Basins Study does not guarantee the accuracy of the data. The NRBS does not assume any liability for actions or consequences resulting from the use of the data; individuals using this data do so entirely at their own risk. The NRBS will not update the data except as deemed necessary for its own purpose.
APPENDIX 1

MASS SPECTRA OF COMPOUNDS IN BLEACHED KRAFT PULP MILL EFFLUENTS
### BSA_BKME 001

![Graph: Mass-to-charge (m/z) vs Abundance](image)

**#1: BSA_BKME 001**

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BSA BKME 002

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#77: BSA BKME 067

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BSA BKME 073

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- 41.10  | 40104     | 58.05   | 1418      | 75.05   | 576       | 87.20   | 341     
- 42.15  | 12976     | 58.95   | 560       | 76.05   | 5301      | 90.00   | 375     
- 43.10  | 59208     | 65.05   | 3488      | 77.10   | 4611      | 91.00   | 413     
- 44.05  | 1459      | 66.15   | 718       | 78.15   | 325       | 91.95   | 293     
- 50.00  | 1525      | 67.00   | 8225      | 79.05   | 979       | 93.05   | 5387    
- 52.65  | 870       | 68.10   | 5175      | 81.15   | 2510      | 94.00   | 659     
- 53.10  | 2211      | 69.10   | 20496     | 82.05   | 3943      | 95.05   | 1633    
- 54.00  | 2526      | 70.10   | 10756     | 83.05   | 10286     | 95.70   | 519     
- 55.05  | 30696     | 71.10   | 24160     | 84.10   | 5941      | 96.10   | 1928    

- **m/z** | **abund.** | **m/z** | **abund.** | **m/z** | **abund.** | **m/z** | **abund.**
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- 99.05  | 3342      | 115.05  | 413       | 129.10  | 272       | 153.10  | 963     
- 102.80 | 456       | 117.85  | 720       | 130.05  | 426       | 154.20  | 2847    
- 103.95 | 11332     | 118.90  | 700       | 132.00  | 1949      | 155.15  | 4668    
- 105.05 | 8274      | 121.00  | 8319      | 133.00  | 732       | 159.20  | 576     
- 106.10 | 1297      | 122.05  | 6631      | 134.05  | 416       | 160.00  | 394     
- 108.20 | 350       | 122.95  | 6916      | 145.95  | 1011      | 161.00  | 197     
- 109.15 | 1180      | 124.10  | 866       | 147.00  | 2393      | 161.40  | 342     
- 111.05 | 2330      | 125.10  | 1718      | 149.00  | 870272    | 162.90  | 519     
- 112.30 | 334       | 125.35  | 906       | 150.00  | 93040     | 163.20  | 406     

**Diagram:**

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- 43 | 5771
- 57 | 121
- 167
- 203222
- 249
- 275
- 323
- 3357
- 395

- **m/z** | **abund.**
- 50 | 100
- 150
- 200
- 250
- 300
- 350

**Diagram:**

- **m/z** | **abund.**
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**Diagram:**

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Full Spectrum # 95 from F:\BSA_BKME.L

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### BSA BKME 088

**Abundance**

![Graph showing abundance distribution](#)

#### #97: BSA BKME 088

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**BSA BKME 094**

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BSA BKME 114

Abundance

#122: BSA BKME 114

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Abundance

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![Graph of BSA BKME 201](image)

**#144: BSA BKME 201**

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#149: BSA BKME 206
Full Spectrum # 149 from F:\BSA_BKME.L

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123.10 | 35 | 142.75 | 277 | 164.80 | 305 | 185.15 | 938
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