Toxicokinetics and Bioaccumulation of Polycyclic Aromatic Compounds in Wood Frog
Tadpoles (*Lithobates sylvaticus*) Exposed to Athabasca Oil Sands Sediment

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Abstract

Many polycyclic aromatic compounds (PACs) are toxic, carcinogenic, and mutagenic. As a result, their effects on aquatic biota and ecosystems are of great concern. Research on PACs in aquatic biota often overlooks the role of amphibians, alkylated PACs, and sediment as an uptake route. In order to study the accumulation and toxicokinetics of PACs following sediment and aqueous exposure, and to compare the bioaccumulation potentials of parent and alkyl PACs, two accumulation-elimination experiments using wood frog tadpoles (*Lithobates sylvaticus*) of Gosner stage 28-32 were conducted (one evaluating exposure to contaminated sediment and water, and the other to contaminated water alone). A complementary field study was then conducted near Fort McMurray, Alberta to assess PAC body burdens in field-collected amphibian larvae, and to determine whether PAC body burdens are related to exposure to sediment and/or water in the field.

The results of our studies showed that PAC concentrations and uptake rates in wood frog tadpoles were highest when they were exposed to PAC-contaminated sediment. Consequently, we determined that the dominant route of exposure of wood frog tadpoles to PACs is sediment rather than water. This finding supports other studies that have shown dietary uptake to be an important route of PAC exposure in other aquatic organisms. In both the laboratory and field study, alkyl PAC concentrations exceeded those of parent PACs in wood frog tadpoles, which also demonstrated petrogenic PAC profiles. Interestingly, parent PACs seemed to have greater bioaccumulation potential than alkyl PACs in the laboratory-exposed wood frog tadpoles (in relation to sediment), possibly due to greater bioavailability or lower metabolism of parent PACs or alternatively, due to a saturation in uptake of alkyl PACs. Nevertheless, only a few compounds, including anthracene, fluoranthene, retene, and C1-

benzofluoranthenes/benzopyrenes, were found to have higher bioaccumulation potentials.

Lithobates sylvaticus tadpoles seemed to be efficient at eliminating and metabolizing both parent and alkyl PACs. However, the elimination of some compounds, such as C4-naphthalenes, was not as efficient.

Furthermore, C3-fluorenes and C2-dibenzothiophenes were isolated as potential markers of amphibian larvae exposure to PAC-contaminated sediment due to their positive correlation with the wetland sediment concentrations. Additional field collections in the Athabasca oil sands are warranted to verify the utility of these markers in the natural environment. Evidently, this thesis highlights the importance of including sediment exposure and alkylated PACs in toxicological and field studies of benthic and epibenthic organisms. The results of this study are the largest, most comprehensive set of toxicokinetic and bioaccumulation information of PACs (52 analytes) in the amphibian larvae *Lithobates sylvaticus* obtained to date.

Résumé

Plusieurs hydrocarbures aromatiques polycycliques (HAP) sont toxiques, cancérigènes et/ou mutagènes; ils causent donc de grandes préoccupations pour les organismes et écosystèmes aquatiques. Bien qu'il y ait de la recherche au sujet des HAP dans les amphibiens, la plupart des études suivent les toxicocinétiques de seulement quelques composés individuels. Ces études négligent habituellement les HAP alkyles, qui sont prédominants dans les régions des sables bitumineux, et se concentrent généralement sur l'eau comme voie d'absorption principale. Afin d'étudier l'accumulation et les toxicocinétiques des HAP suivant leur exposition sédimentaire et aqueuse et de comparer le potentiel de bioaccumulation des HAP parents et alkyles, deux expériences d'accumulation-élimination utilisant des têtards de grenouille des bois (*Lithobates sylvaticus*) du stage Gosner 28-32 ont été menées (la première évaluant l'exposition à des sédiments et de l'eau contaminés et la deuxième évaluant seulement l'exposition à de l'eau contaminée par des HAP). Une étude de terrain complémentaire près de Fort McMurray, Alberta a ensuite été réalisée pour évaluer les concentrations d'HAP dans les larves d'amphibiens de la région des sables bitumineux d'Athabasca et pour déterminer si ces concentrations sont reliées à leur exposition aux sédiments et/ou à l'eau de leurs zones humides.

Les concentrations et les taux d'absorption des HAP dans les têtards de grenouille des bois étaient plus élevés lorsque les têtards étaient exposés aux sédiments contaminés d'HAP que lorsqu'ils n'étaient exposés qu'à de l'eau contaminée. Par conséquent, nous avons déterminé que la voie d'absorption principale des têtards de grenouille des bois aux HAP est par les sédiments plutôt que l'exposition aqueuse. Cette contestation est appuyée par d'autres recherches qui ont démontré que l'absorption alimentaire est une voie importante d'exposition aux HAPs pour certains organismes benthiques. Plus encore, les concentrations d'HAP alkyles sont supérieures

aux concentrations d'HAP parents dans les têtards de grenouille des bois, et cela dans les études de laboratoires et de terrain, ce qui démontre clairement leurs profils pétrogéniques d'HAP. Fait digne de remarque, en laboratoire, les HAP parents semblent avoir un potentiel de bioaccumulation plus élevé que les HAP alkyles dans les têtards de grenouille des bois en comparaison aux sédiments. Ce résultat imprévu est possiblement causé soit par une biodisponibilité plus élevée ou un métabolisme plus lent des HAP parents, ou soit par une saturation d'absorption des HAP alkyles. Néanmoins, seulement quelques composés, dont l'anthracène, le fluoranthène, le retène et les C1-benzofluoranthènes/benzopyrènes, ont présenté des potentiels de bioaccumulation élevés.

Les têtards *Lithobates sylvaticus* semblent être efficaces à éliminer et métaboliser les HAP parents et alkyles. Toutefois, l'élimination de certains composés, tels que les C4-naphtalènes, n'était pas aussi efficace. Plus encore, les C3-fluorènes et les C2-dibenzothiophènes ont été isolés comme marqueurs potentiels d'exposition des larves d'amphibiens aux sédiments contaminés d'HAP à cause de leur corrélation positive avec les concentrations d'HAP dans les sédiments de leurs zones humides. Plus d'études de terrain dans les sables bitumineux d'Athabasca sont nécessaires pour vérifier l'utilité de ces marqueurs dans l'environnement naturel. Évidemment, cette thèse met en lumière l'importance d'inclure l'exposition sédimentaire et les HAP alkyles dans les études toxicologiques et de terrain des organismes benthiques et épibenthiques. Les résultats de cette étude sont les plus vastes et les plus complets des études toxicocinétiques et de bioaccumulation des HAP (52 composés analysés) dans les larves d'amphibiens *Lithobates sylvaticus* menées à ce jour.

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Table of Contents

Abstract	ii
Résumé.....	iv
Acknowledgements	vi
Table of Contents	viii
List of Abbreviations	xi
List of Figures	xiii
List of Tables	xviii
1.0. General Introduction	1
1.1. Introduction	1
1.2. Athabasca Oil Sands.....	2
1.3. Polycyclic Aromatic Compounds (PACs)	3
1.4. Sources and Fate of PACs in Aquatic Ecosystems	4
1.5. Amphibians and Wood Frog Larvae	6
1.6. Metabolism and Elimination of PACs	7
1.7. Bioaccumulation of PACs.....	8
1.8. Study Objectives	10
2.0. Laboratory Study	11
2.1. Introduction	11
2.1.1. Sources of Polycyclic Aromatic Compounds (PACs).....	11
2.1.2. Bioaccumulation of PACs	13
2.1.3. Study Objectives.....	18
2.2. Methodology	19
2.2.1. Tadpole Care.....	19
2.2.2. Experimental Design	19
2.2.3. Analysis of Parent and Alkyl PACs	22
2.2.4. Quantification	24
2.2.5. Percent Organic Carbon and Nitrogen.....	25
2.2.6. Toxicokinetics	26
2.2.7. Statistical Analysis	28
2.3. Results	29

2.3.1. Sediment + Aqueous (192 h) Exposure Experiment	29
2.3.2. Aqueous (24 h) Exposure Experiment.....	36
2.3.3. Sediment + Aqueous (24 h) Exposure Experiment	39
2.3.4. Tadpoles, Sediment, and Water Concentrations.....	42
2.3.5. Toxicokinetics	44
2.4. Discussion	52
2.4.1. Water and Sediment Exposure	52
2.4.2. Parent and Alkyl PACs.....	54
2.5. Conclusion.....	59
3.0. Field Study	60
3.1. Introduction	60
3.1.1. Sources of Polycyclic Aromatic Compounds (PACs).....	60
3.1.2. Athabasca Oil Sands.....	61
3.1.3. Bioaccumulation of PACs	63
3.1.4. Study Objectives.....	64
3.2. Methodology	66
3.2.1. Site Description	66
3.2.2. Sample Collection.....	66
3.2.3. Analysis of Parent and Alkyl PACs	67
3.2.4. Quantification	70
3.2.5. Percent Organic Carbon and Nitrogen.....	71
3.2.6. Toxicokinetics	71
3.2.7. Statistical Analysis	72
3.3. Results	73
3.3.1. Sediment	73
3.3.2. Water	79
3.3.3. Tadpoles.....	80
3.4. Discussion	90
3.5. Conclusion.....	94
4.0. General Conclusion.....	95
References.....	99
Appendix A. List of All Analyzed Parent and Alkyl PACs	108

Appendix B. Method Detection Limits of PACs.....	110
Appendix C. PAC Concentrations in MacKay River Sediment	111
Appendix D. PAC Uptake and Elimination Rates	114
Appendix E. Biota-Sediment Accumulation Factors and Bioconcentration Factors of PACs ...	117
Appendix F. Map of Study Area.....	119
Appendix G. General Characteristics of Wetlands Studied.....	120
Appendix H. Linear Regressions of Wetland Sediment and Water PAC Concentrations.....	121
Appendix I. Linear Regressions of Wetland Sediment and Tadpole Concentrations of Individual PACs	123
Appendix J. Biota-Sediment Accumulation Factors and Bioconcentration Factors of PACs	127
Appendix K. Sediment and Water Quality Guidelines	131

List of Abbreviations

Aq	Aqueous experiment
Alkyl	Alkylated
ANOVA	Analysis of variance
ASE	Accelerated solvent extraction
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BSAF	Biota-sediment accumulation factor
<i>C1-parent</i>	Specified parent PAC with 1 alkyl substitution
<i>C2-parent</i>	Specified parent PAC with 2 alkyl substitutions
<i>C3-parent</i>	Specified parent PAC with 3 alkyl substitutions
<i>C4-parent</i>	Specified parent PAC with 4 alkyl substitutions
CCME	Canadian Council of Ministers of the Environment
DW	Dry weight
DBT	Dibenzothiophene
DOB	Diluted oil sands bitumen
GC-MS	Gas chromatography coupled with mass spectrometry
HAP	Hydrocarbures aromatiques polycycliques (English: Polycyclic aromatic hydrocarbons)
HMW	High molecular weight
ISQG	Interim sediment quality guidelines
LMW	Low molecular weight
k_1	Uptake clearance rate
k_2	Elimination rate constant
k_{tmax}	Uptake rate at time of maximum concentration
K_{ow}	Octanol/water partition coefficient
PAC	Polycyclic aromatic compound
PAH	Polycyclic aromatic hydrocarbon

PEL	Probable effects level
PCC	Pearson's correlation coefficient
SedAq	Sediment + aqueous experiment
Seds	Sediment concentration
SMR	Surface mineable region
TOC	Total organic content
U.S. EPA	United States Environmental Protection Agency
WW	Wet weight

List of Figures

Figure 1.1. Structure of the 16 U.S. EPA Priority PAHs.....	4
Figure 2.1. Ideal uptake and depuration curve of accumulation-elimination experiments, where the concentration of a contaminant in an organism (C_B) increases during exposure to the source of the contaminant (C_S) until it reaches a state of equilibrium. Following termination of exposure, the concentration of the contaminant in the organism decreases exponentially. Subsequently, the k_2 elimination rate and k_1 uptake rate of the contaminant can be calculated (Boethling and Mackay, 2000).....	15
Figure 2.2. Mean ($\pm$ SE) PAC concentration profile (ng g^{-1} , dry weight TOC) of the MacKay River sediment used in all 3 exposure experiments ($n=13$) and the PAC profile of diluted oil sands bitumen (DOB) analyzed and reported by Yang et al. (2011).	30
Figure 2.3. Mean ($\pm$ SE) Σ alkyl PACs and Σ parent PACs (ng g^{-1} , wet weight) in wood frog tadpoles exposed to MacKay River sediment at various time points (hours) during the uptake and depuration phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013. Samples in triplicate.	31
Figure 2.4. Mean ($\pm$ SE) proportion of C1-C2 PACs and C3-C4 PACs (%) of Σ alkyl PACs in wood frog tadpoles exposed to MacKay River sediment at various time points (hours) during the uptake and depuration phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013. Samples in triplicate.	32
Figure 2.5. Mean ($\pm$ SE) PAC concentration profiles (ng g^{-1} , wet weight) of wood frog tadpoles after 0, 24, 48, 96 and 192 hours of exposure to MacKay River sediment. Samples in triplicate and correspond to the uptake phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013. Abbreviations are found in Appendix A.....	33
Figure 2.6. Mean ($\pm$ SE) PAC concentration profiles (ng g^{-1} , wet weight) of wood frog tadpoles after 0, 6, 12, 24, and 48 hours of depuration into clean water tanks during the sediment + aqueous (192 h) exposure experiment conducted in 2013. The PAC profile of 0h corresponds to PAC concentrations in tadpoles that were exposed to MacKay River sediment for 192 hours; the starting point of the depuration phase. Samples in triplicate. Abbreviations are found in Appendix A.....	35
Figure 2.7. Mean ($\pm$ SE) concentration (ng g^{-1} , wet weight) of C4-naphthalenes and C1-dibenzothiophenes in wood frog tadpoles throughout the 192 hour uptake phase and the 48 hour depuration phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013 using MacKay River sediment. Samples in triplicate.	36
Figure 2.8. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g^{-1} , wet weight) in wood frog tadpoles at various time points (hours) during the depuration phase of the aqueous (24 h) exposure experiment conducted in 2014. Samples in triplicate.	37

- Figure 2.9. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g^{-1} , wet weight) in wood frog tadpoles at various time points (hours) during the uptake phase of the aqueous (24 h) exposure experiment conducted in 2014. Samples in triplicate. 38
- Figure 2.10. Mean ($\pm$ SE) Σ parent and Σ alkyl PACs in wood frog tadpoles of two experiments with different exposure routes: the sediment + aqueous (192h) exposure (SedAq) and the aqueous (24h) exposure (Aq). Samples in triplicate. 39
- Figure 2.11. Mean ($\pm$ SE) Σ alkyl PACs and Σ parent PACs (ng g^{-1} , wet weight) in wood frog tadpoles exposed to Athabasca oil sands sediment at various time points (hours) during the depuration phase of the sediment + aqueous (24 h) exposure experiment conducted in 2015. Samples in triplicate. 40
- Figure 2.12. Mean ($\pm$ SE) natural log Σ parent (a) and Σ alkyl (b) PACs (ng g^{-1} , wet weight) in wood frog tadpoles at various time points (hours) during the depuration phase of the sediment + aqueous (192 h) experiment, the aqueous (24 h) experiment, and the sediment + aqueous (24 h) experiment. The exponential equations associated with each depuration experiment and their R^2 and p-values were calculated using SigmaPlot 12. Samples in triplicate. * indicates significant p-values. 41
- Figure 2.13. Log concentrations of individual PACs in wood frog tadpoles in comparison to their concentrations in sediment and water. Tadpole, sediment, and water concentrations are the average log-transformed concentrations of all uptake time points from each of the 3 exposure experiments. Data were analyzed by linear regression and all regressions were found to be significant ($p < 0.05$). 44
- Figure 2.14. Mean ($\pm$ SE) k_1 uptake rates of PACs of the sediment + aqueous (192h) experiment and the aqueous (24 h) experiment ($\text{L g}^{-1} \text{h}^{-1}$). Paired t-test performed in SigmaPlot 12 on log-transformed data was significant ($p = 0.04$). Data were back-transformed. $n = 7$ 45
- Figure 2.15. Mean ($\pm$ SE) k_1 uptake rates of PACs with 2-3 rings and 4-6 rings from the aqueous (24 h) experiment ($\text{L g}^{-1} \text{h}^{-1}$). Student t-test performed in SigmaPlot 12 on log-transformed data ($p = 0.04$) was not significant after Bonferroni correction (critical p-value = 0.003). Data were back-transformed. These data include both parent and alkyl PACs; $n = 16$ for 2-3 ring PACs and $n = 11$ for 4-6 ring PACs. 46
- Figure 2.16. Linear regression of log-transformed bioconcentration factors of PACs and their associated log K_{ow} . The BCFs were calculated from water and tadpole body concentrations at $t = 24\text{h}$ of the sediment + aqueous (192 h) exposure experiment. The linear regression analysis was performed using SigmaPlot 12. $n = 13$ 49
- Figure 2.17. Scatterplot of log-transformed k_2 elimination rate constants of alkyl PACs against their associated log K_{ow} . $\text{PCC} = 0.70$, $p = 0.04$. The k_2 values were calculated using the initial slope (6 h) of the depuration phase of the aqueous (24 h) exposure experiment (h^{-1}). $n = 21$. 50
- Figure 2.19. Log-transformed k_{tmax} uptake rates of individual PACs plotted against their associated molecular weight (g mol^{-1}). The k_{tmax} values were calculated using k_2 elimination

- rates, as well as tadpole and water concentrations at time of maximum PAC concentration of the aqueous (24 h) exposure experiment. n=10. 51
- Figure 3.1. Mean ($\pm$ SE) Σ alkyl PACs ($\mu\text{g g}^{-1}$, dry weight TOC) (solid bars) and mean ($\pm$ SE) Σ parent PACs ($\mu\text{g g}^{-1}$, dry weight TOC) (white bars) in surface sediment samples collected from 5 different wetlands in the Athabasca oil sands region, arranged in increasing distance from an oil sands upgrader. Data sets were analyzed using a one-way ANOVA, where letters indicate significant differences according to Tukey's post-hoc (Σ alkyl PACs: $F_{3,8}=271.59$, $p<0.001$; Σ parent PACs: $F_{3,8}=50.68$, $p<0.001$). Normality was tested using Shapiro-Wilk (Σ alkyl PACs: $p=0.83$; Σ parent PACs: $p=0.82$) and homogeneity of variance using Levene's test (Σ alkyl PACs: $p=0.32$; Σ parent PACs: $p=0.56$). Samples in triplicate..... 74
- Figure 3.2. Mean ($\pm$ SE) Σ PACs ($\mu\text{g g}^{-1}$, dry weight TOC) in surface sediment samples collected in triplicate from 5 wetlands in the Athabasca oil sands region plotted against distance (km) from the nearest upgrader. Gateway Hill Pond was removed because of its status as an opportunistic wetland on reclaimed overburden (data point circled in graph). Data are reported on a log scale and were analyzed by nonlinear regression in SigmaPlot 12. 75
- Figure 3.3. Mean ($\pm$ SE) Σ parent and Σ alkyl PACs ($\mu\text{g g}^{-1}$, dry weight TOC) in surface sediment samples collected in triplicate from 4 wetlands in the Athabasca oil sands region plotted against distance (km) from nearest upgrader. Gateway Hill Pond was removed because of its status as an opportunistic wetland on reclaimed overburden. Data were analyzed by nonlinear regression in SigmaPlot 12 and were not found to be significant. Data are reported on a log scale. 76
- Figure 3.4. Mean ($\pm$ SE) PAC concentration profiles ($\mu\text{g g}^{-1}$, dry weight TOC) of sediment samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region. Wetlands are arranged from nearest to furthest from an oil sands upgrader. Abbreviations are found in Appendix A..... 78
- Figure 3.5. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng L^{-1}) in water samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region in 2014. Wetlands are arranged from nearest to furthest from an oil sands upgrader..... 79
- Figure 3.6. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g^{-1} , wet weight) in wood frog tadpoles collected in triplicate from 5 different wetlands in the Athabasca oil sands region. Wetlands are arranged from nearest to furthest from an oil sands upgrader..... 80
- Figure 3.7. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g^{-1} , wet weight) in wood frog tadpole samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region plotted against distance (km) from nearest upgrader. 81
- Figure 3.8. Log concentrations of PACs in wood frog tadpoles in comparison to their concentrations in sediment (i.) and water (ii.). Tadpole, sediment, and water concentrations are the log-transformed average concentrations of triplicate samples collected from the WF1 Pond. All linear regressions are significant ($p<0.05$). 82

- Figure 3.9. Log concentration of C2-dibenzothiophenes and C3-fluorenes in wood frog tadpoles in comparison to their concentration in sediment. Each point refers to the tadpole and sediment average concentration in one of the 5 wetlands studied in the Athabasca oil sands region. Linear regressions were performed using SigmaPlot 12. 83
- Figure 3.10. Mean ($\pm$ SE) BSAFs of PACs in *Lithobates sylvaticus* tadpoles from 5 wetlands in the Athabasca oil sands region. BSAFs from Gateway Hill (n=43), Jenny Pond (n=42), WF1 Pond (n=40), Pat's Pond (n=33), and Jetliner Pond (n=33) were log-transformed and then analyzed by one-way ANOVA in SigmaPlot 12. One-way ANOVA showed a significant effect of site on BSAFs ($F_{4, 186}=25.52$, $p<0.001$). Different letters indicate significant differences according to Tukey's post-hoc. Means were back-transformed. Normality was tested using Shapiro-Wilk ($p=0.23$) and Levene's test for homogeneity of variance ($p=0.17$). 84
- Figure 3.11. Mean ($\pm$ SE) log bioaccumulation factors (BAFs) of PACs in *Lithobates sylvaticus* tadpoles from 5 wetlands in the Athabasca oil sands region. BAFs from Gateway Hill (n=41), Jenny Pond (n=41), WF1 Pond (n=37), Pat's Pond (n=37), and Jetliner Pond (n=36) were analyzed by one-way ANOVA in SigmaPlot 12. One-way ANOVA showed a significant effect of site on BAFs ($F_{4, 187}=11.10$, $p<0.001$). Different letters indicate significant differences according to Tukey's post-hoc (means were back-transformed). Normality was tested using Shapiro-Wilk ($p=0.23$) and Levene's test for homogeneity of variance ($p=0.05$). 85
- Figure 3.12. Mean ($\pm$ SE) PAC concentration profiles ($\mu\text{g g}^{-1}$, wet weight) of wood frog tadpole samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region. Wetlands are arranged from nearest to furthest from an oil sands upgrader. Abbreviations are found in Appendix A. 87
- Figure 3.13. Linear regression between log-transformed PAC concentration profiles of field and laboratory wood frog tadpoles. Field tadpoles were collected from 5 wetlands in the Athabasca oil sands region in May of 2014 (n=5). Laboratory tadpoles correspond to those exposed to MacKay River sediment for 24 hours during the sediment + aqueous (192 h) exposure experiment conducted in 2013 (n=3). Data were log-transformed and analyzed in SigmaPlot 12. The linear regression was found to be significant ($p<0.001$). Data were normal ($p=0.83$, Shapiro-Wilk) and had constant variance ($p=0.88$). 88
- Figure 3.14. Mean ($\pm$ SE) PAC concentration profile (ng g^{-1} , wet weight) of field wood frog tadpole collected from 5 wetlands in the Athabasca oil sands region in May of 2014 (n=5) and mean ($\pm$ SE) PAC concentration profile of laboratory tadpoles (Figure 2.4) after 24 hours of exposure to MacKay River sediment during the sediment + aqueous (192 h) exposure experiment conducted in 2013 (n=3). 89
- Figure A.1. Log concentrations of PACs in wood frog tadpoles in comparison to their concentrations in sediment (i) and water (ii). Tadpole, sediment, and water concentrations are the log-transformed average concentrations of duplicate or triplicate samples collected from 5 wetlands in the Athabasca oil sands region. All linear regressions were significant ($p<0.05$). 121

Figure A.2. Log concentration of individual PACs in field-collected wood frog tadpoles in comparison to their concentration in the sediment. Each point refers to the tadpole and sediment average concentration in one of the 5 wetlands studied in the Athabasca oil sands region. All equations and statistical details of the linear regressions are listed in Table A.9.

..... 123

List of Tables

Table 2.1. Statistical differences between biota-sediment accumulation factors (BSAF) and bioconcentration factors (BCF) of parent and alkyl PACs of 3 exposure experiments conducted on wood frog tadpoles exposed to MacKay River sediment. Data were log-transformed when normalization was possible. When the p-value of either the Mann-Whitney (M-W) or the student t-test (T-test) was lower than 0.05, the difference between parent and alkyl PACs was considered significant (*). The mean log10 data were back-transformed to mean BSAF and BCF values.....	48
Table A.1. List of all PACs analyzed using GC-MS, their abbreviations, number of rings, and octanol/water coefficients (Log K_{ow}) (Irwin and Service, 1997).	108
Table A.2. Method detection limits of PACs; calculated according to Loconto (2006), with instrument detection limits from 7 replicate blanks and a signal to noise ratio of 3:1 for each matrix studied (tadpoles, sediment, and water).	110
Table A.3. Mean and standard error of PAC concentrations (ng g^{-1} dw and ng g^{-1} dw TOC) in the MacKay River sediment used in all 3 exposure experiments (n=13). TOC normalized concentrations were calculated based on individual TOC values (mean = 0.015 g dw sediment/g TOC).	111
Table A.4. Uptake (k_1 and k_{tmax}) and elimination (k_2) rates determined for PACs measured in wood frog tadpoles during 3 exposure experiments. The k_2 values were determined using the slope of ln elimination concentrations for the sediment + aqueous (192 h) experiment, the aqueous (24 h) experiment, and the sediment + aqueous (24 h) experiment. For the sediment + aqueous (192 h) and the aqueous (24 h) exposure experiments, k_1 and k_{tmax} values were also determined; the initial uptake tadpole concentrations were used for calculating k_1 values, and Equation 3 (Section 2.2.6) for the k_{tmax} values. Log K_{ow} of the PACs (Irwin and Service, 1997) are also included.	114
Table A.5. Polycyclic aromatic compounds with the highest and lowest uptake (k_1 and k_{tmax}) and depuration (k_2) rates during the sediment + aqueous (192 h) experiment, the aqueous (24 h) experiment, and the sediment + aqueous (24 h) experiment. Out of the 52 PACs analyzed, the 3 compounds with the highest k values are listed in descending order of importance (1 st being the highest k value and the 2 nd being the second highest k value) and the 3 compounds with the lowest k values are also listed in descending order of importance (1 st being the lowest k value and the 2 nd being the second lowest k value).....	116
Table A.6. Mean ($\pm$ SE) biota-sediment accumulation factors (BSAFs) of PACs measured in wood frog tadpoles after 24 hours of the sediment + aqueous (24 and 192 h) exposure experiments, as well as the log bioconcentration factors (BCF) determined after 24 hours of the aqueous (24 h) experiment.	117
Table A.7. General characteristics of the 5 wetlands in the Athabasca oil sands region included in this study. Data were provided by Environment and Climate Change Canada (Unpublished, 2016). The pH measurements were obtained by handheld meters in the field in 2014 and the	

conductivity and water hardness measurements were obtained by an accredited analytical laboratory in 2012-14. WF1 Pond was a new site, from which no data had been collected in 2014 (n.a.).	120
Table A.8. Multiple linear regressions between log PAC concentrations in tadpoles, sediment, and water from 5 wetlands in the Athabasca oil sands region. SigmaPlot 12 was used for the equations and statistics. * indicates significant p-values and the independent variables from the linear combination (equation) with which the PAC concentrations in tadpoles can be predicted.	122
Table A.9. Linear regressions between PAC concentrations in wood frog tadpoles and sediment from wetlands in the Athabasca oil sands region. All equations, R^2 , p-values, n (sample size), and power of the regressions were determined using SigmaPlot 12. The compounds with p-values <0.05 (*) are considered to have significant correlations between the tadpoles and sediment.	126
Table A.10. Biota-sediment accumulation factors (BSAF) and log bioaccumulation factors (BAFs) of PACs measured in wood frog tadpoles collected from 5 wetlands in the Athabasca oil sands region.	127
Table A.11. Statistical differences, using Mann-Whitney (M-W) or student t-tests (T-test), between the means or medians of BSAFs of the parent and alkyl PACs in <i>Lithobates sylvaticus</i> tadpoles from 5 wetlands in the Athabasca oil sands region. BSAFs from Gateway Hill Pond, Jenny Pond, WF1 Pond, Pat's Pond, and Jetliner Pond were normalized by log transformation. Shapiro-Wilk (S-W) normality tests were then performed. * indicates a significant difference. The mean log10 data were back-transformed to BSAF values.	129
Table A.12. Statistical differences, using Mann-Whitney (M-W) or student t-tests (T-test), between the means or medians of BSAFs of 2-3 ring or 4-6 ring PACs in <i>Lithobates sylvaticus</i> tadpoles from 5 wetlands in the Athabasca oil sands region. BSAFs from Gateway Hill Pond, Jenny Pond, WF1 Pond, Pat's Pond, and Jetliner Pond were normalized by log transformation. Shapiro-Wilk (S-W) normality tests were then performed. * indicates significant differences. The mean log10 data were back-transformed to BSAF values.	130
Table A.13. Comparison between the PAC concentrations ($\pm$ SE) measured in the water of 5 wetlands from the Athabasca oil sands region ($\mu\text{g L}^{-1}$) and the current Canadian water quality guidelines based on the protection of freshwater life for 9 PACs ($\mu\text{g L}^{-1}$) (CCME, 2010). * indicates concentration exceeding water quality guidelines.	131
Table A.14. Comparison between the PAC concentrations ($\pm$ SE) measured in the surface sediment of 5 wetlands from the Athabasca oil sands region ($\mu\text{g kg}^{-1}$ dw) and the current Canadian Interim sediment quality guidelines (ISQGs) and probable effect levels (PELs) for 13 PACs in freshwater sediments ($\mu\text{g kg}^{-1}$ dw) (CCME, 2010). * indicates concentration exceeding sediment quality guidelines.	131

1.0. General Introduction

1.1. Introduction

Contaminants associated with the Athabasca oil sands and the oil industry may have adverse effects on individual organisms as well as entire ecosystems. In particular, polycyclic aromatic compounds (PACs) are of great concern to aquatic biota and ecosystems (Eisler, 1987, Laender et al., 2011, Wu et al., 2011, Ikenaka et al., 2013). Not only are PACs toxic, carcinogenic, and/or mutagenic organic compounds (Sanz-Lazaro et al., 2008, IARC, 1998, OMOE, 1990), but due to their hydrophobicity, some PACs also have a tendency to bioaccumulate in organisms (Hellou et al., 2012, Leppanen, 1999, Schwarzenbach et al., 2003). Recent evidence suggests that some alkyl PACs have significant bioaccumulation potential (Wayland et al., 2008), which may increase their hazard to aquatic life and human health.

Developing amphibians are particularly susceptible to PAC contamination; in addition to being sensitive and having multiple pathways of uptake, frog tadpoles are obligate-aquatic animals just as snowmelt critically contributes to PAC inputs in many wetlands (Hayes et al., 2006, Quaranta et al., 2009, Kelly et al., 2009). Nonetheless, very few toxicokinetic PAC studies have been conducted in amphibians. This thesis examines for the first time (1) the bioaccumulation potential of parent and alkyl PACs in tadpoles under experimental treatments to assess their toxicokinetics from sediment and aqueous exposures, and (2) the bioaccumulation of parent and alkyl PACs in tadpoles under natural conditions in wetlands from the Athabasca oil sands, in order to determine the potential for PACs to accumulate in a predominant species of this aquatic food web.

1.2. Athabasca Oil Sands

The Alberta oil sands is the third largest reserve of oil in the world, containing about 166 billion barrels of proven oil (NRCAN, 2013, Gouvernement of Alberta, 2016). These oil sands deposits underlie 142 200 km² of boreal forest in three major areas of northern Alberta, Canada: Athabasca, Cold Lake, and Peace River. The Athabasca oil sands region is the largest of the three oil sands regions, and the only one where deposits are shallow enough (within 75 m of the earth's surface) to be mined in open pits rather than extracted through in-situ methods. This surface mineable region (SMR) corresponds to only 20% of potential oil, but covers an area of approximately 4 800 km² (NRCAN, 2013). In 2014, Alberta produced approximately 2.3 million barrels per day of crude bitumen, with surface mining accounting for 45% of the production (Gouvernement of Alberta, 2016).

In the next 15 years, crude oil production from the Alberta oil sands is predicted to rise, even after the disruption of the 2016 Fort McMurray wildfires, from 2.37 million barrels per day in 2015 to 3.67 million barrels per day by 2030 (CAPP, 2016). Evidently, oil sands operations will continue to extract this unconventional oil as global oil demands keep rising. Correspondingly, the numerous environmental problems associated with oil sands extraction, including disruption of quantity and quality of habitats for aquatic and terrestrial organisms, accumulation of waste in large tailings ponds, emission of greenhouse gases, as well as contamination from harmful chemical substances, will continue to impact the surrounding environment and ecosystems (JOSM, 2016). Indeed, oil sands mining, processing, and upgrading emits and releases large quantities of PACs and metals to the atmosphere and the surrounding area. Kelly *et al.* (2009) even found substantial loadings of airborne PACs in snow and snowmelt as far as 50 km away from Athabasca oil sands upgraders.

1.3. Polycyclic Aromatic Compounds (PACs)

PACs are a group of organic contaminants that are ubiquitously found in the environment; they include polycyclic aromatic hydrocarbons (PAHs) and dibenzothiophenes (DBTs). Parent PAHs have two or more unsubstituted benzene (aromatic) rings fused together, whereas alkyl PAHs are those with various degrees of alkyl substitutions added to the main aromatic ring structures (Neff, 1979). DBTs are organosulfur compounds consisting of two benzene rings fused to a central thiophene ring, with or without alkyl substitutions (Kropp and Fedorak, 1998). Since DBTs are sulfur-containing analogs of PAHs, DBTs and PAHs are collectively referred to as polycyclic aromatic compounds (PACs).

Parent and alkyl PACs not only differ in structure, but also in physical and chemical properties. These differences in turn affect their transport, exposure pathways, fate, and effects. For instance, with their higher modeled hydrophobicity, alkyl PACs have generally been found to be more toxic than their corresponding parent counterparts, while heavier parent PACs are frequently associated with higher carcinogenicity (OMOE, 1990, Manzetti, 2013, Hawthorne et al., 2007, Lin et al., 2015). Nonetheless, there is a lack of understanding and monitoring of alkylated PAHs and DBTs. The majority of research has concentrated on the 16 parent PAHs designated by the United States Environmental Protection Agency (U.S. EPA) as priority pollutants (Figure 1.1), considerably underestimating the toxicity of petrochemical mixtures (Turcotte, 2008). Thus, in order to determine more conclusive differences and trends between parent and alkyl PACs, more inclusive assessments of PACs are needed.

Figure 1.1. Structure of the 16 U.S. EPA Priority PAHs.

1.4. Sources and Fate of PACs in Aquatic Ecosystems

The primary sources of PACs are pyrogenic and petrogenic. Only a minimal amount are biogenic; produced by plants and microbial activity (Neff, 1979, Tsibart and Gennadiev, 2013). Pyrogenic PACs are typically higher molecular weight (HMW) and/or unsubstituted parent compounds derived from the incomplete combustion of organic matter such as petroleum, coal, and wood. Conversely, petrogenic PACs are often lower molecular weight (LMW) and/or alkylated compounds formed at lower temperatures during diagenesis and the formation of petroleum. Therefore, alkyl PACs are associated with bitumen, crude oil, their refined products, and the production of these substances. For instance, the Athabasca oil sands in northeastern

Alberta are characterized by a dominance of petrogenic, alkylated PACs (Kelly et al., 2009). These alkylated PACs can be released by leaching from industrial tailings ponds and inadvertent releases of refined oil products, as well as from natural sources such as leaching and seepage from oil-rich rocks and sediment, and weathering and erosion of exposed oil sands deposits (although the latter source can also be increased by surface mining) (Headley et al., 2001). There is evidence that open-pit surface mining of bitumen, as in the case of the Athabasca oil sands, may be contributing significantly to PAC contamination of aquatic ecosystems (Kurek et al., 2013, Kelly et al., 2009).

PAHs constitute 1 to 3% of crude oil, of which 90% consists of alkyl PAHs (Bence et al., 1996). Considering the importance of alkylated PACs in petrochemicals, as well as their importance in the environment and their potential adverse effects, it is imperative to study the contamination of both parent and alkyl PACs in aquatic ecosystems near oil sands mining and installations. Furthermore, the potential for significant bioaccumulation of alkyl PACs in aquatic organisms should be assessed. To our knowledge, no study concerning the bioaccumulation potential and toxicokinetics of parent and alkyl PACs in amphibian larvae exposed to bitumen-rich oil sands sediment and/or water has been undertaken. This would be indispensable in interpreting PAC residues (Hellou et al., 2012); for instance, it would help us determine whether higher PAC body burdens in amphibian larvae are a result of higher levels of exposure or a product of bioaccumulation. Finally, distinguishing which PACs have the greatest bioaccumulation potential will help determine those of greater concern and of greater importance in monitoring.

1.5. Amphibians and Wood Frog Larvae

Wood frog tadpoles (*Lithobates sylvaticus* - formerly known as *Rana sylvatica*) were chosen as model organisms to study the bioaccumulation and toxicokinetics of parent and alkyl PACs in aquatic organisms because of their frequent contact with sediment substrate, their lower efficiency in metabolizing PACs in comparison to higher trophic organisms, their sufficient biomass for analysis, and their ubiquity in the Athabasca oil sands region. Furthermore, they have a broad distribution, living in and/or near ephemeral wetlands in deciduous, coniferous, and mixed forests throughout Canada and the United States. *Lithobates sylvaticus* typifies biphasic amphibian life history and its larval stage can provide important information on uptake of PACs via both water and sediment.

Indeed, developing amphibian larvae can accumulate PACs from water through dermal absorption and across respiratory surfaces, and from sediment through dermal and dietary absorption; since some are in contact or ingest sediment while feeding on detritus material. Correspondingly, wood frog tadpoles are epibenthic omnivorous amphibian larvae that primarily feed on plant detritus and algae, as well as eggs and larvae of various amphibians. In addition, *Lithobates sylvaticus* tadpoles are an important food source for many species of fish, waterfowl, wading birds, and larger invertebrates, making them a vital component of freshwater ecosystems. Not only are they directly important to food webs, they also contribute to energy and nutrient transfers within and between aquatic and terrestrial ecosystems. Many studies have shown that amphibians are particularly sensitive to contaminant exposure and environmental changes during early development (premetamorphosis/Gosner stage <42) (Hatch and Burton, 1998, Stanley, 2008, Gross et al., 2009). Therefore, PACs can be damaging to amphibian larvae, especially in terms of growth, proper development, and survival (Marquis et al., 2006). As a result of their

ecological distribution, close association with sediment, sensitivity to contaminants, and ecological importance, *Lithobates sylvaticus* tadpoles are useful in monitoring and studying the bioaccumulation of PACs.

1.6. Metabolism and Elimination of PACs

After tadpoles accumulate PACs, these organic contaminants may be transformed and/or incorporated into tissues, or eliminated through metabolism and/or excretion (Newman, 2010, Livingstone, 1998). Recent investigations have revealed metabolism as the most important elimination route of PACs in certain organisms (Dimitrova et al., 2010). PACs are mainly transformed and metabolized through the cytochrome P450 enzyme system. During phase I, the compounds are oxidized by the cytochrome P450 monooxygenases to more reactive intermediates (phenols and epoxides). In phase II, biotransformation enzymes transform the intermediates via conjugation into more polar compounds, rendering them more water soluble and amenable to transport out of cells and subsequently, out of the organism (elimination) (Hellou et al., 2012, Shimada, 2006, Manzetti, 2013, van der Oost et al., 2003). With intermediate levels of total cytochrome P450 and inducible cytochrome P4501A activity compared to those of invertebrates and vertebrates of higher trophic levels (e.g., mammals) (Marty et al., 1992, Ertl and Winston, 1998), fish and amphibians are known to metabolize parent PACs (Livingstone, 1998, Berrojalbiz et al., 2009, Garrigues et al., 2004, Gewurtz et al., 2000, Neff, 1979, Ueda et al., 2011). However, the efficiency of amphibians at metabolizing alkyl PACs and DBTs is still unknown. If the metabolism and elimination of alkyl PACs is slower than that of their parent counterparts, this could result in greater bioaccumulation of alkylated PACs as well as increases in their associated adverse effects.

1.7. Bioaccumulation of PACs

Bioaccumulation is characterized as the net accumulation of a contaminant from all possible routes of uptake (Boethling and Mackay, 2000). In aquatic ecosystems, sources of PAC uptake include food, sediment, water, and fine suspended particles in water and air (Berrojalbiz et al., 2009, Newman, 2010). Due to their hydrophobic (lipophilic) nature, PACs preferentially associate with the organic phases of suspended particulates and sediment, and can easily penetrate through lipophilic membranes of organisms and accumulate in their tissues (Leppanen, 1999). Consequently, aquatic organisms that live in close proximity to the sediment surface of aquatic ecosystems are especially susceptible to PAC contamination and exposure. For instance, Landrum and Scavia (1983) estimated that sediment-associated anthracene contributed to 77% of *Hyalella azteca*'s steady-state body burden. This important route of uptake inevitably leads to higher risks for aquatic organisms that ingest and/or are in contact with sediment.

Biota-sediment accumulation factors (BSAFs) have been used to describe the accumulation of PACs in various aquatic organisms, especially in benthic invertebrates and fish (Iannuzzi et al., 2011, Thomann and Komlos, 1999, Van Geest et al., 2011, Thorsen et al., 2004). BSAFs are the lipid and organic carbon normalized ratios of PAC concentrations in organisms relative to those in the sediment in which they reside (Boethling and Mackay, 2000). Wayland *et al.* (2008) used BSAFs as a first-level screening tool to compare bioaccumulation potential of PACs in larval and adult insects from different types of wetlands within the Athabasca oil sands region. BSAFs of alkyl PACs were consistently greater than the theoretical BSAF value of 1 (when PAHs partition equally between the lipids in organisms and the organic carbon in sediment) and greater than those of parent PACs; suggesting important bioaccumulation potential of alkyl PACs. This preliminary oil sands study concerning bioaccumulation of PACs

in benthic invertebrates warrants further field and laboratory investigations of PAC body burdens, bioaccumulation potentials, and toxicokinetics in other aquatic organisms.

Despite extensive research conducted thus far on bioaccumulation of PACs (Gewurtz et al., 2000, Meador et al., 1995, Camus et al., 2003, Berrojalbiz et al., 2009), more information on the toxicokinetics and bioaccumulation potential of parent and alkyl PAHs and DBTs is needed (Jensen et al., 2012, EPA, 2000). Most studies only investigated a few select parent PACs and typically solely consider sediments spiked with PACs (Van Geest et al., 2010, Berrojalbiz et al., 2009, Jensen et al., 2012, Yakan et al., 2011). Even though in nature, PACs are found as complex mixtures (Muijs and Jonker, 2009). Accumulation-elimination experiments have successfully determined uptake and elimination kinetics of organic contaminants in aquatic organisms, including zooplankton, oligochaetes, mussels, fish, and amphibians (Landrum and Scavia, 1983b) (Leppanen, 1999, Landrum and Scavia, 1983b, Klok et al., 2012, Berrojalbiz et al., 2009, Garrigues et al., 2004, Leney et al., 2006). However, a more comprehensive accumulation-elimination experiment evaluating the toxicokinetics and bioaccumulation potential of both parent and alkyl PACs present in field-collected oil sands sediment would be very valuable. This would provide us with a greater understanding of complex mixtures of PACs and provide us with important kinetic parameters of alkyl PACs, which have scarcely been experimentally determined and are often required in bioaccumulation studies. Interest in alkyl PACs is growing within bioaccumulation studies because of their importance in petrogenic sources and some of their high toxicity and carcinogenicity, thus their toxicokinetics will certainly be of value to future PAC research.

1.8. Study Objectives

The overall objectives of this study are threefold. The first objective is to determine the bioaccumulation potential of parent and alkyl PACs in wood frog tadpoles through exposure to complex mixtures of PACs in both sediment and water, in order to help interpret PAC residues measured in animal tissues in natural environments. Based on recent evidence suggesting greater bioaccumulation potential for alkyl PACs (Wayland et al., 2008), we postulate that biota-sediment accumulation factors for alkyl PACs, determined through two accumulation-elimination experiments (one evaluating exposure to contaminated sediment and water, and the other to contaminated water alone), will be greater than those of their unsubstituted parent counterparts. Subsequently, toxicokinetic parameters for both the uptake and depuration phases will be established for each PAC.

The second objective is to assess whether PAC body burdens in field-collected amphibian larvae are related to their sediment and/or water exposure in the field. We hypothesize that wood frog tadpoles of the Athabasca oil sands region are bioaccumulating PACs, especially alkyl PACs, and that this correlates with the PAC concentrations in the sediment and water of the natural wetlands inhabited by the tadpoles.

The third objective is to determine whether there are compounds that could be potential markers of PAC exposure. Not only do we expect to find that alkyl PACs are bioaccumulating to a greater extent than parent PACs in Athabasca oil sands wood frog tadpoles, we also anticipate the identification of several alkylated compounds which could serve as good markers of PAC contamination in natural environments.

2.0. Laboratory Study

2.1. Introduction

Oil demands continue to rise due to the ever increasing global population and its unrelenting reliance on fossil fuels. Consequently, petrochemical companies are turning to unconventional sources of oil in order to meet the growing global demand for fuel. In the Alberta oil sands, which includes the Athabasca, Cold Lake, and Peace River deposits, crude oil production is predicted to rise from 2.37 million barrels per day in 2015 to 3.67 million barrels per day by 2030 (CAPP, 2016). Oil sands extraction, whether through surface mining or in-situ methods, can have many effects on the environment, including degradation and fragmentation of wildlife habitat, accumulation of waste in large tailings ponds, emission of greenhouse gases, as well as emission and releases of various contaminants to the environment. Specifically, the contamination from xenobiotics associated with the extraction and processing of unconventional oil is threatening the health of surrounding aquatic organisms and ecosystems (Lee et al., 2015, Dew et al., 2015, Huang et al., 2016, Kurek et al., 2013, Kirk et al., 2014, Tolton et al., 2012, Kelly et al., 2009). In particular, polycyclic aromatic compounds (PACs), which include polycyclic aromatic hydrocarbons (PAHs) and dibenzothiophenes (DBTs), are organic compounds that have been found to be toxic, carcinogenic, and/or mutagenic to aquatic biota (Sanz-Lazaro et al., 2008, CCME, 1999, OMOE, 1990, Turcotte, 2008, Yang et al., 2010, Ikenaka et al., 2013, Ball and Truskewycz, 2013, Eisler, 1987).

2.1.1. Sources of Polycyclic Aromatic Compounds (PACs)

Although PACs are ubiquitous in the environment and can be formed naturally from forest fires, volcano eruptions, and diagenesis, the majority of these hydrophobic compounds are released by anthropogenic sources (Zhang and Tao, 2009). Anthropogenic PACs can be

pyrogenic or petrogenic, depending on the condition under which they are formed (Lima et al., 2005). Pyrogenic PACs are mainly comprised of higher molecular weight (HMW) and unsubstituted parent PACs formed at high temperatures during the incomplete combustion of organic material such as wood and coal. Conversely, petrogenic PACs mostly consist of lower molecular weight (LMW) and alkylated PACs formed at lower temperatures, such as during the formation of petroleum. Therefore, alkyl PACs are often associated with bitumen, crude oil, their refined products, and their production.

In the environment, PACs are typically found as complex mixtures, of both pyrogenic and petrogenic PACs. The ratio of pyrogenic to petrogenic PACs changes according to the primary sources of these compounds. For instance in the Athabasca oil sands region, where PACs are mostly of petrogenic origin, LMW and alkylated PACs dominate most media, including air (Zhang et al., 2015b), snow (Kelly et al., 2009, Manzano et al., 2016), sediment (Headley et al., 2002, Kurek et al., 2013), and biota (Wayland et al., 2008, Lundin et al., 2015). In addition to being more abundant in certain regions, alkylated PACs are generally found to be more toxic than their unsubstituted counterparts (OMOE, 1990, Manzetti, 2013, Hawthorne et al., 2007, Lin et al., 2015) and have been shown to have significant bioaccumulation potential in certain aquatic organisms (Wayland et al., 2008). However, most research involving PACs and aquatic biota only investigate a few parent PACs, rarely assessing what happens when organisms are exposed to alkyl PACs and DBTs, and complex mixtures of PACs (Jensen et al., 2012, Yakan et al., 2011, Lu et al., 2004, Ball and Truskewycz, 2013). Considering the importance of alkylated PACs in petrochemicals, in the environment, as well as their toxicity, and other potential adverse effects, it is imperative to study the uptake, elimination, and bioaccumulation of both parent and alkyl PACs in aquatic organisms.

In aquatic ecosystems, sources of PACs include food, sediment, water, and fine suspended particles in water and air (Newman, 2010, Berrojalbiz et al., 2009). Due to their hydrophobic (lipophilic) nature, PACs preferentially associate with the organic phases of suspended particles and the sediment. In addition, PACs can easily partition through lipophilic membranes of organisms and accumulate in their tissues (Leppanen, 1999). Consequently, aquatic organisms that live in close proximity to the sediment substrate of aquatic ecosystems are especially susceptible to PAC contamination and exposure. For this reason, many studies on uptake and elimination kinetics of PACs have been conducted on benthic organisms, including amphipods, oligochaetes, and mussels (Berrojalbiz et al., 2009, Garrigues et al., 2004, Klok et al., 2012, Landrum and Scavia, 1983a, Leppanen, 1999). To our knowledge, one paper explored the toxicokinetics of PACs in the amphibian larvae *Pleurodeles waltl* (Spanish ribbed newt) (Garrigues et al., 2004), but there have been no studies in anuran larvae.

2.1.2. Bioaccumulation of PACs

In order to examine the bioaccumulation and toxicokinetics of parent and alkyl PACs in aquatic organisms, wood frog tadpoles (*Lithobates sylvaticus* - formerly known as *Rana sylvatica*) were chosen for this laboratory study since they are highly exposed to bottom sediment in nature, they are less efficient at metabolizing PACs in comparison to higher trophic organisms, they can provide sufficient biomass for analysis, they fulfil important ecological roles (e.g. prey, predators, nutrient cycling, and indicators of wetland quality), and they are ubiquitous in the Athabasca oil sands region. Furthermore, the larval stage of *Lithobates sylvaticus* can provide important information on uptake of PACs via water and sediment. As epibenthic omnivorous amphibian larvae, wood frog tadpoles can accumulate PACs not only from water through dermal absorption and across respiratory surfaces, but also from sediment through

dietary absorption since they can ingest sediment while feeding on detritus material. Some studies have investigated the relative importance of exposure routes for PACs in aquatic organisms (Lu et al., 2004, Landrum and Scavia, 1983b, Leppanen and Kukkonen, 2000), however most have used invertebrates such as amphipods and worms, and none to our knowledge have used amphibians. Therefore, we designed a study with two accumulation-elimination exposures (one evaluating exposure to contaminated sediment and water, and the other to contaminated water alone) in order to isolate the contribution of aqueous and sediment uptake of PACs in an amphibian.

Following the uptake of PACs, tadpoles can either transform and/or accumulate them in their tissues, or eliminate them through metabolism and/or excretion (Livingstone, 1998, Newman, 2010). In accumulation-elimination experiments, uptake and depuration of PACs typically follow the uptake and depuration curves in Figure 2.1. However, there are many factors affecting the extent to which PACs accumulate in organisms, including the ratio between rate of uptake and depuration, the properties and bioavailability of individual compounds, the mobility of the organism, and the capacity of the organism to metabolize PACs (Thorsen et al., 2004). This study will be investigating the toxicokinetics and accumulation of PACs in wood frog tadpoles in order to learn more about the extent to which these factors influence the bioaccumulation of PACs in anuran larvae.

Figure 2.1. Ideal uptake and depuration curve of accumulation-elimination experiments, where the concentration of a contaminant in an organism (C_B) increases during exposure to the source of the contaminant (C_S) until it reaches a state of equilibrium. Following termination of exposure, the concentration of the contaminant in the organism decreases exponentially. Subsequently, the k_2 elimination rate and k_1 uptake rate of the contaminant can be calculated (Boethling and Mackay, 2000).

There are three principal pathways currently proposed for metabolic activation of PACs: the pathway via bay region dihydrodiol epoxide by cytochrome P450 enzymes, the pathway via radical cation by one-electron oxidation, and the ortho-quinone pathway by dihydrodiol dehydrogenase (Xue and Warshawsky, 2005). PACs are mainly transformed and metabolized through the cytochrome P450 enzyme system. Cytochrome P450 is a family of hundreds of isoenzymes that are present in all eukaryotes and some prokaryotes. In phase I, PACs are oxidized by the cytochrome P450 monooxygenases to more reactive intermediates (phenols and epoxides). Some of these activated phase I metabolites have been linked with PAC toxicity and DNA adduct formation (Incardona et al., 2006, Stowers and Anderson, 1985). During phase II, biotransformation enzymes transform the intermediates via conjugation into more polar compounds, rendering them more water soluble and amenable to transport out of cells and

subsequently, out of the organism (elimination) (Hellou et al., 2012, Shimada, 2006, Manzetti, 2013, van der Oost et al., 2003). Phase II is primarily performed by conjugation enzymes such as uridine diphosphate glucuronosyltransferases, sulfotransferases, and glutathione-S-transferase. Although there are few studies on the metabolism of PACs in amphibians, Ueda *et al.* (2011) did find that amphibians relied strongly on sulfur conjugation for phase II transformation of pyrene. In addition, it has been determined that, like mammals, it is through the cytosolic aryl hydrocarbon receptor that induction of cytochrome P4501A also occurs in fish and amphibians (Marty et al., 1989, Di Giulio et al., 1995). Moreover, fish and amphibians have levels of total cytochrome P450 and inducible cytochrome P4501A activity between those of invertebrates and vertebrates of higher trophic levels (e.g., mammals) (Marty et al., 1992, Ertl and Winston, 1998). In other words, these aquatic vertebrates are more efficient at metabolizing PACs than invertebrates but not as efficient as mammals. Correspondingly, fish and amphibians are known to metabolize parent PACs (Berrojalbiz et al., 2009, Garrigues et al., 2004, Gewurtz et al., 2000, Neff, 1979, Ueda et al., 2011); however, the efficiency of amphibians at metabolizing alkyl PACs and DBTs is still unknown.

Moreover, bioaccumulation is characterized as the net accumulation of a contaminant from all possible routes of uptake (Boethling and Mackay, 2000). The bioaccumulation potential of organic contaminants in aquatic organisms has often been described using bioconcentration factors (BCFs) and biota-sediment accumulation factors (BSAFs) (Iannuzzi et al., 2011, Thomann and Komlos, 1999, Van Geest et al., 2011, Thorsen et al., 2004). BCFs refers to the concentration of a PAC in an organism's tissue divided by its equilibrium concentration in water, whereas BSAFs are the lipid and organic carbon normalized ratios of PAC concentrations in organisms relative to those in the sediment in which they reside (Boethling and Mackay, 2000).

BSAFs can be used as first-level screening tools to compare the bioaccumulation potentials of different compounds in benthic organisms (Wong et al. 2001, Wayland et al. 2008). For instance, Wayland *et al.* (2008) compared the bioaccumulation potential of PACs in larval and adult insects from different types of wetlands within the Athabasca oil sands region. They found that the BSAFs of alkyl PACs were consistently greater than the theoretical BSAF value of 1 (when PACs partition equally between the lipid of organisms and the organic carbon of sediment) and greater than the BSAFs of parent PACs; suggesting important bioaccumulation potential of alkyl PACs. It would be of great value to assess whether alkyl PACs also tend to have greater bioaccumulation potential than parent PACs in amphibian larvae. Furthermore, future research on PACs in aquatic organisms would benefit from knowing which individual compounds have the greatest uptake rates, the lowest elimination rates, and the greatest bioaccumulation potentials; this information would also be valuable for many monitoring programs, especially those concerned about aquatic ecosystems and contamination from petrogenic PACs.

2.1.3. Study Objectives

The first objective of this laboratory study is to determine the bioaccumulation potential of parent and alkyl PACs in wood frog tadpoles through exposure to complex mixtures of PACs in both sediment and water to help interpret PAC residues measured in animal tissues in natural environments. Based on recent evidence (Wayland et al., 2008), we hypothesize that biota-sediment accumulation factors for alkyl PACs, determined through accumulation-elimination experiments using *Lithobates sylvaticus* tadpoles, will be greater than those of their unsubstituted parent counterparts. Subsequently, toxicokinetic parameters for both the uptake and depuration phases will be established for each PAC. We hypothesize that if wood frog tadpoles are less efficient at eliminating alkyl PACs, these compounds will have, in addition to higher bioaccumulation potentials, also lower elimination rates than those of parent PACs.

The second objective is to investigate whether sediment or aqueous exposure is more important for PAC uptake and accumulation in amphibian larvae. Lu *et al.* (2004) suggest that the relative importance of sediment ingestion to aqueous exposure increases with hydrophobicity of PACs. Accordingly, we predicted that wood frog tadpoles exposed to PAC-contaminated sediment would uptake more HMW and alkylated compounds, compared to wood frog tadpoles from the aqueous-only exposure, due to their higher log K_{ow} and greater affinity to organic matter and sediment particles. This novel laboratory study will provide vital information on the uptake, accumulation, and elimination of parent and alkyl PACs in amphibian larvae exposed to PAC-contaminated sediment.

2.2. Methodology

2.2.1. Tadpole Care

Under the BL-2202 Animal Care Protocol, *Lithobates sylvaticus* tadpoles were reared at the University of Ottawa's Aquatic Facility starting from induced breeding of field-collected adult wood frogs (collected by Dr. Vance Trudeau's lab near Ottawa, Ontario). All eggs, and subsequently tadpoles, were kept in a flow-through tank containing 14°C Aquatic Facility water (domestic municipal water with the chloramines removed). Following facility protocols, the photoperiod consisted of 12:12 L:D and the tadpoles were fed daily (sera[®] micron powdered algae prior to development of mouthparts, and crushed Nasco Frog Brittle afterwards). When aquarium tanks were used during transfers, they were cleaned using Accel[®] PREvention Concentrate disinfectant (1:40 dilution) to ensure optimal health of the tadpoles.

2.2.2. Experimental Design

A total of 3 exposure experiments were conducted between May 2013 and June 2015 using *Lithobates sylvaticus* tadpoles and sediment from the Athabasca oil sands region. Sediment was collected in May of 2013, 2014, and 2015 from the MacKay River in Fort McKay, Alberta; a First Nation community situated 58 km north of Fort McMurray, Alberta. The MacKay River flows mostly northeast, through the surface mineable bituminous sands area until it reaches the Athabasca River. Each year, five gallons of superficial sediment were collected 1 m from the water's edge upstream of Range Rd 110A Bridge (N 57°10'1.84" W 111°38'6.10"). Oil sheens were visible on the river's surface, especially along its banks. The sediment had an average total carbon content of $1.5 \pm 0.1\%$ (SE, n=10). The clastic fraction of this sediment consists of approximately 50% sand, 30% silt, and 20% clay (RAMP, 2004).

2.2.2.1. Sediment + Aqueous Exposure Experiments

A 10-day sediment + aqueous exposure experiment was performed in June 2013 using 30 2 L round tanks (solvent-washed with acetone and hexane) filled with 375 g ww of MacKay River sediment (density of 1600 kg/m³) and 1.3 L of water, following the standard 1:4 sediment:water ratio (Van Geest et al., 2010). Each tank housed 10-12 tadpoles of Gosner stage 28-30 (for sample post-dissection target wet weight of 1-2 g) that had fasted for 24 hours prior to exposure. The water was renewed every 24 hours (60%) and kept at 19°C ± 1°C throughout the experiment. The experiment lasted 240 hours, for which the first 192 hours corresponded to the uptake phase and the last 48 hours, the depuration phase. Using a sequential harvesting design, tadpoles from 3 replicate tanks (10-12 tadpoles per tank) were collected after 0, 6, 12, 24, 48, 96, and 192 hours of exposure to the MacKay River sediment. The remaining tadpoles were transferred into tanks filled with clean water only. Sampling of tadpoles occurred from these depuration tanks after 6, 12, 24, and 48 hours of elimination. Once euthanized using tricaine methanesulfonate (AquaLife TMS, Syndel Laboratories Ltd, B.C., Canada), tadpoles were either dissected immediately or stored at 4°C until dissection was possible (within 24 hours). Sediment and water samples were also collected at each applicable time point. To compare elimination rates following a shorter uptake phase, a second sediment + aqueous exposure experiment was conducted in 2015, this time with a 24 hour uptake phase. This sediment + aqueous (24 h) exposure experiment also included measurements of elimination rates of compounds that were absent in the first experiment. The wood frog tadpoles of this experiment were of Gosner stage 28-32.

2.2.2.2. Aqueous Exposure Experiment

The previous two exposure experiments provided us with toxicokinetic information related to uptake via sediment and water, together. However, in order to determine uptake via sediment and water separately, an aqueous-only exposure was conducted using a screen that prevented tadpoles from contacting the sediment, exposing them only to the contaminants in the water.

A 37.9 L aquarium (61.0 x 22.9 x 33.0 cm) was filled with 24 L of Aquatic Facility water and 6 L of MacKay River sediment collected only 36 hours prior to the experimental set-up. The tadpoles were separated from the substrate by craft netting installed 3 cm above the sediment using copper piping to ensure exposure to PACs would be via aqueous exposure only (the tadpoles were not able to contact nor ingest the sediment). Prior to installation, the netting and pipes were soaked in water for several days in order to avoid any leaching in the exposure tank. Moreover, the water in the exposure tank was left to equilibrate with the sediment for a week before introducing 180 tadpoles of Gosner stage 29-32 to the aquarium for 24 hours (the time point after peak concentration of most PACs analyzed in the SedAq (192h) experiment). These tadpoles were then transferred into a clean water flow-through tank for 3, 6, 12, and 24 hours of elimination. At each of these depuration time points, water samples and 3 sets of 10-12 tadpoles were taken as replicates from the tank. Another 144 tadpoles were then added to the contaminated, preconditioned water. These last 144 wood frog tadpoles corresponded to the uptake phase of the aqueous (24 h) exposure experiment. After 6, 12, and 24 hours, 33-36 tadpoles were removed and dissected according to the same procedures as the sediment + aqueous experiments.

2.2.3. Analysis of Parent and Alkyl PACs

2.2.3.1. Tadpoles

After collection, the wood frog tadpoles were individually dissected to remove the entire gastrointestinal tract to ensure that the PAC concentrations measured would only correspond to the PACs accumulated in the tadpole bodies themselves and not be derived from ingested sediment still present in the gastrointestinal tract. The dissected tadpoles were then finely cut with stainless-steel micro-dissecting scissors, homogenized, and mixed with HydromatrixTM (Varian Inc, Palo Alto, CA, USA) to remove excess water. Each tadpole sample was spiked with 100 μL of ^{13}C -labeled standard (Cambridge Isotope Laboratories Inc., Andover, MA, USA), a mixture of the 16 U.S. EPA Priority PAHs (Figure 1.1). The samples were extracted using accelerated solvent extraction (ASE-350, Dionex Corporation, Sunnyvale, CA, USA) and optimal settings: a 50:50 acetone and hexane solvent mixture, a pressure of 1500 psi, a heating time of 7 min at 150°C, two static cycles of 5 min, and a 50% flush. To remove excess lipids found in biological tissue (NOAA, 1998), an 18 hour 6M potassium hydroxide digest was subsequently performed on the extracts (Martinez et al., 2004). PACs remaining in the co-extracted water were separated by liquid-liquid extraction, through 3 additions of 10 mL of hexane. Samples were then evaporated to 2 mL (solvent exchanged to 2,2,4-trimethylpentane) under a gentle stream of nitrogen (TurboVap[®], Biotage, Charlotte, NC, USA) in order to be centrifuged (4000 rpm for 10 minutes) for removal of any remaining particulates. Samples were once again evaporated, to 1 mL for fractionation.

Clean-up and fractionation was adapted from U.S. EPA method 3630C (EPA, 1996a) for the use of 6 mL (1 g) SupelcleanTM LC solid-phase extraction cartridges packed with silica gel (45 μm , 0.8 $\text{cm}^3 \text{g}^{-1}$, 475 $\text{m}^2 \text{g}^{-1}$). Correspondingly, preconditioning included acetone, pentane,

and 2,2,4-trimethylpentane additions, and analytes were eluted with 7 mL of a 2:3 (v/v) mixture of methylene chloride/pentane. Finally, recovered samples were solvent exchanged to 2,2,4-trimethylpentane, evaporated to 1 mL, and spiked with 10 μ L p-terphenyl-D₁₄ as an internal standard before being quantified by gas chromatography coupled with mass spectrometry (GC-MS). Excluding pesticide-grade pentane, all solvents used in this study were high-grade Optima[®] solvents from Fisher Chemical.

In addition to PAC analysis, total lipid content of the tadpole samples was determined. Following the Smedes Method (Smedes, 1999), an Ultra-Turrax[®] homogenizer (IKA[®] T10 basic) was used to blend 100 mg aliquots of wet biota twice with 800 μ L 2-propanol, 1 mL cyclohexane, and 1.1 mL of distilled water. The supernatant material was air-dried in pre-weighed aluminum weighing dishes for approximately 24 hours and lipid content calculated based on differences of weight. Ingestion rates were also calculated. By carefully observing the gastrointestinal tracts of each tadpole from the 12 h uptake time point (n=22), the ingested sediment was delicately separated from the rest of the gastrointestinal tract (there was a clear separation in the bowel content between pre- and post-exposure). Ingested sediment was then completely air dried and weighed. Weights of ingested sediment represent 12 hours of ingestion by the tadpoles, and ingestion rates were calculated with units of $\text{mg g tadpole}^{-1} \text{ h}^{-1}$.

2.2.3.2. Sediment

Analysis of PACs in sediment followed the same procedures as those for tadpole samples. However, prior to homogenization, samples were centrifuged (3000 rpm for 30 mins), decanted, and subsampled for determination of percent dry weight and elemental organic carbon analysis. In order to eliminate all sulfur interferences, activated powdered copper (Laboratory

grade, Fisher Chemical) was added to each sample at a copper to sediment weight ratio of 1:2 before accelerated solvent extraction. Samples were rinsed with pentane prior to elution on the SPE columns (EPA, 1996b).

2.2.3.3. Water

For freely dissolved PAC analysis, water samples were filtered and eluted within a week of collection. 500 mL of water were filtered through pre-weighed glass microfiber filters (WhatmanTM, GF/C, 1.2 μm , $\varnothing$ 42.5 mm) and then loaded on preconditioned Oasis[®] HLB 6cc (200 mg) extraction cartridges. Following Oasis[®] HLB extraction method for PAHs in drinking water (Waters Corporation, 2008), the extraction cartridges were first conditioned with 5 mL each of methylene chloride, methanol, and LC/MS grade water. Then the water samples, which included LC/MS grade water samples as method blanks, were spiked with 100 μL of ^{13}C -standard (Cambridge Isotope Laboratories Inc., Andover, MA, USA) and loaded on to the extraction cartridges using a vacuum manifold and a flow rate of approximately 15 mL min^{-1} . Analytes were eluted with 8 mL methylene chloride. After the addition of 2 mL of 2,2,4-trimethylpentane, samples were evaporated to 1 mL and subsequently, quantified by gas chromatography coupled with mass spectrometry.

2.2.4. Quantification

All tadpole, sediment, and water samples were analyzed by gas chromatography (Agilent 6890) coupled with mass spectrometry, on the electron impact mode (Agilent 5973, Agilent Technologies, Santa Clara, CA, USA). Injections of 1 μL were made in splitless mode at 280°C on a DB5-MS 30 m x 0.25 μm x 0.25 μm Agilent column. Initial oven temperature of 60°C was held for 2 min then increased at a rate of 6°C min^{-1} to a final temperature of 300°C, which was

held for 10 min. A constant flow rate of 35 cm sec⁻¹ of helium was used as the carrier gas for a total run time of 52 min. In addition, the electron impact mass spectrometer's transfer line temperature was set at 310°C, its source temperature at 230°C, and its quadrupole temperature at 150°C.

All quantified parent and alkyl PACs and their abbreviations are listed in Appendix A. Their method limits of quantification, which were based on a signal to noise ratio of 3:1, are detailed in Appendix B. All samples were blank corrected to remove background PAC contamination, and for quality assurance, reference material was extracted with each set of samples. Reference material consisted of 10 mg of NYNJ-1944 Standard Reference Material[®] (New York-New Jersey waterway sediment, National Institute of Standards and Technology, USA) for sediment samples, and 100 mg of 2974a Organics Standard Reference Material[®] (Freeze-dried *Mytilus edulis* mussel tissue, National Institute of Standards and Technology, USA) for tadpole samples. Concentrations of PACs were calculated using the isotope dilution method of quantification in order to correct for any variability in extraction and analytical methods as well as lower recovery rates (Mechlinska et al., 2010). Average PAC recovery rates (average $\pm$ SD) were $80 \pm 15\%$ for tadpole samples, $110 \pm 60\%$ for sediment samples, and $50 \pm 10\%$ for water samples.

2.2.5. Percent Organic Carbon and Nitrogen

Percent organic carbon and nitrogen were determined in all sediment samples at the G.G Hatch Stable Isotope Laboratory at the University of Ottawa. After acid desiccation using nitric acid, samples were flash combusted in a Micro Cube elemental analyser (Elementar, Germany) at a temperature of 1800°C. They were carried by helium through reducing/oxidizing columns

before being trapped within a single "trap and purge" adsorption column. Detection was accomplished by a thermal conductivity detector, with a routine analytical precision (2 sigma) of $\pm 0.1\%$.

2.2.6. Toxicokinetics

A first-order (two-component) toxicokinetic model was applied to each PAC analyzed (Boethling and Mackay, 2000, Newman, 2010). This model involves first-order uptake from a source (sediment or water) into one compartment (tadpoles), and first-order elimination from that compartment:

$$\frac{dC_b}{dt} = k_1 C_s - k_2 C_b \quad (2)$$

Where C_b is the PAC concentration in the tadpoles (ng g^{-1} , wet weight), C_s is the PAC concentration in the exposure source (ng g^{-1} for sediment or ng L^{-1} for water), k_1 is the uptake clearance rate ($\text{g g}^{-1} \text{ h}^{-1}$ for sediment or $\text{L g}^{-1} \text{ h}^{-1}$ for water), and k_2 is the elimination rate constant (h^{-1}). In the case of the sediment + aqueous (24 and 192 h) exposure experiments, sediment was the main source of PAC, whereas water was the primary source for the aqueous-only (24 h) exposure experiment. Uptake via water is assumed to be negligible in the sediment + aqueous exposures since statistical testing found uptake from water to be significantly lower than uptake from sediment and water together (will be presented in Section 2.3.5.).

From this toxicokinetic model, the depuration phase can be characterized by the following equation:

$$\frac{dC_b}{dt} = -k_2 C_b \quad (3)$$

Correspondingly, linear regression analyses were used to determine elimination and uptake rates. For elimination rate constants (k_2), linear regressions were performed on natural log-transformed PAC concentrations of tadpole body tissue versus time of tadpole sampling during the depuration phase. The slope of each PAC (12 to 24 h) corresponded to its k_2 value (Equation 2). For the SedAq (192h) and the Aq (24h) experiments, the slope of 24 hours of depuration was used whereas the slope of 12 hours was used for the SedAq (24h) since the depuration phase was shorter than 24 hours.

To calculate the uptake rate (k_1), linear regressions were performed on PAC concentrations in tadpole body tissue (ng g^{-1} , wet weight) versus duration of exposure for 3 to 6 h of uptake. At the time of maximum concentration, PACs typically have reached steady-state/equilibrium thus, dC_b/dt should equal zero. Consequently, Equation 4 was used to also determine a k_{max} uptake rate for each detected PAC. Equation 4 was adapted from Equation 1:

$$k_{\text{tmax}} = \left(\frac{C_b}{C_s} \right) \cdot k_2 \quad (4)$$

Where k_{tmax} is the uptake rate at time of maximum PAC concentration, k_2 is the elimination rate previously calculated using Equation 3, C_b is the PAC concentration in tadpoles at time of maximum concentration (ng g^{-1} , wet weight), and C_s is the mean PAC concentration in the exposure source during the uptake phase; concentration in the sediment for the sediment + aqueous (24 and 192 h) exposures (ng g^{-1} , dry weight) and the mean PAC concentration in water for the aqueous (24 h) exposure (ng L^{-1}).

The bioaccumulation potential of each parent and alkyl PAC was also assessed. For the sediment + aqueous (24 and 192 h) exposure experiments, biota-sediment accumulation factors (BSAFs) were calculated at $t=24$ h (Equation 7). This bioaccumulation factor describes the

bioaccumulation of each PAC in the wood frog tadpoles relative to the PAC concentration in the MacKay River sediment:

$$BSAF = \left(\frac{C_b}{C_s} \right) \cdot \left(\frac{TOC}{L} \right) \quad (7)$$

Where C_b is the concentration of PACs in the organism (g PAC/kg organism), C_s is the concentration of PACs in the sediment (g PAC/kg dry weight sediment), TOC is the total organic carbon content in the sediment (g organic carbon/g dry weight sediment), and L is the lipid content in the organism (g lipid/g organism) (Boethling and Mackay, 2000). On the other hand, for the aqueous (24 h) exposure experiment the bioconcentration factor (BCF) was calculated instead (Equation 8):

$$BCF = \frac{C_b}{C_w} \quad (8)$$

Where C_b is the concentration of PACs in the organism (g PAC/kg organism) and C_w is the concentration of PACs measured in water (ng L⁻¹). BCFs are usually reported on a log scale.

2.2.7. Statistical Analysis

Simple linear regressions, multiple linear regressions, and Pearson correlations were used to analyze the relationships between toxicokinetic parameters, log K_{ow} and molecular weight of PACs. Student or paired T-tests and Mann-Whitney tests were performed to find differences between type of PACs (parent or alkyl) and their number of rings (2-3-rings or 4-6-rings). An alpha value of 0.05 was used in Post Hoc tests. Shapiro-Wilk and Levene's tests were used to verify the normality of distribution and the homogeneity of variance, with a p-value of 0.05. When required, the data were log-transformed prior to statistical analysis, and the Bonferroni correction was performed. All statistical analyses were done using SigmaPlot 12 software.

2.3. Results

2.3.1. Sediment + Aqueous (192 h) Exposure Experiment

The sediment collected from the MacKay River, to which wood frog tadpoles were exposed for each of the 3 experiments conducted in this study, had a total PAC concentration of $520 \pm 50 \mu\text{g g}^{-1}$ dry weight TOC ($\pm\text{SE}$, $n=13$) and a characteristic petrogenic PAC profile (Figure 2.2). Higher concentrations of alkylated PAC compounds, especially those with 2 or 3 alkyl substitutions (C2- and C3-), indicate a petrogenic source of PACs and a profile typical of oil sands sediment (Headley et al., 2002, Yang et al., 2011). When comparing the chemical profile of the MacKay River sediment to that of a diluted oil sands bitumen sample from Yang *et al.* (2011) (Figure 2.2), it is clear that they follow a similar PAC profile. Σ Alkyl PACs of the MacKay River sediment averaged $510 \pm 50 \mu\text{g g}^{-1}$ dw TOC compared to $10 \pm 0.5 \mu\text{g g}^{-1}$ dw TOC for Σ parent PACs. Values for individual PAC concentrations in the MacKay River sediment can be found in Appendix C, but the most abundant parent PACs were (in descending order) benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, benzo[b]fluoranthene, and fluoranthene. The alkyl PACs that accounted for most of the total concentration of PACs were C2-, C3-, C4-dibenzothiophenes (45%), C2-, C3-, C4-phenanthrenes/anthracenes (30%), and C2-, C3-, C4-fluoranthenes/pyrenes (15%).

Figure 2.2. Mean ($\pm$ SE) PAC concentration profile (ng g^{-1} , dry weight TOC) of the MacKay River sediment used in all 3 exposure experiments ($n=13$) and the PAC profile of diluted oil sands bitumen (DOB) analyzed and reported by Yang *et al.* (2011).

The wood frog tadpoles used for the sediment + aqueous (192 h) exposure experiment had an average weight of 0.275 g ww and total length of $26.5 \pm 3.9 \text{ mm}$ ($\pm$ SD, $n=332$); no significant growth was measured between the start and end of the experiment. In addition, the wood frog tadpoles had an average lipid content of $1.53 \pm 0.07\%$ ($\pm$ SE, $n=38$) on a wet weight basis and a mortality rate of 1.0%. The average ingestion rate of sediment during the exposure was $3.96 \pm 0.37 \text{ mg g}^{-1} \text{ h}^{-1}$ ($\pm$ SE) ($n=22$) and the tadpoles contained background levels of PACs of $290 \pm 75 \text{ ng g}^{-1}$ (Σ PACs $\pm$ SE, ww). Σ PACs reached up to $1048 \pm 260 \text{ ng g}^{-1}$ in tadpoles during the 192 h uptake phase of the experiment. As seen in Figure 2.3, the highest Σ alkyl ($1015 \pm 260 \text{ ng g}^{-1}$) and Σ parent ($65 \pm 25 \text{ ng g}^{-1}$) PAC concentrations were measured in tadpoles exposed to the MacKay River sediment for 12 and 48 hours, respectively. Σ alkyl PACs were on average an order of magnitude higher than those of Σ parent PACs throughout the experiment.

After the initial uptake and increase in PAC concentrations, both Σ parent and Σ alkyl PACs decreased, and even more so after the tadpoles were transferred to clean water for the depuration phase (Figure 2.3). Σ alkyl PACs in the tadpole bodies returned to background levels after 6 to 12 hours of depuration, whereas Σ parent PACs reached background levels after 6 hours.

Figure 2.3. Mean ($\pm$ SE) Σ alkyl PACs and Σ parent PACs (ng g⁻¹, wet weight) in wood frog tadpoles exposed to MacKay River sediment at various time points (hours) during the uptake and depuration phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013. Samples in triplicate.

Both the concentration and the proportion of alkylated PACs in the tadpoles changed through the course of the exposure experiment. At $t=0$, compounds with 1 or 2 alkyl substitutions corresponded to $52 \pm 2\%$ of Σ alkyl PACs and those with 3 or 4 alkyl substitutions, $48 \pm 2\%$. Subsequently, the proportion of C3- and C4-alkyl PACs significantly increased (t-test, $p < 0.04$) (Figure 2.4). Notably, at the end of the uptake phase ($t=192$ h), C3- and C4-alkyl PACs corresponded to $60 \pm 3\%$ of Σ alkyl PACs in the tadpoles and at the end of the depuration phase ($t=240$ h), they corresponded to $91 \pm 4\%$. The C3- and C4-alkyl PACs that were present at $t=240$ h were C4-naphthalenes, C4-phenanthrenes/anthracenes, and C3-fluoranthenes/pyrenes. Nonetheless, C4-naphthalenes accounted for most of the alkyl PACs that remained at the end of

the depuration phase; averaging $84 \pm 3\%$ of Σ alkyl PACs at $t=240$ h.

Figure 2.4. Mean ($\pm$ SE) proportion of C1-C2 PACs and C3-C4 PACs (%) of Σ alkyl PACs in wood frog tadpoles exposed to MacKay River sediment at various time points (hours) during the uptake and depuration phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013. Samples in triplicate.

Not only did the tadpoles accumulate a greater total amount of alkyl PACs in their bodies than total parent PACs, but also higher concentrations of individual groups of alkyl PACs (C1-, C2-, C3-, and C4-) compared to their corresponding unsubstituted counterparts (Figure 2.5). For instance, the concentrations of C1-, C2-, C3-, and C4-dibenzothiophenes were much higher than the parent dibenzothiophene throughout the majority of the uptake phase of the experiment (Figure 2.5). Overall, during the uptake phase, the most abundant parent compounds in the tadpoles were (in descending order) benzo[a]pyrene, chrysene and phenanthrene, and the most abundant alkyl PACs were C2-, C3-, C4-dibenzothiophenes and C4-naphthalenes. However, at $t=24$ h, which corresponds to the time point between peak uptake concentrations, the most abundant parent compounds in the tadpoles were (in descending order) benzo[a]pyrene, anthracene, and pyrene, while C3-, C4-dibenzothiophenes, C3-fluorenes, and C4-naphthalenes were the most abundant alkyl PACs (Figure 2.5).

Figure 2.5. Mean ($\pm$ SE) PAC concentration profiles (ng g⁻¹, wet weight) of wood frog tadpoles after 0, 24, 48, 96 and 192 hours of exposure to MacKay River sediment. Samples in triplicate and correspond to the uptake phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013. Abbreviations are found in Appendix A.

As Figure 2.6 demonstrates, the compounds that were present in the wood frog tadpoles after 48 hours of elimination in clean water tanks were (in descending order): C4-naphthalenes, C4- and C1-phenanthrenes/anthracenes, pyrene, C1-dibenzothiophenes, C3- and C1-fluoranthenes/pyrenes, C1-benzo[a]anthracenes/chrysenes, and acenaphthene. However, only C4-naphthalenes, and possibly C1-dibenzothiophenes, seem to be resistant in the tadpoles from the SedAq (192 h) experiment when examining individual PAC concentration graphs (Figure 2.7).

Figure 2.6. Mean (±SE) PAC concentration profiles (ng g⁻¹, wet weight) of wood frog tadpoles after 0, 6, 12, 24, and 48 hours of depuration into clean water tanks during the sediment + aqueous (192 h) exposure experiment conducted in 2013. The PAC profile of 0h corresponds to PAC concentrations in tadpoles that were exposed to MacKay River sediment for 192 hours; the starting point of the depuration phase. Samples in triplicate. Abbreviations are found in Appendix A.

Figure 2.7. Mean ($\pm$ SE) concentration (ng g⁻¹, wet weight) of C4-naphthalenes and C1-dibenzothiophenes in wood frog tadpoles throughout the 192 hour uptake phase and the 48 hour depuration phase of the sediment + aqueous (192 h) exposure experiment conducted in 2013 using MacKay River sediment. Samples in triplicate.

2.3.2. Aqueous (24 h) Exposure Experiment

For the aqueous (24 h) exposure experiment, the first tadpoles to be introduced to the aquarium with PAC-contaminated water were the depuration phase tadpoles (Figure 2.8). After 24 hours of exposure, total PAC concentrations in these tadpoles reached 775 ng g⁻¹ (Σ parent: 65 $\pm$ 15 ng g⁻¹, Σ alkyl: 710 $\pm$ 165 ng g⁻¹). Once transferred into a flow-through tank, concentrations of PACs in the tadpoles decreased exponentially, much like in the SedAq (192h) experiment. Both parent and alkyl PAC concentrations decreased to one third of their initial concentration within 24 hours of depuration (Σ parent: 20 $\pm$ 2 ng g⁻¹, Σ alkyl: 220 $\pm$ 105 ng g⁻¹). The wood frog tadpoles used for the aqueous (24 h) exposure experiment had an average weight of 0.158 g ww, a total length of 23.2 $\pm$ 2.4 mm ($\pm$ SD, n=230), and a mortality rate of 1.5%.

Figure 2.8. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g⁻¹, wet weight) in wood frog tadpoles at various time points (hours) during the depuration phase of the aqueous (24 h) exposure experiment conducted in 2014. Samples in triplicate.

After the first set of tadpoles were removed from the exposure aquarium, the second set of tadpoles were introduced for the uptake phase of the experiment (Figure 2.9). These tadpoles contained relatively low background levels of PACs (165 ± 10 ng g⁻¹ ww Σ PACs). Similarly to the SedAq (192 h) experiment, the highest Σ alkyl tadpole PAC concentrations (410 ± 50 ng g⁻¹) were measured after 12 hours of exposure to the contaminated water. Σ alkyl PAC concentrations were once again at least an order of magnitude higher than those of Σ parent PACs throughout the uptake phase. Moreover, Σ parent PAC concentrations were as variable as in the SedAq (192 h) exposure experiment. Indeed, in contrast to Σ alkyl PACs, Σ parent PAC concentrations of both experiments did not follow an ideal uptake curve, as presented in the introduction of this chapter (Figure 2.1). The highest Σ parent PAC concentration (30 ± 5 ng g⁻¹) was measured after only 3 hours of exposure, and the lowest was measured after 6 hours of exposure (10 ± 2 ng g⁻¹).

Figure 2.9. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g^{-1} , wet weight) in wood frog tadpoles at various time points (hours) during the uptake phase of the aqueous (24 h) exposure experiment conducted in 2014. Samples in triplicate.

The average total PAC concentration in the water to which the wood frog tadpoles were exposed to during the Aq (24 h) experiment was $1350 \pm 435 \text{ ng L}^{-1}$ ($\pm$ SE, $n=6$). This is much higher (2 to 6 times higher) than the total water PAC concentration measured during the SedAq (192 h) experiment ($240 \pm 35 \text{ ng L}^{-1}$, $n=5$). Yet, Σ PACs in tadpoles from the Aq (24 h) experiment reached lower concentrations than those from the SedAq (192 h) experiment ($420 \pm 50 \text{ ng g}^{-1}$ vs. $1050 \pm 50 \text{ ng g}^{-1}$ ww at $t=12\text{h}$) (Figure 2.10). Nevertheless, for both the sediment and aqueous experiments, alkyl PACs accounted for 94% and 86% of PAC concentrations in the water, respectively.

Figure 2.10. Mean ($\pm$ SE) Σ parent and Σ alkyl PACs in wood frog tadpoles of two experiments with different exposure routes: the sediment + aqueous (192h) exposure (SedAq) and the aqueous (24h) exposure (Aq). Samples in triplicate.

2.3.3. Sediment + Aqueous (24 h) Exposure Experiment

After 24 hours of exposure to MacKay River sediment, wood frog tadpoles from the sediment + aqueous (24 h) exposure experiment had Σ parent PAC concentrations of 14 ± 2 ng g⁻¹ and Σ alkyl PAC concentrations of 150 ± 20 ng g⁻¹. During this exposure, water concentrations averaged 6 ± 1 ng L⁻¹ for Σ parent and 85 ± 15 ng L⁻¹ for Σ alkyl PACs. Interestingly, PACs were also detectable in the water during the depuration phase. In fact, Σ alkyl PAC concentrations in the flow-through depuration tank increased from 20 ± 5 ng L⁻¹ at $t = 0$ h to 30 ± 5 ng L⁻¹ at $t = 12$ h, while Σ parent PACs increased from 2 to 6 ± 1 ng L⁻¹ during that time. The wood frog tadpoles used for the sediment + aqueous (24 h) exposure experiment had an average weight of 0.306 g ww, a total length of 29.1 ± 2.7 mm ($\pm$ SD, $n=81$), and a mortality rate of 0%.

Much like in the previous two exposure experiments, concentrations of parent and alkyl PACs in the tadpole bodies decreased exponentially during the depuration phase of the sediment + aqueous (24 h) exposure experiment (Figure 2.11 and 2.12). Figure 2.12 shows this exponential decrease in the depuration concentrations of all 3 exposure experiments.

Figure 2.11. Mean ($\pm$ SE) Σ alkyl PACs and Σ parent PACs (ng g⁻¹, wet weight) in wood frog tadpoles exposed to Athabasca oil sands sediment at various time points (hours) during the depuration phase of the sediment + aqueous (24 h) exposure experiment conducted in 2015. Samples in triplicate.

Figure 2.12. Mean ($\pm$ SE) natural log Σ parent (a) and Σ alkyl (b) PACs (ng g^{-1} , wet weight) in wood frog tadpoles at various time points (hours) during the depuration phase of the sediment + aqueous (192 h) experiment, the aqueous (24 h) experiment, and the sediment + aqueous (24 h) experiment. The exponential equations associated with each depuration experiment and their R^2 and p-values were calculated using SigmaPlot 12. Samples in triplicate. * indicates significant p-values.

2.3.4. Tadpoles, Sediment, and Water Concentrations

In order to compare concentrations of PACs in the wood frog tadpoles to those from the sediment and/or water concentrations they were exposed to, data of individual compounds and groups of compounds were log-transformed and analyzed by linear regression using SigmaPlot 12. PAC concentrations in tadpoles showed significant positive correlations with sediment PAC concentrations in both SedAq (192 h) and SedAq (24 h) exposure experiments (Figure 2.13). The linear regression models between the tadpoles and sediment PAC concentrations explained 41 and 40% of the variability, respectively.

Although the importance of the linear regression between tadpole and sediment PAC concentrations and the regression between tadpole and water PAC concentrations were rather similar in the SedAq (192 h) experiment ($p < 0.001$, $R^2 = 0.41$ and $p < 0.001$, $R^2 = 0.39$), under multiple linear regression analysis, only the sediment PAC concentration was found to be a good predictor of the PAC concentrations in the wood frog tadpoles ($p < 0.001$ compared to $p = 0.06$ for the water PAC concentration). The overall model fit was $R^2 = 0.62$ (Tadpole PAC = $-0.09 + (0.48 * \text{Sediment PAC}) + (0.33 * \text{Water PAC})$). Conversely, PAC concentrations in the wood frog tadpoles of the SedAq (24 h) experiment seem to be more associated with the PAC concentrations of the sediment ($p < 0.001$, $R^2 = 0.40$) than with those of the water ($p = 0.03$, $R^2 = 0.19$); however, neither water nor sediment PAC concentrations were good predictors of the tadpole PAC concentrations in the SedAq (24 h) experiment when considering multiple linear regression analysis ($p > 0.05$). This is possibly related to the fact that the relationship between water and sediment PAC concentration was not as strong for the SedAq (24 h) experiment ($p = 0.02$, $R^2 = 0.16$) than for the SedAq (192 h) experiment ($p < 0.001$, $R^2 = 0.41$).

Only tadpole and water PAC concentrations were analyzed by linear regression for the Aq (24 h) experiment since aqueous exposure was the only exposure route. For this experiment, the PAC concentrations in tadpoles could be predicted by the following formula: Tadpole PAC = $-0.45 + (0.81 * \text{Water PAC})$ ($p < 0.001$, $R^2 = 0.37$).

Figure 2.13. Log concentrations of individual PACs in wood frog tadpoles in comparison to their concentrations in sediment and water. Tadpole, sediment, and water concentrations are the average log-transformed concentrations of all uptake time points from each of the 3 exposure experiments. Data were analyzed by linear regression and all regressions were found to be significant ($p < 0.05$).

- a.i SedAq (192 h) PAC concentrations in tadpoles vs. sediment
- a.ii SedAq (192 h) PAC concentrations in tadpoles vs. water
- a.iii SedAq (192 h) PAC concentrations in water vs. sediment
- b.i SedAq (24 h) PAC concentrations in tadpoles vs. sediment
- b.ii SedAq (24 h) PAC concentrations in tadpoles vs. water
- b.iii SedAq (24 h) PAC concentrations in water vs. sediment
- c.i Aq (24 h) PAC concentrations in tadpoles vs. water

2.3.5. Toxicokinetics

Following first-order toxicokinetics, the uptake and elimination rates were determined for each PAC detected in each of the 3 exposure experiments (Appendix D). The average ($\pm$ SD) k_2 elimination rates were $0.092 \pm 0.053 \text{ h}^{-1}$ in the SedAq (192 h) experiment, $0.070 \pm 0.042 \text{ h}^{-1}$ in the Aq (24 h) experiment, and $0.077 \pm 0.054 \text{ h}^{-1}$ in the SedAq (24 h). Although the k_2 averages and values measured in sediment + aqueous (24 and 192 h) exposures were greater than the aqueous (24 h) exposure, a one-way ANOVA found no significant differences between the means of the experiments ($p = 0.319$). On the other hand, using a paired t-test, k_1 uptake rates were found to be significantly higher for compounds in the SedAq (192 h) experiment than the Aq (24 h) experiment ($p < 0.001$) (Figure 2.14). In fact, the mean k_1 uptake rate from the SedAq (192 h) experiment ($1.72 \text{ L g}^{-1} \text{ h}^{-1}$) was ~ 2 orders of magnitude greater than that of the Aq (24 h)

experiment ($0.01 \text{ L g}^{-1} \text{ h}^{-1}$). In addition, the mean of k_{tmax} uptake rates from the SedAq (192 h) exposure (0.38) was significantly greater than the mean k_{tmax} value of the Aq (24h) exposure (0.03) ($p < 0.001$). Evidently, uptake of PACs is significantly greater in wood frog tadpoles from the sediment + aqueous exposure experiment than the aqueous-only experiment.

Figure 2.14. Mean ($\pm$ SE) k_1 uptake rates of PACs of the sediment + aqueous (192h) experiment and the aqueous (24 h) experiment ($\text{L g}^{-1} \text{ h}^{-1}$). Paired t-test performed in SigmaPlot 12 on log-transformed data was significant ($p=0.04$). Data were back-transformed. $n=7$.

As Table A.4 in Appendix D summarizes, the compounds with the highest k_{tmax} accumulation rates (k_1 at t_{max}) were C3- and C4- dibenzothiophenes in the SedAq (192 h) experiment, and benzo[k]fluoranthene and C4-naphthalenes in the Aq (24 h) exposure. With regards to the k_1 uptake rates, the highest values were for C3-dibenzothiophenes and C2-fluorenes in the SedAq (192 h) experiment, and C3-phenanthrenes/anthracenes and C1-fluorenes in the Aq (24 h) experiment. Evidently, alkylated dibenzothiophenes were among the most rapidly accumulated compounds during the sediment + aqueous exposure experiment, but not the aqueous-only experiment. Moreover, the compounds with the lowest k_2 elimination rate constants were (in ascending order): C4-naphthalenes, pyrene, and C2-

benz[a]anthracenes/chrysenes in the SedAq (192 h) experiment, and 1-, 9/4-methylphenanthrene and C3-naphthalenes in the Aq (24 h) experiment. Interestingly, the compounds that remained after 48 h of depuration in the SedAq (192 h) experiment have some of the lowest k_2 elimination rates, including C4-naphthalene (0.006 h^{-1}), pyrene (0.013 h^{-1}), and C1-dibenzothiophenes (0.087 h^{-1}). However, k_2 values were generally not that different among individual PACs (Appendix D).

To determine any differences in accumulation and elimination rates (k_1 , k_{tmax} , and k_2) between PACs, compounds were divided by type (parent and alkyl) and by their number of rings (2-3 rings and 4-6 rings). Using student t-tests, no significant differences in mean uptake rates and mean elimination rates were found between parent and alkyl PACs. However, mean k_1 uptake rate of the Aq (24 h) experiment was found to be higher for PACs with 2-3 rings than those with 4-6 rings ($p=0.04$), but after correcting for multiple comparisons (Bonferroni correction), the difference was not significant (Figure 2.15).

Figure 2.15. Mean ($\pm$ SE) k_1 uptake rates of PACs with 2-3 rings and 4-6 rings from the aqueous (24 h) experiment ($\text{L g}^{-1} \text{h}^{-1}$). Student t-test performed in SigmaPlot 12 on log-transformed data ($p=0.04$) was not significant after Bonferroni correction (critical p -value = 0.003). Data were back-transformed. These data include both parent and alkyl PACs; $n=16$ for 2-3 ring PACs and $n=11$ for 4-6 ring PACs.

Differences in biota-sediment accumulation factors (BSAFs) and bioconcentration factors (BCFs) between types of PACs were also analyzed. Significant differences were found between BSAFs of parent and alkyl PACs, and BCFs of 2-3 and 4-6 ring PACs. In fact, the BSAFs in wood frog tadpoles after 24 hours of exposure were significantly greater for parent PACs than for alkyl PACs (Table 2.1). This was true for both the SedAq (192 h) experiment ($p=0.02$) and the SedAq (24 h) experiment ($p=0.04$). Interestingly, the parent PACs also had a higher median BSAF compared to the alkyl PACs after 12 hours of exposure in the SedAq (192 h), but this difference was not significant.

Although parent PACs seem to have greater BSAFs than alkyl PACs, when looking at individual BSAFs (Table A.6 in Appendix E), only a few individual PACs had notable bioaccumulation potential. After 24 hours of exposure, anthracene (1.47 ± 0.73), retene (1.06 ± 0.72), and C1-benzofluoranthenes/benzopyrenes (2.63 ± 2.30) from the SedAq (192 h) experiment had the highest BSAFs ($\text{BSAF} \pm \text{SE}$), while fluoranthene (2.35 ± 1.26) was the only PAC with a BSAF greater than 1 in the SedAq (24 h) experiment. As for BCFs, which measure the bioconcentration of PACs in tadpoles relative to PACs in water, the mean BCF value after 12 hours of exposure in the Aq (24 h) experiment was significantly higher for alkyl PACs than for parent PACs ($p=0.02$) (Table 2.1).

Table 2.1. Statistical differences between biota-sediment accumulation factors (BSAF) and bioconcentration factors (BCF) of parent and alkyl PACs of 3 exposure experiments conducted on wood frog tadpoles exposed to MacKay River sediment. Data were log-transformed when normalization was possible. When the p-value of either the Mann-Whitney (M-W) or the student t-test (T-test) was lower than 0.05, the difference between parent and alkyl PACs was considered significant (*). The mean log10 data were back-transformed to mean BSAF and BCF values.

	SedAq (192 h)	SedAq (192 h)	SedAq (24 h)	Aq (24 h)	Aq (24 h)
	Median BSAF (t=12h)	Mean BSAF (t=24h)	Median BSAF (t=24h)	Mean BCF (t=12h)	Mean BCF (t=24h)
Statistical Test	M-W	T-test	M-W	T-test	T-test
Parent PACs	0.65 <i>n</i> =7	0.34* <i>n</i> =7	0.12* <i>n</i> =4	2.04* <i>n</i> =6	3.55 <i>n</i> =5
Alkyl PACs	0.10 <i>n</i> =13	0.09* <i>n</i> =28	0.01* <i>n</i> =21	2.58* <i>n</i> =27	3.10 <i>n</i> =24
p-value	0.11	0.02	0.04	0.02	0.21

As a measure of PAC hydrophobicity, log K_{ow} was compared to uptake and elimination rates (log k_1 , log k_{tmax} and log k_2) and accumulation factors (log BSAF and log BCF). A significant linear relationship was found between log K_{ow} and log BCF values of the SedAq (192 h) experiment ($F_{1, 11}=6.73$, $p=0.03$) (Figure 2.16). When looking at only alkyl PACs, a significant correlation was found between log K_{ow} and log k_2 elimination rates of the SedAq (24 h) experiment ($PCC=0.70$, $p=0.04$) (Figure 2.17).

Figure 2.16. Linear regression of log-transformed bioconcentration factors of PACs and their associated log K_{ow}. The BCFs were calculated from water and tadpole body concentrations at t=24h of the sediment + aqueous (192 h) exposure experiment. The linear regression analysis was performed using SigmaPlot 12. n=13.

Figure 2.17. Scatterplot of log-transformed k_2 elimination rate constants of alkyl PACs against their associated $\log K_{ow}$. $PCC=0.70$, $p=0.04$. The k_2 values were calculated using the initial slope (6 h) of the depuration phase of the aqueous (24 h) exposure experiment (h^{-1}). $n=21$.

In addition, a significant correlation was found between molecular weight of PACs and their k_{tmax} in the Aq (24 h) experiment ($\text{PCC}=0.72$, $p=0.02$) (Figure 2.18). However, since molecular weight can only be known for individual compounds, this positive correlation only applies to individual PACs and not to any of the alkyl groups (Appendix A).

Figure 2.18. Log-transformed k_{tmax} uptake rates of individual PACs plotted against their associated molecular weight (g mol^{-1}). The k_{tmax} values were calculated using k_2 elimination rates, as well as tadpole and water concentrations at time of maximum PAC concentration of the aqueous (24 h) exposure experiment. $n=10$.

2.4. Discussion

2.4.1. Water and Sediment Exposure

Since most PACs in aquatic environments largely reside in sediment (Wang et al., 2011, Lotufo, 1998), potentially posing a greater risk to benthic and epibenthic organisms, one of the main objectives of this laboratory study was to assess the relative importance of sediment and aqueous exposure in *Lithobates sylvaticus* tadpoles through two accumulation-elimination experiments (one evaluating exposure to contaminated sediment and water, and the other to contaminated water alone). To our knowledge, this is the first study of its kind using amphibian larvae.

The wood frog tadpoles in the sediment + aqueous (192 h) experiment reached higher total PAC concentrations ($1050 \pm 50 \text{ ng g}^{-1}$ at $t=12\text{h}$) than those from the aqueous (24 h) experiment ($420 \pm 50 \text{ ng g}^{-1}$ at $t=12\text{h}$). Hence, accumulation of PACs was much higher in the wood frog tadpoles that were in contact with the MacKay River sediment than those that were not, even though the water PAC concentrations during the aqueous-only exposure were 2 to 6 times greater than those of the sediment + aqueous experiments. Evidently, when PAC-contaminated sediments are present, the dominant route of exposure for wood frog tadpoles is sediment exposure. Accordingly, uptake rates of individual PACs were significantly higher in tadpoles that were simultaneously exposed to PACs from sediment and water than those that were exposed to PACs from water only. It is important to note that the wood frog tadpoles available for the aqueous (24 h) experiment were smaller than those from the sediment + aqueous experiments. This could have led to lower uptake/accumulation of PACs in these tadpoles. In order to try to compensate for the size difference, an additional tadpole was added per replicate tank in the aqueous (24 h) experiment. Nevertheless, under multiple linear

regression analysis, only the sediment PAC concentrations were found to be a good predictor of PAC concentrations in the wood frog tadpoles of the sediment + aqueous (192 h) experiment ($p < 0.001$ compared to $p = 0.061$ for the water PAC concentration). The overall model fit was $R^2 = 0.62$ (Tadpole PAC = $-0.09 + (0.48 * \text{Sediment PAC}) + (0.33 * \text{Water PAC})$). Therefore, the greater importance of sediment exposure compared to aqueous exposure was not only as we predicted, but also supports the results of studies that have similarly highlighted the prominence of sediment in the accumulation of PACs in other aquatic organisms.

Notably, Landrum and Scavia (1983a) estimated that sediment-associated anthracene contributed up to 77% of the steady-state body burden of the benthic amphipod *Hyaella azteca*. In addition, through two experiments using spiked sediment and the freshwater oligochaete *Lumbriculus variegatus*, Leppanen *et al.* (2000) found that the proportion of parent (not yet biotransformed) benzo[a]pyrene in tissues was larger in feeding animals because of faster uptake rates of benzo[a]pyrene in these feeding animals compared to non-feeding animals. Even more, the contribution of ingested material in the accumulation of PACs in aquatic organisms has been shown to increase with molecular weight and hydrophobicity (Lu *et al.*, 2004, Thomann *et al.*, 1992, Meador *et al.*, 1995). When assessing the relative importance of sediment pore water and ingested sediment particles to total uptake of PACs, Lu *et al.* (2004) found that sediment-bound phenanthrene accounted for less than 20% of the total uptake in the oligochaete *Ilyodrilus templetoni*, while the estimated uptake from sediment ingestion for the more hydrophobic benzo[a]pyrene accounted for more than 95% of the total uptake. As most of the PACs measured in the MacKay River exposure sediment were alkylated PACs with $\log K_{ow}$ higher than 5, it is not surprising that the contribution of sediment exposure was greater than the contribution from water exposure in the accumulation of PACs in our wood frog tadpoles. Evidently, it is important

to consider sediment exposure when assessing the uptake and bioaccumulation of PACs in benthic and epibenthic organisms, including vertebrates such as anuran larvae, in aquatic ecosystems.

2.4.2. Parent and Alkyl PACs

By means of three laboratory exposure experiments, this study was able to determine whether sediment or aqueous PAC exposure is more important for amphibian larvae, as well as investigate whether parent or alkyl polycyclic aromatic compounds have greater bioaccumulation potentials. Wood frog tadpoles from all exposure experiments accumulated much more total alkyl PACs than parent PACs, as well as higher concentrations of the alkylated compounds compared to their respective parent homologues. Correspondingly, the wood frog tadpoles had clear petrogenic PAC profiles, closely resembling the PAC profile of the MacKay River sediment to which they were exposed. These petrogenic profiles are typical of sediment and biota found in oil-contaminated or bitumen-rich areas (Wayland *et al.*, 2008, Headley *et al.*, 2002, Yang *et al.*, 2011).

Counter to our predictions, biota-sediment accumulation factors were significantly greater for parent PACs than alkyl PACs in wood frog tadpoles of this study's sediment + aqueous exposure experiments. We expected alkyl PACs to have greater bioaccumulation than parent PACs due to their higher bioavailability and persistence. For example, Wayland *et al.* (2008) found biota-accumulation factors were higher for alkyl PACs than for their parent counterparts, in both insect larvae and adult insects of various Athabasca oil sands wetlands. Likewise, Thorsen *et al.* (2004) measured BSAF values greater than 1 for petrogenic PAHs in mussels and clams, compared to BSAF values less than 1 for most pyrogenic PAHs; indicating

that petrogenic PACs were more bioavailable than pyrogenic. However, our study suggests that in amphibian larvae, unsubstituted parent PACs have greater bioaccumulation potential than alkylated PACs.

There are a few possible explanations for the unexpected difference in biota-sediment accumulation factors. Firstly, higher concentrations of alkyl PACs in the MacKay River sediment could have induced higher metabolism of these alkylated compounds in the tadpoles compared to their parent counterparts. However, parent and alkyl PACs are thought to be metabolized through similar pathways; largely through the cytochrome P450 enzyme system (Manzetti, 2013, Shimada, 2006, van der Oost et al., 2003). Secondly, while accumulation of alkyl PACs was higher than parent PACs in the wood frog tadpoles (10 times higher than parent PACs), the alkyl PAC concentrations in the sediment they were exposed to was comparably much higher than those of parent PACs (up to 50 times higher than parent PACs). Consequently, the tadpoles would have had to accumulate much more alkyl PACs to reach comparable BSAF values to those of parent PACs. Since we did not see a significant difference in k_1 uptake rates between parent and alkyl PACs, it could be suggested that the high sediment concentration of alkyl PACs could have led to low BSAFs because of a saturation in uptake of alkyl PACs in these tadpoles. Nonetheless, BSAF values are usually not dependent on the concentration of PACs in sediment (Kraaij et al., 2001).

BSAF are usually more strongly influenced by metabolism and sediment desorption kinetics (Kraaij et al., 2001, Lamoureux and Brownawell, 1999). Therefore, the more likely explanation for greater bioaccumulation potential of parent PACs than alkyl PACs in wood frog tadpoles is their differences in bioavailability and/or metabolism. Our exposure experiments show that wood frog tadpoles start to eliminate and/or metabolize alkylated PACs as soon as 12-

24 hours after initial exposure to PAC-laden sediment, and that elimination/metabolism seems to be greater for alkyl PACs than for parent PACs. Our study was designed to investigate elimination of PACs during the depuration phase but not necessarily quantify the metabolism of these two types of compounds. Nonetheless, Malmquist *et al.* (2015, 2013) studied the transformation potential of several alkyl PACs relative to their unsubstituted compounds and found that the metabolism of alkyl PACs was more efficient than parent PACs in the benthic polychaete worm *Nereis diversicolor*. When hydrophobic organic compounds such as PACs have similar affinities for lipids and organic carbon, BSAFs are assumed to approach a value of 1. As other factors become more important, including elimination and metabolism, BSAF values start to decline and deviate further from this value of 1 (Moermond *et al.*, 2005). Therefore, our lower BSAF values for alkyl PACs could be a result of tadpoles being more efficient at metabolizing these alkylated compounds. However, it is important to note that we did not find significantly greater k_2 elimination rates for alkyl PACs than parent PACs, which could have helped support this postulation. Further investigations in the timing and extent of metabolism of parent and alkyl PACs in tadpoles are warranted.

Moreover, it is well-known that bioavailability of a contaminant can also greatly influence its bioaccumulation potential (Thorsen *et al.*, 2004, Kraaij *et al.*, 2001). Yet, research on the bioavailability of PACs scarcely includes compounds with alkyl substitutions. According to Thorsen *et al.* (2004), the type of PAC entering aquatic systems influences bioavailability in different ways, with source being an important factor on the bioavailability of PACs. By calculating BSAF values for 38 individual PACs in freshwater mussel and marine clam, Thorsen *et al.* (2004) found that petrogenic PACs (mostly alkyl PACs) tended to be more bioavailable than pyrogenic PACs (mostly parent PACs), regardless of organism and location. However, there

were several exceptions to this classification. Accordingly, our contrasting results of parent PACs (pyrogenic PACs) having higher BSAF values rather than alkyl PACs (petrogenic PACs), could be explained by these exceptions. For example, in the case of MacKay River sediment, the alkylated PACs may have been more tightly sorbed to the bitumen-sand particulate phase and not as readily available for equilibrium partitioning as the unsubstituted PACs. This is different than the Thorsen *et al.* (2004) study, where most of the petrogenic PACs were assumed to be associated with neat or dissolved petroleum. Consequently, the higher bioavailability of parent PACs in our exposure experiments could have led to increased bioaccumulation potential of parent PACs in wood frog tadpoles, compared to the more sediment-bound and bitumen-associated alkylated PACs.

Nevertheless, both parent and alkyl PACs generally did not have elevated biota-sediment accumulation factors in our exposure experiments. Only a few compounds, including anthracene, fluoranthene, retene, and C1-benzofluoranthenes/benzopyrenes, had BSAFs above 1, and only C4-naphthalenes seemed to remain in wood frog tadpoles after 48 hours of depuration. Consequently, *Lithobates sylvaticus* tadpoles seem to be efficient at eliminating and metabolizing both parent and alkyl PACs. In fact, Livingstone (1998) found that vertebrates, such as fish, were much more effective than invertebrates at metabolizing PACs due to their higher levels of cytochrome P450. Correspondingly, we would expect PACs to also be readily metabolized by amphibians and wood frog tadpoles (Leppanen, 1999). However, as the first study to our knowledge to assess uptake, elimination, and bioaccumulation of parent and alkyl PACs in anuran larvae, not only were we able to uncover how efficient wood frog tadpoles are at eliminating both parent and alkyl PACs, we also found that elimination of these hydrophobic compounds starts as early as 12 to 24 hours after exposure. Notwithstanding, there is great value

in discovering that certain compounds such as C4-naphthalenes are resistant in wood frog tadpoles, especially when interpreting body residues of amphibian larvae in natural ecosystems.

Interestingly, background levels of PACs in the wood frog tadpoles of this study (at $t=0$) seemed to vary considerably between experiments and were higher than expected. Σ PACs of wood frog tadpoles at $t=0$ h were $290 \pm 75 \text{ ng g}^{-1}$ in the 2013 SedAq (192 h) experiment and $165 \pm 10 \text{ ng g}^{-1}$ in the 2014 Aq (24 h) experiment. Unfortunately, there was a shortage of tadpoles in 2015 due to extreme temperature fluctuations and thus, the background PAC concentration in the wood frog tadpoles of the 2015 SedAq (24 h) experiment remains unknown, but it was likely lower than $170 \pm 25 \text{ ng g}^{-1}$, their Σ PAC concentration at $t=24$ h. There are no data on typical tadpole PAC concentrations in uncontaminated wetlands in the literature to compare with, but we were able to measure PACs in tadpoles from 5 wetlands in the Athabasca oil sands region in 2014 and their average total concentrations ranged from 100 to 195 ng g^{-1} ww (see Chapter 3). The tadpoles in this laboratory study might have had higher background PAC concentrations since they were transferred into aquarium tanks during their fasting period (24 h) prior to each exposure experiment. Their feces, that contained parent and alkyl PACs, could have somewhat contaminated the water in those transfer tanks, and to varying extent between years of study. Nonetheless, PAC concentrations at $t=0$ h were low enough in the wood frog tadpoles to observe clear uptake and accumulation of PACs once they were introduced to their exposure tanks.

2.5. Conclusion

This laboratory study revealed the uptake and elimination kinetics of parent and alkyl PACs in *Lithobates sylvaticus* tadpoles exposed to natural complex mixtures of PACs in sediment from the Athabasca oil sands area. Although there were no significant differences in uptake or elimination rates between parent and alkyl PACs, there was still measurable uptake of both types of compounds, with alkyl PACs reaching particularly high concentrations in the wood frog tadpoles of all three exposure experiments. Indeed, the wood frog tadpoles had clear petrogenic PAC profiles, closely resembling that of the MacKay River sediment to which they were exposed. However, our results suggest that unsubstituted parent PACs have greater bioaccumulation potential than alkylated PACs in wood frog tadpoles. We believe this could be a result of greater bioavailability of the parent PACs, greater metabolism of the alkyl PACs, or alternatively, due to a saturation in uptake of alkyl PACs. Nevertheless, *Lithobates sylvaticus* tadpoles seem to be efficient at metabolizing and eliminating both parent and alkyl PACs. Only a few compounds, including anthracene, fluoranthene, retene, and C1-benzofluoranthenes/benzopyrenes, were found to have higher bioaccumulation potentials, and only C4-naphthalenes seemed resistant to elimination.

Through two accumulation-elimination experiments, this study also determined the dominant route of exposure for wood frog tadpoles. Indeed, when PAC-contaminated sediment is present, the dominant route of exposure is sediment rather than aqueous exposure. This supports the suggestions that dietary uptake may dominate over aqueous uptake for some lipophilic compounds such as PACs in benthic organisms. Evidently, this laboratory study highlights how imperative it is to include sediment exposure as well as alkylated PACs in toxicological studies of benthic and epibenthic organisms.

3.0. Field Study

3.1. Introduction

Maintaining the health of freshwater ecosystems is vital to all aquatic and terrestrial organisms, including humans. In addition to water and food, freshwater ecosystems provide us with an immeasurable amount of ecosystem services (Pelster et al., 2008). As the global population and global demands continue to rise, the protection and conservation of these ecosystems from human impact will become more imperative as well as more difficult. Aquatic organisms face the challenge of trying to avoid the effects from contamination by xenobiotics. Polycyclic aromatic compounds (PACs) are a group of organic compounds of particular concern since they are ubiquitous in the environment and have been shown to be toxic, carcinogenic, and/or mutagenic to many aquatic organisms, including amphibians (Sanz-Lazaro et al., 2008, CCME, 1999, OMOE, 1990, Turcotte, 2008, Yang et al., 2010, Ikenaka et al., 2013, Ball and Truskewycz, 2013, Eisler, 1987).

3.1.1. Sources of Polycyclic Aromatic Compounds (PACs)

PACs include polycyclic aromatic hydrocarbons (PAHs) and dibenzothiophenes (DBTs). Parent and alkyl PACs have various structural configurations consisting of two or more benzene rings fused together; alkyl PACs have additional alkyl substitutions attached to the central aromatic ring structure. PACs are produced primarily from pyrogenic and petrogenic sources (Tsibart and Gennadiev, 2013, Neff, 1979). Pyrogenic PACs are typically high molecular weight (HMW) compounds and/or unsubstituted parent PACs derived from the incomplete combustion of organic matter at high temperatures (e.g., wood, coal, and petroleum) (Lima et al., 2005). Thus, pyrogenic PACs may originate naturally from wildfires and volcanic eruptions, or anthropogenically from vehicle engines, agricultural burning, waste incinerators, industrial

smelters, and other industrial operations that use fossil fuels. Conversely, petrogenic PACs are often low molecular weight (LMW) compounds and/or alkyl substituted PACs formed from the slow breakdown of organic matter at lower temperatures during diagenesis and the formation of petroleum (Baird et al., 2007). Consequently, petrogenic PACs can be released by leaching from industrial tailings ponds, blowing of dust particles from surface mines, inadvertent releases and spills of oil products, as well as from natural sources such as erosion, leaching, and seeps from oil-rich sediment and rocks (Headley et al., 2001)

3.1.2. Athabasca Oil Sands

The Alberta oil sands are the third largest oil reserve in the world, containing about 166 billion barrels of proven oil (Gouvernement of Alberta, 2016, NRCAN, 2013). These oil sands deposits underlie 142 200 km² of boreal forest in three major areas of northern Alberta, Canada (Athabasca, Cold Lake, and Peace River). The Athabasca oil sands region is the largest of these regions, and the only one where deposits are shallow enough (within 75 m of the earth's surface) to be mined in open pits rather than extracted through in-situ methods. This surface mineable region (SRM) corresponds to only 20% of potential oil, but still covers an area of approximately 4 800 km² (NRCAN, 2013). In 2014, Alberta produced approximately 2.3 million barrels per day of crude bitumen, with surface mining accounting for 45% of the production (Gouvernement of Alberta, 2016). From 2015 to 2030, crude oil production from the oil sands is predicted to increase from 2.37 million barrels per day to 3.67 million b/d (CAPP, 2016). Evidently, oil sands operations continue to extract this unconventional oil as global demands keep rising. Correspondingly, there are numerous environmental problems associated with this extraction, including the reduction in quantity and quality of habitats for aquatic and terrestrial organisms, accumulation of waste in large tailings ponds, increase in emission of greenhouse gases, and

contamination from harmful chemical substances, which will all continue to impact the surrounding environment and ecosystems (JOSM, 2016).

In particular, oil sands mining, processing, and upgrading emits and releases large quantities of PACs to the atmosphere and surrounding area. Kelly *et al.* (2009) found substantial loadings of airborne PACs in snow as far as 50 km away from Athabasca oil sands upgraders. Research has also shown that the deposition and flux of PACs in the Athabasca oil sands region have increased through time. Notably, using lake sediment records, Kurek *et al.* (2013) were able to report present-day PAC levels as ~2.5–23 times greater than ~1960 levels (prior to oil sands development), and Manzano *et al.* (2016) estimated a remarkable twofold increase in total snow loadings of PACs between 2008 and 2014.

This increase in PAC contamination, especially of C1-C4 alkylated PACs (Kurek *et al.*, 2013), is inevitably affecting aquatic ecosystems and organisms. For example, aquatic organisms collected near the Athabasca oil sands operations have been shown to accumulate PACs and have adverse effects to these hydrophobic compounds (Hersikorn and Smits, 2011, Wayland *et al.*, 2008, Tolton *et al.*, 2012, Tetreault *et al.*, 2003, Colavecchia *et al.*, 2004). Wetlands are vulnerable to PAC contamination since PACs can enter these shallow freshwater ecosystems through wet and dry deposition, as well as through important snowmelt inputs during spring (Pelster *et al.*, 2008). Due to their hydrophobicity, PACs tend to associate with the organic phases of suspended particles and ultimately accumulate in the sediment at the bottom of wetlands. Consequently, organisms residing in the benthic substrate of wetlands may be at higher risk of exposure to PACs. Benthic and epibenthic invertebrates have been the subject of numerous studies on PAC contamination of freshwater ecosystems (Ganshorn, 2002, Gewurtz *et*

al., 2000, Hellou et al., 2012, Wayland et al., 2008), but few studies have considered amphibians (Gupta, 2009, Hersikorn and Smits, 2011, Garrigues et al., 2004, Marquis et al., 2006).

3.1.3. Bioaccumulation of PACs

Amphibians are a class of vertebrates that generally undergo dramatic metamorphosis from an aquatic larvae to a terrestrial or a semi-aquatic adult. Certain anuran larvae, such as *Lithobates sylvaticus* tadpoles, are epibenthic and can uptake PACs not only from water through dermal absorption and across respiratory surfaces, but also from sediment through dietary absorption since they can ingest sediment while feeding on detritus material (McDiarmid and Altig, 1999). Due to their frequent contact with sediment substrate, lower efficiency in metabolizing PACs in comparison to higher trophic organisms, sufficient biomass for analysis, important ecological roles, and ubiquity in the Athabasca oil sands region, we chose to study the bioaccumulation of parent and alkyl PACs in wood frog tadpoles inhabiting wetlands near Athabasca oil sands mines and upgraders.

Bioaccumulation is the net accumulation of a contaminant from all possible routes of uptake (Boethling and Mackay, 2000). Bioaccumulation factors (BAFs) and biota-sediment accumulation factors (BSAFs) are often used to describe the bioaccumulation potential of contaminants in field aquatic organisms (Thorsen et al., 2004, Moermond et al., 2005, Brunson et al., 1998, Zhang et al., 2015a, Hellou et al., 2012, Lotufo, 1998, Vigano et al., 2007). BAFs account for all routes of uptake in an aquatic organism under field conditions, whereas BSAFs are the lipid and organic carbon normalized ratios of PAC concentrations in aquatic organisms relative to those in the sediment in which they reside (Boethling and Mackay, 2000). Using BSAFs as a screening tool, Wayland *et al.* (2008) was able to compare the bioaccumulation

potential of parent and alkyl PACs in larval and adult insects from different types of wetlands within the Athabasca oil sands region. They found that the BSAFs of alkyl PACs were consistently greater than the theoretical BSAF value of 1, which is when PACs partition equally between organism lipids and sediment organic carbon, and greater than the BSAFs of parent PACs; this suggests important bioaccumulation potential of alkyl PACs. Consequently, it would be of great value to assess whether the bioaccumulation potential of alkyl PACs tends to also be higher in other important wetland organisms, such as amphibian larvae.

3.1.4. Study Objectives

The first objective of this field study is to evaluate whether parent or alkyl PACs are more abundant and have greater bioaccumulation potential in wood frog tadpoles inhabiting wetlands. PACs will be concurrently measured in *Lithobates sylvaticus* tadpoles, sediments, and water from various wetlands in the Athabasca oil sands region. Based on recent evidence (Wayland et al., 2008) and the predominance of alkyl PACs in other media studied in this region, we expect alkyl PACs to be more abundant than parent PACs in wood frog tadpoles, especially if alkyl PACs have greater bioaccumulation potentials than their unsubstituted counterparts.

The second objective of this field study is to determine whether the PACs measured in wood frog tadpoles are more closely related to concentrations found in the sediment or the water of the wetlands. We predict that PAC concentrations in tadpoles will depend more on sediment PAC concentrations if sediment is the primary source of uptake. Notably, food availability in the natural environment is variable and thus wood frog tadpoles may leave the benthic zone to forage. Consequently, PAC concentrations in tadpoles may be more closely related to PAC concentrations in the water.

The third objective of this study is to investigate if there is a relationship between PAC concentrations and distance from oil sands operations, in either of the three media measured (water, sediment, and tadpoles). The atmospheric deposition of PACs has been shown to decrease exponentially from oil sands upgraders (Kelly et al., 2009, Manzano et al., 2016). Furthermore, most PACs preferentially associate with the particulate phase. As a result, we predict that proximity to oil sands upgraders will be positively correlated to PAC concentrations in the sediment and perhaps, to a lesser extent, PAC concentrations in the water and wood frog tadpoles.

The fourth objective of this study is to reveal potential markers of PAC contamination in wood frog tadpoles that could facilitate research and governmental efforts to monitor the effects and extent of petrogenic PAC contamination in wild amphibian populations.

3.2. Methodology

3.2.1. Site Description

Lithobates sylvaticus tadpoles, sediment, and water were collected from 5 wetlands in the Athabasca oil sands region on May 2014: Jenny Pond (N 57°8'8.72" W 111°36'3.60"), Gateway Hill Pond (N 56°59'51.51" W 111°33'43.90"), WF1 Pond (N 57°12'6.88" W 111°38'42.02"), Jetliner Pond (N 56°38'45.97" W 111°06'41.55"), and Pat's Pond (N 57°30'39.24" W 111°23'54.66") (see map in Appendix F). Pat's Pond, Jenny Pond, and WF1 Pond are naturally occurring wetlands, whereas Jetliner Pond is an old borrow pit, and Gateway Hill Pond is an opportunistic wetland formed on reclaimed overburden from oil sands mining. All wetland sites are situated north of Fort McMurray and located inside the oil sands surface mineable region (SMR), except for Jetliner Pond, which is situated south of the SMR. Pat's Pond was selected as a reference wetland because it is one of the furthest sites from oil sands mines and upgraders (28.9 km; see Appendix F). Pat's Pond is also the most northerly wetland; this is important because prevailing winds are from the north-west, making Pat's Pond less affected by airborne emissions from oil sands operations.

3.2.2. Sample Collection

Lithobates sylvaticus (formerly known as *Rana sylvatica*) tadpoles were collected in triplicate (5-10 tadpoles per replicate) in each of the 5 wetlands near Fort McMurray, Alberta. Collections were made near the ponds' edges, in the vicinity of semi-permeable membrane devices (SPMDs) that were deployed a month earlier by Environment and Climate Change Canada. The tadpoles were euthanized on-site using tricaine methanesulfonate (AquaLife TMS, Syndel Laboratories Ltd, B.C., Canada), following procedures approved by the University of Ottawa Animal Care Committee (Protocol BL-2202). Samples were kept on ice until all tadpoles

were identified as *Lithobates sylvaticus* (Wood frog) and then frozen at -20°C. All tadpoles had a minimum length of 25 mm; distinguishing them from *Pseudacris maculata* (Boreal Chorus frog), the other predominant frog species occupying the area. Amphibian collections were completed under the Alberta Environment and Sustainable Resource Development Research Permit 55377 and Collection Licence 55378.

Concurrently with the tadpoles, sediment and water samples were collected from each wetland. Triplicate samples of surface sediment were collected from the top 5 cm of the sediment at the bottom of each pond, in the vicinity of the tadpole collections, using a shovel and centrifuge tubes. Sediment samples were kept at 4°C until they arrived at the University of Ottawa, where they were frozen at -20°C. Triplicate samples of 500 mL of water were also collected in the vicinity of the tadpole and sediment samples, using 500 mL solvent washed plastic bottles. Water samples were stored at 4°C until they were analyzed for within one week of collection for PACs at the University of Ottawa. All samples were kept covered and in the dark to minimize photodegradation.

3.2.3. Analysis of Parent and Alkyl PACs

3.2.3.1. Tadpoles

Wood frog tadpoles were individually dissected before being finely cut with stainless-steel micro-dissecting scissors. Specifically, the entire gastrointestinal tract was removed from each tadpole to ensure that the PAC concentrations measured would only correspond to the PACs accumulated in the tadpole bodies themselves and not from ingested sediment still present in the gastrointestinal tract. Following homogenization, tadpole samples were mixed with Hydromatrix™ (Varian Inc, Palo Alto, CA, USA) to remove excess water, and then spiked with

100 μL of ^{13}C -standard (Cambridge Isotope Laboratories Inc., Andover, MA, USA), a mixture of 16 U.S. EPA Priority PAHs (Figure 1.1). The samples were extracted using accelerated solvent extraction (ASE-350, Dionex Corporation, Sunnyvale, CA, USA) and optimal settings: a 50:50 acetone and hexane solvent mixture, a pressure of 1500 psi, a heating time of 7 min at 150°C , two static cycles of 5 min, and a 50% flush. To remove excess lipids found in biological tissue (NOAA, 1998), an 18 hour 6M potassium hydroxide digest was subsequently performed on the extracts (Martinez et al., 2004). PACs remaining in the co-extracted water were separated by liquid-liquid extraction, through 3 additions of 10 mL of hexane. Samples were then evaporated to 2 mL (solvent exchanged to 2,2,4-trimethylpentane) under a gentle stream of nitrogen (TurboVap[®], Biotage, Charlotte, NC, USA) and then centrifuged (4000 rpm for 10 minutes) for removal of any remaining particulates. Samples were once again evaporated, to 1 mL for fractionation.

Clean-up and fractionation was adapted from U.S. EPA method 3630C for the use of 6 mL (1 g) Supelclean[™] LC solid-phase extraction (SPE) cartridges packed with silica gel (45 μm , $0.8\text{ cm}^3\text{ g}^{-1}$, $475\text{ m}^2\text{ g}^{-1}$). SPEs were preconditioned using acetone, pentane, and 2,2,4-trimethylpentane additions and analytes were eluted with 7 mL of a 2:3 (v/v) mixture of methylene chloride/pentane. Finally, recovered samples were solvent exchanged to 2,2,4-trimethylpentane, evaporated to 1 mL, and spiked with 10 μL p-terphenyl- D_{14} as an internal standard before being quantified by gas chromatography coupled with mass spectrometry. Excluding pesticide-grade pentane, all solvents used in this study were high-grade Optima[®] solvents from Fisher Chemical.

3.2.3.2. Sediment

Analysis of PACs in sediment followed the same procedures as those for tadpole samples. However, prior to homogenization, samples were centrifuged (3000 rpm for 30 mins), decanted, and subsampled for determination of percent dry weight and elemental organic carbon. Before accelerated solvent extraction, activated powdered copper (Laboratory grade, Fisher Chemical) was added to each sample at a copper to sediment weight ratio of 1:2 to eliminate sulfur interferences. Samples were rinsed with pentane prior to elution on the SPE columns (EPA, 1996b).

3.2.3.3. Water

Water samples were filtered and eluted within a week of collection. 500 mL of water were filtered through pre-weighed glass microfiber filters (Whatman™, GF/C, 1.2 µm, Ø 42.5 mm) and then loaded on preconditioned Oasis® HLB 6cc (200 mg) extraction cartridges. Following Oasis® HLB extraction method for PAHs in drinking water (Waters Corporation, 2008), the extraction cartridges were first conditioned with 5 mL each of methylene chloride, methanol, and LC/MS grade water. Then, the water samples and method blanks (LC/MS grade water samples) were spiked with 100 µL of ¹³C-standard (Cambridge Isotope Laboratories Inc., Andover, MA, USA) and loaded on to the extraction cartridges using a vacuum manifold and a flow rate of approximately 15 mL min⁻¹. Analytes were eluted with 8 mL methylene chloride. After the addition of 2 mL of 2,2,4-trimethylpentane, samples were evaporated to 1 mL and subsequently, quantified by gas chromatography coupled with mass spectrometry.

3.2.4. Quantification

All tadpole, sediment, and water samples were analyzed by gas chromatography (Agilent 6890) coupled with mass spectrometry, on the electron impact mode (Agilent 5973, Agilent Technologies, Santa Clara, CA, USA). Injections of 1 μL were made in splitless mode at 280°C on a DB5-MS 30 m x 0.25 μm x 0.25 μm Agilent column. The initial oven temperature of 60°C was held for 2 min and then increased at a rate of 6°C min⁻¹ to a final temperature of 300°C, which was held for 10 min. A constant flow rate of 35 cm sec⁻¹ of helium was used as the carrier gas for a total run time of 52 min. The electron impact mass spectrometer's transfer line temperature was set at 310°C, its source temperature at 230°C, and its quadrupole temperature at 150°C.

All quantified parent and alkyl PACs and their abbreviations are listed in Appendix A. Their method limits of quantification, which were based on a signal to noise ratio of 3:1, are detailed in Appendix B. All samples were blank corrected to remove background PAC contamination, and for quality assurance, reference material was extracted with each set of samples. Reference material consisted of 10 mg of NYNJ-1944 Standard Reference Material[®] (New York-New Jersey waterway sediment, National Institute of Standards and Technology, USA) for sediment samples, and 100 mg of 2974a Organics Standard Reference Material[®] (Freeze-dried mussel tissue (*Mytilus edulis*), National Institute of Standards and Technology, USA) for tadpole samples. Concentrations of PAHs and DBTs were calculated using the isotope dilution method of quantification in order to correct for any variability in the extraction, analytical methods, and lower recovery rates (Mechlinska et al., 2010). Average recovery rates (average $\pm$ SD) were 80 $\pm$ 15% for tadpole samples, 110 $\pm$ 60% for sediment samples, and 50 $\pm$ 10% for water samples.

3.2.5. Percent Organic Carbon and Nitrogen

Percent organic carbon and nitrogen were determined in all sediment samples at the G.G Hatch Stable Isotope Laboratory at the University of Ottawa. After acid desiccation using nitric acid, samples were flash combusted in a Micro Cube elemental analyser (Elementar, Germany) at a temperature of 1800°C. They were carried by helium through reducing/oxidizing columns before being trapped within a single "trap and purge" adsorption column. Detection was accomplished by a thermal conductivity detector, with a routine analytical precision (2 sigma) of +/-0.1%.

3.2.6. Toxicokinetics

The bioaccumulation potential of each parent and alkyl PAC was assessed. For each wetland, biota-sediment accumulation factors (BSAFs) and bioaccumulation factors (BAFs) were calculated (Newman, 2010). BSAFs describe the bioaccumulation of each PAC in the benthic wood frog tadpoles relative to the PAC concentration in the sediment of their respective wetlands (Equation 7):

$$BSAF = \left(\frac{C_b}{C_s} \right) \cdot \left(\frac{TOC}{L} \right) \quad (7)$$

Where C_b is the concentration of PACs in the organism (g PAC/kg organism), C_s is the concentration of PACs in the sediment (g PAC/kg dry weight sediment), TOC is the total organic carbon content in the sediment (g organic carbon/g dry weight sediment), and L is the lipid content in the organism (g lipid/g organism) (Boethling and Mackay, 2000).

On the other hand, BAFs describe the bioaccumulation of each PAC in the wood frog tadpoles from all uptake routes under field condition, in relation to the PAC concentration in the water of their wetland (Equation 8):

$$BAF = \frac{C_b}{C_w} \quad (8)$$

Where C_b is the concentration of PACs in the organism (g PAC/kg organism) and C_w is the concentration of PACs measured in water (ng L⁻¹). BAFs are typically reported on a log scale.

3.2.7. Statistical Analysis

Simple linear regressions, multiple linear regressions, and nonlinear regressions (exponential) were used to analyze the relationships between individual toxicokinetic and environmental parameters. Student or paired t-tests and Mann-Whitney tests were performed on the toxicokinetic parameters to find differences between type of PACs (parent or alkyl) and their number of rings (2-3-rings or 4-6-rings). An alpha value of 0.05 was used in Post Hoc tests. Shapiro-Wilk and Levene's tests were used to verify the normality of distribution and the homogeneity of variance, with a p-value of 0.05. When required, the data were log-transformed prior to statistical analysis to meet parametric assumptions and the Bonferroni correction was performed when multiple comparisons were made. All statistical analyses were done using SigmaPlot 12 software.

3.3. Results

3.3.1. Sediment

Of the 5 wetlands sampled in the Athabasca oil sands region, Gateway Hill Pond had the highest total PAC sediment concentration ($465 \pm 20 \mu\text{g g}^{-1}$, dw TOC). It was more than an order of magnitude greater than all other wetlands, including Jenny Pond, which had the second highest total PAC sediment concentration ($40 \pm 5 \mu\text{g g}^{-1}$, dw TOC). Alkyl PACs accounted for 72-98% of ΣPACs in the sediment of the 5 wetland sites. One-way ANOVA showed significant differences in ΣPACs among sites, as well as significant differences in $\Sigma\text{alkyl PACs}$ ($p < 0.001$) (Figure 3.1). The highest $\Sigma\text{alkyl PAC}$ sediment concentration was found at Gateway Hill Pond ($455 \pm 25 \mu\text{g g}^{-1}$, dw TOC), while the lowest were found at Pat's Pond and Jetliner Pond (3.5 and $2.5 \pm 0.5 \mu\text{g g}^{-1}$ dw TOC, respectively). Pat's Pond and Jetliner Pond's $\Sigma\text{alkyl PAC}$ sediment concentrations were not significantly different from one another ($p > 0.05$). In terms of $\Sigma\text{parent PACs}$ in sediment (Figure 3.1), both Gateway Hill and WF1 wetlands had significantly greater parent PAC concentrations (10 ± 1 and $7 \pm 1 \mu\text{g g}^{-1}$ dw TOC, respectively) than Jenny Pond, Pat's Pond, and Jetliner Pond ($\Sigma\text{parent PACs} < 2 \mu\text{g g}^{-1}$, dw TOC each) ($p < 0.001$).

Figure 3.1. Mean ($\pm$ SE) Σ alkyl PACs ($\mu\text{g g}^{-1}$, dry weight TOC) (solid bars) and mean ($\pm$ SE) Σ parent PACs ($\mu\text{g g}^{-1}$, dry weight TOC) (white bars) in surface sediment samples collected from 5 different wetlands in the Athabasca oil sands region, arranged in increasing distance from an oil sands upgrader. Data sets were analyzed using a one-way ANOVA, where letters indicate significant differences according to Tukey's post-hoc (Σ alkyl PACs: $F_{3,8} = 271.59$, $p < 0.001$; Σ parent PACs: $F_{3,8} = 50.68$, $p < 0.001$). Normality was tested using Shapiro-Wilk (Σ alkyl PACs: $p = 0.83$; Σ parent PACs: $p = 0.82$) and homogeneity of variance using Levene's test (Σ alkyl PACs: $p = 0.32$; Σ parent PACs: $p = 0.56$). Samples in triplicate.

Σ PAC concentrations in wetland sediments show a significant negative exponential relationship with increasing distance (measured from Google Earth using the 5 operating upgraders shown in Appendix F) to the nearest upgrader ($p=0.04$); the relationship remains significant even after excluding Gateway Hill Pond ($p=0.01$), which is a reclaimed site where overburden could be an important source of PACs (Figure 3.2). This indicates that Σ PAC sediment concentrations exponentially decrease with distance from the nearest upgrader. Nevertheless, Σ parent and Σ alkyl PACs of sediment from Jenny Pond, WF1 Pond, Pat's Pond, and Jetliner Pond were not found to have independently any significant exponential relationship with distance from the nearest upgrader (Figure 3.3).

Figure 3.2. Mean ($\pm$ SE) Σ PACs ($\mu\text{g g}^{-1}$, dry weight TOC) in surface sediment samples collected in triplicate from 5 wetlands in the Athabasca oil sands region plotted against distance (km) from the nearest upgrader. Gateway Hill Pond was removed because of its status as an opportunistic wetland on reclaimed overburden (data point circled in graph). Data are reported on a log scale and were analyzed by nonlinear regression in SigmaPlot 12.

Figure 3.3. Mean ($\pm$ SE) Σ parent and Σ alkyl PACs ($\mu\text{g g}^{-1}$, dry weight TOC) in surface sediment samples collected in triplicate from 4 wetlands in the Athabasca oil sands region plotted against distance (km) from nearest upgrader. Gateway Hill Pond was removed because of its status as an opportunistic wetland on reclaimed overburden. Data were analyzed by nonlinear regression in SigmaPlot 12 and were not found to be significant. Data are reported on a log scale.

Using mean concentrations of individual and grouped PACs, sediment PAC profiles were generated for each of the 5 studied wetlands (Figure 3.4). The PAC profiles show the predominance of alkyl PACs compared to their parent compounds in the sediment of most wetlands studied. In fact, as stated before, these alkylated compounds accounted for 72-98% of PACs detected in sediment from Gateway Hill (98%), Jenny Pond (96%), WF1 (72%), Pat's Pond (76%), and Jetliner Pond (76%). The following 2 alkylated homologue groups accounted for the majority of the PACs detected in the sediment samples (average of all 5 wetlands $\pm$ SE): alkyl phenanthrenes/anthracenes ($28 \pm 10\%$) and alkyl dibenzothiophenes ($23 \pm 9\%$). The unsubstituted PACs with the highest concentrations in Jenny Pond, WF1 Pond, and Pat's Pond were naphthalene and phenanthrene, whereas benzo[a]pyrene and phenanthrene were the most

concentrated in Gateway Hill sediment and benzo[k]fluoranthene and diben[a,h]anthracene in Jetliner Pond sediment.

According to Figure 3.4, sediment from Gateway Hill Pond had a characteristic petrogenic profile, similar to bitumen, as well as the MacKay River sediment previously used in the laboratory study (Figure 2.1). Indeed, the higher concentrations of alkylated compounds, especially those with 2 or 3 alkyl substitutions (C2- and C3-) indicate a petrogenic source of PACs and a profile typical of oil sands sediment (Yang et al., 2011, Headley et al., 2002). The sediment from the other 4 wetlands do not have such a clear petrogenic profile, and also have lower concentrations of PACs (Figure 3.4). Nonetheless, high concentrations of C3-phenanthrenes/anthracenes were found in Jenny Pond sediment ($25 \pm 3 \mu\text{g g}^{-1}$, dw TOC), and relatively high concentrations of naphthalene ($4 \pm 0.4 \mu\text{g g}^{-1}$, dw TOC), phenanthrene ($1.5 \pm 0.9 \mu\text{g g}^{-1}$, dw TOC), and C2-fluorenes ($6 \pm 0.3 \mu\text{g g}^{-1}$, dw TOC) were found in WF1 Pond sediment.

Figure 3.4. Mean (±SE) PAC concentration profiles (µg g⁻¹, dry weight TOC) of sediment samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region. Wetlands are arranged from nearest to furthest from an oil sands upgrader. Abbreviations are found in Appendix A.

3.3.2. Water

Σ PAC concentrations in water samples collected in May 2014 ranged from 45 to 1765 ng L⁻¹. WF1 Pond had the highest Σ PAC water concentration (1765 ± 670 ng L⁻¹), whereas Jetliner Pond had the lowest (45 ng L⁻¹) (Figure 3.5). Water from Jetliner was analyzed by Environment and Climate Change Canada. Although Gateway Hill had the highest Σ alkyl sediment PAC concentration, it did not have the highest Σ alkyl water PAC concentration. The highest concentration of Σ alkyl PACs was measured in water from WF1 Pond (1735 ± 665 ng L⁻¹). Moreover, neither total, parent, nor alkyl PAC concentrations in water showed any significant exponential or linear relationship with distance to the nearest active upgrader ($p > 0.1$). Other characteristics such as pH, conductivity, and water hardness of the 5 wetlands can be found in Appendix G.

Figure 3.5. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng L⁻¹) in water samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region in 2014. Wetlands are arranged from nearest to furthest from an oil sands upgrader.

3.3.3. Tadpoles

Wood frog tadpoles collected in the Athabasca oil sands were of Gosner stage 29-38, were similar in size, and were collected in triplicate whenever possible (with 5-10 tadpoles per sample). Wood frog tadpoles from Gateway Hill and WF1 wetlands had higher Σ PAC concentrations (195 ± 20 and 145 ± 75 ng g⁻¹ ww, respectively) than those from Jenny Pond (110 ± 5 ng g⁻¹ ww), Pat's Pond (100 ± 2 ng g⁻¹ ww), and Jetliner Pond (100 ± 6 ng g⁻¹ ww), but this difference was not significant. Notably, Σ PAC concentrations in tadpoles varied less between sites than for sediment and water. Gateway Hill tadpoles had the highest Σ alkyl PAC concentrations but one of the lowest Σ parent PAC concentrations (Figure 3.6). Conversely, Jenny Pond had the lowest Σ alkyl PAC concentrations, but the highest Σ parent PAC concentrations. Wood frog tadpoles from Pat's Pond had the lowest Σ parent PAC concentrations.

Figure 3.6. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g⁻¹, wet weight) in wood frog tadpoles collected in triplicate from 5 different wetlands in the Athabasca oil sands region. Wetlands are arranged from nearest to furthest from an oil sands upgrader.

Although Σ alkyl PAC concentrations in wood frog tadpoles tend to decrease with distance from the nearest upgrader (Figure 3.7), both Σ alkyl and Σ parent PAC concentrations in the collected tadpoles do not show any significant linear or exponential relationship with distance from upgrader. In addition, removing the Gateway Hill tadpole concentrations (as previously done for sediment and water) further weakens the relationships between Σ alkyl and Σ parent PACs and distance.

Figure 3.7. Mean ($\pm$ SE) Σ alkyl and Σ parent PACs (ng g⁻¹, wet weight) in wood frog tadpole samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region plotted against distance (km) from nearest upgrader.

In order to compare concentrations of PACs in the wood frog tadpoles to those from the sediment and/or water collected from the same wetlands, data of individual compounds and groups of compounds were log-transformed and analyzed by linear regression in SigmaPlot 12. Tadpole PAC concentrations from all 5 wetlands showed significant positive correlations with both their associated sediment and water PAC concentrations (Figure A.1 in Appendix H).

Regressions for WF1 Pond are shown in Figure 3.8 as examples of the correlations found between sediment, water, and tadpoles of each wetland studied. Interestingly, the linear regressions of PAC concentrations in sediment and wood frog tadpoles of Gateway Hill, WF1 Pond, and Pat's Pond explained more of the variance than those with water PAC concentrations (Figure A.1; a.i, a.ii, c.i, c.ii, d.i, d.ii). Conversely, the linear regressions of Jenny Pond and Jetliner Pond tadpoles with water PAC concentrations seem to be more predictive of tadpole concentrations than those with sediment PAC concentrations (Figure A.1; b.i, b.ii, e.i, e.ii). The relative importance of sediment and water exposure for each wetland was confirmed by multiple linear regression analysis (Table A.8 in Appendix H).

Figure 3.8. Log concentrations of PACs in wood frog tadpoles in comparison to their concentrations in sediment (i.) and water (ii.). Tadpole, sediment, and water concentrations are the log-transformed average concentrations of triplicate samples collected from the WF1 Pond. All linear regressions are significant ($p < 0.05$).

Linear regressions were also performed between concentrations in wood frog tadpoles and sediment for each individual PAC, with the intention of uncovering potential markers of PAC contamination in natural wetlands. The linear regression graphs and statistical details of each compound can be found in Appendix I (Figure A.2 and Table A.9). Most PACs in the field-collected tadpoles had positive correlations with their concentrations in the wetland sediment,

except for 3,6-dimethylphenanthrene and C1-phenanthrenes/anthracenes. The compounds with the highest R^2 values were: C3-fluorenes (0.96), C1-benzo[a]anthracenes/chrysenes (0.87), C2-dibenzothiophenes (0.80), and C4-fluoranthenes/pyrenes (0.69). The linear regressions of C3-fluorenes ($p < 0.01$) and C2-dibenzothiophenes ($p < 0.05$) were significant (Figure 3.9). However, both relationships did not pass the Bonferonni correction (critical p -value = 0.002). Linear regressions were also performed against PAC concentrations in water, but none were found to be significant.

Figure 3.9. Log concentration of C2-dibenzothiophenes and C3-fluorenes in wood frog tadpoles in comparison to their concentration in sediment. Each point refers to the tadpole and sediment average concentration in one of the 5 wetlands studied in the Athabasca oil sands region. Linear regressions were performed using SigmaPlot 12.

Field-derived BAFs and BSAFs were calculated for each of the 5 wetlands studied for the purpose of assessing the bioaccumulation behaviour of PACs (Table A.10 in Appendix J). One-way ANOVA found a significant effect of site on BSAF ($F_{4, 186} = 25.52$, $p < 0.001$) and BAF ($F_{4, 187} = 11.10$, $p < 0.001$). Mean BSAFs were significantly different between wetlands except for WF1 and Jetliner Pond. Gateway Hill Pond had the lowest mean BSAF, whereas Jetliner Pond had the

highest (Figure 3.10). Jetliner Pond also had the highest mean BAF, while Jenny Pond had the lowest (Figure 3.11).

Figure 3.10. Mean ($\pm$ SE) BSAFs of PACs in *Lithobates sylvaticus* tadpoles from 5 wetlands in the Athabasca oil sands region. BSAFs from Gateway Hill (n=43), Jenny Pond (n=42), WF1 Pond (n=40), Pat's Pond (n=33), and Jetliner Pond (n=33) were log-transformed and then analyzed by one-way ANOVA in SigmaPlot 12. One-way ANOVA showed a significant effect of site on BSAFs ($F_{4, 186}=25.52$, $p<0.001$). Different letters indicate significant differences according to Tukey's post-hoc. Means were back-transformed. Normality was tested using Shapiro-Wilk ($p=0.23$) and Levene's test for homogeneity of variance ($p=0.17$).

Figure 3.11. Mean ($\pm$ SE) log bioaccumulation factors (BAFs) of PACs in *Lithobates sylvaticus* tadpoles from 5 wetlands in the Athabasca oil sands region. BAFs from Gateway Hill (n=41), Jenny Pond (n=41), WF1 Pond (n=37), Pat's Pond (n=37), and Jetliner Pond (n=36) were analyzed by one-way ANOVA in SigmaPlot 12. One-way ANOVA showed a significant effect of site on BAFs ($F_{4, 187}=11.10$, $p<0.001$). Different letters indicate significant differences according to Tukey's post-hoc (means were back-transformed). Normality was tested using Shapiro-Wilk ($p=0.23$) and Levene's test for homogeneity of variance ($p=0.05$).

Statistical analyses showed BSAFs of alkyl PACs were generally higher than parent PACs, but this difference was only significant in tadpoles from Pat's Pond (Table A.11 in Appendix J). Moreover, BSAFs were found to be significantly higher ($p<0.05$) in 2-3 ring PACs than 4-6 ring PACs in all wetlands except Gateway Hill Pond (Table A.12 in Appendix J). PACs with 2-3 rings also had higher BAF means than those with 4-6 rings in all the wetlands, although this difference was not significant. Only one significant difference in BAFs related to type of PAC was found; notably, parent PACs had significantly higher BAFs than alkyl PACs in wood frog tadpoles from Jetliner Pond ($p=0.04$).

The tadpole PAC profiles in Figure 3.12 demonstrate that the PAC compounds with 2-3 rings, notably naphthalenes, fluorenes, phenanthrenes, anthracenes, and dibenzothiophenes, are considerably more present and abundant in the field-collected wood frog tadpoles than those with 4-6 rings. Correspondingly, independent student t-tests showed significantly higher mean

concentrations of 2-3 ring PACs than 4-6 ring PACs at all sites ($p < 0.001$) except for Jetliner Pond. Nonetheless, high concentrations of alkylated benzo[fluoranthenes/benzopyrenes and C4-benzo[a]anthracenes/chrysenes (4-6 ring PACs) were found in most tadpole samples.

The tadpole PAC profiles were also dominated by C1-C4 alkyl PACs (Figure 3.12). These alkylated compounds accounted for 91-97% of all PACs detected in field-collected wood frog tadpoles. Overall, C3- and C4- alkyl PACs were twice as abundant in body tissues as their C1- and C2- homologues. The following four alkylated homologue groups accounted for the majority of the PACs detected in wood frog tadpoles: fluorenes (23-43%), naphthalenes (14-34%), dibenzothiophenes (16-25%), and phenanthrenes/anthracenes (9-21%). In descending order of accumulation, the three most frequently detected unsubstituted PACs at all sampling sites were: naphthalene, anthracene, and phenanthrene.

The PAC profiles of the wood frog tadpoles are similar between the 5 wetlands in the Athabasca oil sands region (Figure 3.12). Nonetheless, Gateway Hill and WF1 Pond tadpoles had higher concentrations of alkylated dibenzothiophenes; approximately 30% higher than all other wetlands. Also, tadpoles collected from WF1 Pond had the highest concentrations of C4-benzo[a]anthracenes/chrysenes and C1-C2 benzo[fluoranthenes/benzopyrenes.

Figure 3.12. Mean ($\pm$ SE) PAC concentration profiles ($\mu\text{g g}^{-1}$, wet weight) of wood frog tadpole samples collected in triplicate from 5 different wetlands in the Athabasca oil sands region. Wetlands are arranged from nearest to furthest from an oil sands upgrader. Abbreviations are found in Appendix A.

Wood frog tadpoles collected in the Athabasca oil sands region had very similar PAC profiles to the wood frog tadpoles that were exposed to MacKay River sediment (see Chapter 2) (Figure 3.13 and 3.14). This is evidenced by a significant positive correlation between PAC concentration in field and laboratory tadpoles (Field = $-0.39 + (0.60 * \text{Lab})$, $R^2=0.45$, $p<0.001$) (Figure 3.13).

Figure 3.13. Linear regression between log-transformed PAC concentration profiles of field and laboratory wood frog tadpoles. Field tadpoles were collected from 5 wetlands in the Athabasca oil sands region in May of 2014 ($n=5$). Laboratory tadpoles correspond to those exposed to MacKay River sediment for 24 hours during the sediment + aqueous (192 h) exposure experiment conducted in 2013 ($n=3$). Data were log-transformed and analyzed in SigmaPlot 12. The linear regression was found to be significant ($p<0.001$). Data were normal ($p=0.83$, Shapiro-Wilk) and had constant variance ($p=0.88$).

PACs tend to increase in concentration with increasing degree of alkylation in both field and laboratory tadpoles (Figure 3.14). However, in laboratory-exposed tadpoles, total concentrations of alkylated dibenzothiophenes ($325 \pm 65 \text{ ng g}^{-1} \text{ ww}$) and alkylated fluoranthenes/pyrenes ($60 \pm 15 \text{ ng g}^{-1} \text{ ww}$) were much higher than in field-collected tadpoles (30 ± 5 and $3 \pm 0.5 \text{ ng g}^{-1} \text{ ww}$, respectively). Conversely, alkylated fluorenes were more dominant in

field-collected tadpoles, accounting for $30 \pm 5\%$ of Σ PACs compared to $15 \pm 5\%$ in laboratory-exposed tadpoles (Figure 3.14).

Figure 3.14. Mean ($\pm$ SE) PAC concentration profile (ng g⁻¹, wet weight) of field wood frog tadpole collected from 5 wetlands in the Athabasca oil sands region in May of 2014 (n=5) and mean ($\pm$ SE) PAC concentration profile of laboratory tadpoles (Figure 2.4) after 24 hours of exposure to MacKay River sediment during the sediment + aqueous (192 h) exposure experiment conducted in 2013 (n=3).

3.4. Discussion

By concurrently measuring PACs in *Lithobates sylvaticus* tadpoles, sediment, and water of 5 wetlands in the Athabasca oil sands region, this field study evaluated whether parent or alkyl PACs were more abundant in tadpoles from these wetlands, and whether these PACs were more closely related to concentrations in the sediment or the water. Comparable to our laboratory results from Chapter 2, concentrations of alkyl PACs were much higher than those of parent PACs in all three media analyzed. In fact, alkyl PACs accounted for $94 \pm 4\%$ of Σ PACs concentrations in wood frog tadpoles (mean $\pm$ SD), $85 \pm 12\%$ of Σ PACs in sediment samples and $90 \pm 7\%$ of Σ PACs in water from the 5 wetlands. In addition, the tadpoles collected from the 5 wetlands seem to follow petrogenic PAC profiles; 2 and 3 ring PACs were the most abundant and the concentration of PACs increased with the degree of alkylation. The BAFs and BSAFs measured in this study corroborate the tadpole PAC profiles, suggesting a predominant petrogenic source of PACs. Indeed, the BAFs and BSAFs of 2-3 ring PACs were generally greater than those with 4-6 rings, although only significantly for the BSAFs. Therefore, wood frog tadpoles from these Northeastern Alberta wetlands seem to preferentially accumulate 2-3 ring PACs, especially in comparison to their concentrations in the sediment (BSAFs), and potentially also preferentially accumulating alkyl PACs.

Since wood frog tadpoles are often benthic, feeding on various detritus materials and preferring shallower waters, it was of great interest to also assess whether PACs in the tadpole bodies are more related to those from the sediment or those from the water, and whether there would be a clear main exposure pathway in the 5 wetlands studied. Tadpole PAC concentrations from all 5 wetlands studied showed significant positive relationships with both sediment and water PAC concentrations, but no clear dominant exposure pathway or trend among the wetlands

was found. This is possibly due to the low number of sites in this field study; unfortunately, tadpoles were only found in sufficient numbers in 5 of the 9 wetlands surveyed. Nonetheless, linear regression models with sediment PAC concentrations accounted for a greater amount of variability in Gateway Hill and WF1 Pond wood frog tadpole PAC concentrations than models with water PAC concentrations, while linear regressions with water PAC concentrations were more predictive of tadpole concentrations for Jenny Pond and Jetliner Pond.

This field study was able to reveal two potential markers of PAC exposure in *Lithobates sylvaticus* tadpoles in natural wetlands: C3-fluorenes and C2-dibenzothiophenes. In fact, positive correlations between tadpole and sediment concentrations were found for C3-fluorenes ($Tads = 0.387 + (0.319 * Seds)$, $R^2 = 0.96$, $p < 0.01$) and C2-dibenzothiophenes ($Tads = 0.0410 + (0.187 * Seds)$, $R^2 = 0.80$, $p < 0.05$). However, after correcting for multiple comparisons, using the Bonferonni correction, both relationships were found to not be significant. These two compounds are alkylated PACs that have a log K_{ow} of 5.50 and have k_2 elimination rates slightly above average, 0.10 h^{-1} for C3-FLU and 0.11 h^{-1} for C2-DBT, as shown in Chapter 2. Although elimination rates of these potential markers are not the lowest of the studied PACs, which would have suggested persistence and helped explain their correlation with sediment concentrations, they did have relatively high k_1 uptake rates (4.89 and $2.87 \text{ L g}^{-1} \text{ h}^{-1}$, respectively). In fact, both k_1 uptake rates are above the average $2.62 \text{ L g}^{-1} \text{ h}^{-1}$, and the k_1 of C3-fluorenes is close to the maximum uptake rate measured in the laboratory study ($5.72 \text{ L g}^{-1} \text{ h}^{-1}$). Correspondingly, the uptake rates of these two compounds are sufficient enough to ensure their accumulation by wood frog tadpoles from most sediments; this could help explain their potential as good markers for PAC contamination. Notably, an increase in the number and range of wetlands studied is warranted to confirm the relationship between C3-fluorenes and C2-dibenzothiophenes

concentrations in tadpoles and sediment. Nonetheless, this is the first known study of its kind and will be of value for future wood frog tadpole studies, as well as monitoring programs assessing PAC contamination in areas with petrogenic sources of PACs.

Our field study was also able to preliminarily determine whether there is a distance gradient of PAC contamination in relation to oil sand upgraders in amphibian larvae, sediment, and/or water. Only Gateway Hill Pond had a clear petrogenic sediment PAC profile, most likely due to its history as an opportunistic wetland formed over reclaimed overburden from oil sands mining; this overburden would have been rich in bitumen and related petrogenic PACs. Although there was a significant exponential relationship between Σ PAC sediment concentrations and distance to nearest upgrader, individually, Σ parent and Σ alkyl PACs of sediment from Jenny Pond, WF1 Pond, Pat's Pond and Jetliner Pond did not show any significant relationship with distance from the nearest upgrader. Moreover, neither total, parent, nor alkyl PAC concentrations in water showed significant negative relationships with distance to the nearest active upgrader. Σ PAC concentrations were highest in the sediment and water from Gateway Hill and WF1 Pond, however the trend was not significant nor was it pronounced for the Σ PAC concentrations in the wood frog tadpoles. Therefore, under this preliminary field study, emissions from oil sands mining and upgrading were not a clear source of PACs to the water and tadpoles of these 5 surrounding wetlands. Correspondingly, PAC concentrations in the water of our 5 wetlands did not exceed Canadian freshwater quality guidelines (CCME, 2010), except for fluoranthene in the water of Gateway Hill Pond (Table A.13 in Appendix K). On the other hand, Canadian interim sediment quality guidelines (ISQGs) were exceeded by 3 of the 13 PACs currently included in the CCME guidelines (CCME, 2010) in the Gateway Hill Pond (i.e. acenaphthene, phenanthrene, and benzo[a]pyrene) (Table A.14 in Appendix K). Consequently, despite the relatively low PAC

levels in the tadpoles and water collected in the Athabasca oil sands region, there is still concern for the health of benthic organisms such as wood frog tadpoles in these wetlands. Especially considering the fact that current CCME guidelines only include 13 of the 52 analytes we measured.

3.5. Conclusion

This field study demonstrated that alkyl PACs were much more abundant than parent PACs in the sediment, water, and wood frog tadpoles of each of the 5 wetlands studied in the Athabasca oil sands region. In addition, BSAFs of 2-3 rings PACs were significantly greater than those with 4-6 rings. Accordingly, all wood frog tadpole samples seem to follow petrogenic PAC profiles. Moreover, this field study discerned C3-fluorenes and C2-dibenzothiophenes as potential markers of amphibian larvae exposure to PAC-contaminated sediment.

Although this field study found no clear dominant exposure pathway among all the wetlands studied, PAC concentrations in wood frog tadpoles from each of the 5 wetlands showed significant correlations with both sediment and water PAC concentrations. Understanding how complex mixtures of PACs are taken up by amphibian larvae in natural wetlands provides valuable information on the bioaccumulation of PACs and the interpretation of body burdens of field-collected tadpoles. PAC concentrations in water and tadpoles of the 5 wetlands were not significantly correlated with their distance from oil sands upgrading facilities. However, Σ PAC concentrations in sediment did exponentially decrease with distance from the nearest oil sands upgrader. Additionally, the concentration of a few PACs in the wetland sediment exceeded Canadian ISQGs for freshwater sediments. Evidently, this field study shows promise for additional field collections in the Athabasca oil sands in order to contribute to greater knowledge on the uptake, bioaccumulation, and potential as markers of parent and alkyl PACs in benthic organisms.

4.0. General Conclusion

Although many studies have examined the toxicokinetics and bioaccumulation of PACs in aquatic organisms, this thesis is, to our knowledge, the first to evaluate the accumulation and elimination of both parent and alkyl PACs in amphibian larvae. The results of this study are the largest, most comprehensive set of toxicokinetic and bioaccumulation information of PACs (52 analytes) in the amphibian larvae *Lithobates sylvaticus* obtained to date. As predicted, both the laboratory and field studies showed that concentrations of alkyl PACs were much higher than parent PACs in wood frog tadpoles. Accordingly, tadpoles exposed to PAC-contaminated sediment in the laboratory as well as those collected from 5 wetlands in the Athabasca oil sands region had clear petrogenic PAC profiles. However, in relation to the PAC concentrations measured in the sediment, parent PACs seem to have greater bioaccumulation potentials than alkyl PACs in the laboratory-exposed wood frog tadpoles. The higher BSAF values for parent PACs could be attributed to higher bioavailability or lower metabolism in these unsubstituted compounds, or alternatively, to a saturation of uptake of alkyl PACs.

In field-collected wood frog tadpoles, parent and alkyl PACs had similar bioaccumulation potentials; however, BSAFs of the 2-3 ring PACs (often associated with petrogenic sources) were significantly higher than 4-6 ring PACs in the tadpoles from the Athabasca oil sands region. Nevertheless, *Lithobates sylvaticus* tadpoles seem to be efficient at eliminating and metabolizing both parent and alkyl PACs. Only a few compounds, including anthracene, fluoranthene, retene, and C1-benzofluoranthenes/benzopyrenes, were found to have higher bioaccumulation potentials, and other compounds, such as C4-naphthalenes, were not as efficiently eliminated.

This study is one of the few that targets complex mixtures of contaminants rather than individual compounds. In addition, this study uniquely evaluated the exposure of PACs in amphibian larvae from both water and sediment. Through two accumulation-elimination experiments (one evaluating exposure to contaminated sediment and water, and the other to contaminated water alone), this study was able to determine the dominant route of exposure for wood frog tadpoles that are in contact with sediment. PAC concentrations and uptake rates in wood frog tadpoles were highest when tadpoles were exposed to PAC-contaminated sediment. Consequently, we determined that the dominant route of exposure of wood frog tadpoles to PACs is sediment rather than aqueous. Similar results have been observed in other studies where dietary uptake was more important than aqueous uptake for select PACs in benthic organisms.

Despite not finding a clear dominant exposure pathway for the wood frog tadpoles collected in the Athabasca oil sands region, the field study showed that PAC concentrations in wood frog tadpoles could be predicted by the concentrations of PACs in both the water and the sediment of each of the 5 wetlands. The significant relationships between PAC concentrations in tadpoles and environmental parameters are important in understanding how complex mixtures of PACs are taken up by amphibian larvae in natural wetlands. These relationships also provide valuable information on the bioaccumulation of PACs and the interpretation of PAC residues in field-collected tadpoles.

This field study will also help with the interpretation of tadpole PAC residues by identifying possible markers of exposure. Indeed, C3-fluorenes and C2-dibenzothiophenes were isolated as potential markers of amphibian larvae exposure to PAC-contaminated sediment because of their significant relationship with the concentrations in wetland sediment. The

toxicokinetics obtained in the laboratory study only help to explain part of this relationship; consequently, additional field collections in the Athabasca oil sands are needed to confirm the potential for using C3-fluorenes and C2-dibenzothiophenes as indicators of exposure to PAC-contaminated sediment in future monitoring programs and amphibian studies.

Similar to other studies in the Athabasca oil sands region (Kelly et al., 2009, Manzano et al., 2016), our field study showed that Σ PAC concentrations in wetland sediments decreased exponentially with distance from oil sands operations. This supports the statement that oil sands mining and upgrading facilities are important sources of PAC contamination to surrounding snow and freshwater ecosystems in the Athabasca oil sands region (Kelly et al., 2009, Kurek et al., 2013, Manzano et al., 2016). Accordingly, Canadian ISQGs were exceeded by 3 of the 13 PACs in the sediment of Gateway Hill Pond (acenaphthene, phenanthrene, and benzo[a]pyrene). On the other hand, PAC concentrations in collected water and tadpoles did not show significant relationships with distance from oil sands operations. Even though most PAC concentrations in the water of our 5 wetlands did not exceed Canadian freshwater guidelines, fluoranthene concentrations in the water of Gateway Hill Pond did exceed guidelines. Therefore, although there was little evidence of significant contamination from the upgrading facilities to the water of the 5 wetlands studied, some PACs in wetland sediment and water are reaching quality guidelines for the protection of freshwater life. Notably, these guidelines do not include alkyl PACs; potentially underestimating the impact of these compounds.

Despite lower PAC levels in the wood frog tadpoles collected in the Athabasca oil sands region, our laboratory and field studies showed that sediment can be an important source of PAC exposure to amphibian larvae and thus there is concern for the health of benthic organisms in these wetlands, especially from Gateway Hill Pond. Extending field collections to: other

wetlands, longer-term monitoring programs, and effect-level studies is warranted. Evidently, this thesis highlights how imperative it is to include sediment exposure, as well as alkylated PACs in toxicological studies and field studies of benthic and epibenthic organisms.

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Appendix A. List of All Analyzed Parent and Alkyl PACs

Table A.1. List of all PACs analyzed using GC-MS, their abbreviations, number of rings, and octanol/water coefficients (Log K_{ow}) (Irwin and Service, 1997).

Type of compound		Compound	Symbol	Log K _{ow}	# Rings
Parent PACs	Low molecular weight (LMW)	Naphthalene	NAPH	3.37	2-3 rings
		Acenaphthylene	ACY	4.07	2-3 rings
		Acenaphthene	ACE	3.92	2-3 rings
		Fluorene	FLU	4.18	2-3 rings
		Phenanthrene	PHE	4.57	2-3 rings
		Anthracene	ANT	4.54	2-3 rings
	High molecular weight (HMW)	Fluoranthene	FLA	5.22	4-6 rings
		Pyrene	PYR	5.18	4-6 rings
		Benz[a]anthracene	BaA	5.91	4-6 rings
		Chrysene	CRY	5.86	4-6 rings
		Benzo[b]fluoranthene	BbF	5.80	4-6 rings
		Benzo[k]fluoranthene	BkF	6.00	4-6 rings
		Benzo[a]pyrene	BaP	6.04	4-6 rings
		Indeno[1,2,3-cd]pyrene	IDP	7.00	4-6 rings
		Dibenz[a,h]anthracene	DBA	6.75	4-6 rings
		Benzo[g,h,i]perylene	BgP	6.50	4-6 rings
		Dibenzothiophene	DBT	4.49	2-3 rings
Alkyl PACs	Individual PACs	2-Methylnaphthalene	2-MN		2-3 rings
		1-Methylnaphthalene	1-MN		2-3 rings
		3-Methylphenanthrene	3-MP		2-3 rings
		2-Methylphenanthrene	2-MP		2-3 rings
		9/4-Methylphenanthrene	9/4-MP		2-3 rings
		1-Methylphenanthrene	1-MP		2-3 rings
		3,6-Dimethylphenanthrene	3,6-DMP		2-3 rings
		2,6-Dimethylphenanthrene	2,6-DMP		2-3 rings
		1,7-Dimethylphenanthrene	1,7-DMP		2-3 rings
		1,8-Dimethylphenanthrene	1,8-DMP		2-3 rings
		Retene	RET		2-3 rings

Type of compound		Compound	Symbol	Log K _{ow}	# Rings
Alkyl PACs	Grouped PACs	C2-Naphthalenes	C2-NAPH	4.37	2-3 rings
		C3-Naphthalenes	C3-NAPH	5.00	2-3 rings
		C4-Naphthalenes	C4-NAPH	5.55	2-3 rings
		C1-Fluorenes	C1-FLU	4.97	2-3 rings
		C2-Fluorenes	C2-FLU	5.20	2-3 rings
		C3-Fluorenes	C3-FLU	5.50	2-3 rings
		C1-Phenanthrenes/Anthracenes	C1-PHE/ANT	5.14	2-3 rings
		C2-Phenanthrenes/Anthracenes	C2-PHE/ANT	5.51	2-3 rings
		C3-Phenanthrenes/Anthracenes	C3-PHE/ANT	6.00	2-3 rings
		C4-Phenanthrenes/Anthracenes	C4-PHE/ANT	6.51	2-3 rings
		C1-Dibenzothiophenes	C1-DBT	4.86	2-3 rings
		C2-Dibenzothiophenes	C2-DBT	5.50	2-3 rings
		C3-Dibenzothiophenes	C3-DBT	5.73	2-3 rings
		C4-Dibenzothiophenes	C4-DBT		2-3 rings
		C1-Fluoranthenes/Pyrenes	C1-FLA/PYR	5.72	4-6 rings
		C2-Fluoranthenes/Pyrenes	C2-FLA/PYR		4-6 rings
		C3-Fluoranthenes/Pyrenes	C3-FLA/PYR		4-6 rings
		C4-Fluoranthenes/Pyrenes	C4-FLA/PYR		4-6 rings
		C1-Benz[a]anthracenes/Chrysenes	C1-BaA/CRY	6.42	4-6 rings
		C2-Benz[a]anthracenes/Chrysenes	C2-BaA/CRY	6.88	4-6 rings
		C3-Benz[a]anthracenes/Chrysenes	C3-BaA/CRY	7.44	4-6 rings
		C4-Benz[a]anthracenes/Chrysenes	C4-BaA/CRY	8.00	4-6 rings
		C1-Benzofluoranthenes/Benzopyrenes	C1-BFLA/BPYR		4-6 rings
		C2-Benzofluoranthenes/Benzopyrenes	C2-BFLA/BPYR		4-6 rings

Appendix B. Method Detection Limits of PACs.

Table A.2. Method detection limits of PACs; calculated according to Loconto (2006), with instrument detection limits from 7 replicate blanks and a signal to noise ratio of 3:1 for each matrix studied (tadpoles, sediment, and water).

Compound	Tadpoles MDL (ng g⁻¹)	Sediment MDL (ng g⁻¹)	Water MDL (ng L⁻¹)
Naphthalene	3.09	0.527	1.15
Acenaphthylene	n.d.	n.d.	0.08
Acenaphthene	0.24	0.053	0.32
Fluorene	0.27	0.050	0.45
Phenanthrene	0.24	0.033	0.25
Anthracene	0.22	0.020	n.d.
Fluoranthene	0.97	0.016	0.31
Pyrene	0.30	0.010	0.26
Benz[a]anthracene	0.57	0.015	0.28
Chrysene	0.23	0.015	0.26
Benzo[b]fluoranthene	0.22	0.008	0.49
Benzo[k]fluoranthene	0.10	0.003	0.40
Benzo[a]pyrene	0.93	0.047	n.d.
Indeno[1,2,3-cd]pyrene	0.14	0.006	n.d.
Dibenz[a,h]anthracene	0.19	0.009	n.d.
Benzo[g,h,i]perylene	0.09	0.015	n.d.

^{n.d.} Compounds not detected in all 7 blanks, therefore not able to calculate a method detection limit.

Appendix C. PAC Concentrations in MacKay River Sediment

Table A.3. Mean and standard error of PAC concentrations (ng g^{-1} dw and ng g^{-1} dw TOC) in the MacKay River sediment used in all 3 exposure experiments ($n=13$). TOC normalized concentrations were calculated based on individual TOC values (mean = $0.015 \text{ g dw sediment/g TOC}$).

Compound	Mean PAC concentration in sediment		Mean PAC concentration in sediment	
	(ng g^{-1} , dw)	SE	(ng g^{-1} , dw TOC)	SE
Naphthalene	0.06	0.02	4.07	1.13
Acenaphthylene	0.31	0.05	20.33	3.62
Acenaphthene	3.19	0.14	209.80	9.56
Fluorene	3.23	0.06	209.57	2.92
Phenanthrene	12.11	0.25	783.63	13.40
Anthracene	2.54	0.08	168.10	5.79
Fluoranthene	9.20	0.92	639.61	64.61
Pyrene	10.33	0.61	684.39	41.45
Benz[a]anthracene	6.32	0.87	417.26	60.90
Chrysene	17.22	0.63	1092.44	33.03
Benzo[b]fluoranthene	14.17	0.57	920.51	36.25
Benzo[k]fluoranthene	3.34	0.51	207.60	32.97
Benzo[a]pyrene	12.26	0.69	816.52	48.91
Indeno[1,2,3-cd]pyrene	4.21	0.22	268.82	12.23
Dibenz[a,h]anthracene	1.64	0.18	100.51	10.56
Benzo[g,h,i]perylene	23.21	1.06	1531.51	71.19
Dibenzothiophene	12.33	0.18	808.37	12.33
2-Methylnaphthalene	0.41	0.05	25.50	3.11
1-Methylnaphthalene	0.29	0.04	17.71	2.25
3-Methylphenanthrene	14.18	0.35	927.14	22.44
2-Methylphenanthrene	12.57	0.17	819.77	10.18

Compound	Mean PAC concentration in sediment	SE	Mean PAC concentration in sediment	SE
	(ng g ⁻¹ , dw)		(ng g ⁻¹ , dw TOC)	
9/4-Methylphenanthrene	27.80	0.34	1828.46	25.97
1-Methylphenanthrene	14.24	0.14	932.12	9.53
3,6-Dimethylphenanthrene	68.45	0.88	4519.38	69.68
2,6-Dimethylphenanthrene	31.46	0.92	2061.57	59.57
1,7-Dimethylphenanthrene	15.34	0.70	1010.53	46.69
1,8-Dimethylphenanthrene	24.94	0.40	1644.37	28.53
Retene	30.93	2.25	2106.95	160.72
C2-Naphthalenes	14.83	0.26	984.35	20.87
C3-Naphthalenes	80.51	2.00	5435.20	161.92
C4-Naphthalenes	222.25	6.12	15034.99	485.90
C1-Fluorenes	38.52	0.62	2570.52	52.50
C2-Fluorenes	177.77	2.69	11836.77	229.28
C3-Fluorenes	370.73	4.37	24761.93	440.05
C1-Phenanthrenes/Anthracenes	89.45	1.73	5916.15	122.70
C2-Phenanthrene/Anthracenes	332.07	5.19	22253.29	529.22
C3-Phenanthrene/Anthracenes	550.44	11.24	37160.10	1024.89
C4-Phenanthrene/Anthracenes	1684.48	51.80	112893.11	4099.81
C1-Dibenzothiophenes	227.47	3.84	15177.49	295.60
C2-Dibenzothiophenes	684.21	8.45	45511.53	798.63
C3-Dibenzothiophenes	1686.01	26.52	112898.57	2331.70
C4-Dibenzothiophenes	1167.91	23.42	77467.91	1746.30
C1-Fluoranthenes/Pyrenes	142.47	8.96	9642.76	624.74
C2-Fluoranthenes/Pyrenes	344.43	22.30	23331.94	1568.52
C3-Fluoranthenes/Pyrenes	383.71	23.26	25919.16	1633.95
C4-Fluoranthenes/Pyrenes	401.55	22.92	27356.70	1618.38

Compound	Mean PAC concentration in sediment		Mean PAC concentration in sediment	
	(ng g⁻¹, dw)	SE	(ng g⁻¹, dw TOC)	SE
C1-Benz[a]anthracenes/Chrysenes	55.50	3.39	3422.94	169.96
C2-Benz[a]anthracenes/Chrysenes	87.61	5.63	6531.45	366.79
C3-Benz[a]anthracenes/Chrysenes	75.66	5.39	5728.98	357.38
C4-Benz[a]anthracenes/Chrysenes	36.54	5.21	2125.03	310.57
C1-Benzofluoranthenes/Benzopyrenes	28.66	1.92	1789.58	98.69
C2-Benzofluoranthenes/Benzopyrenes	10.22	0.60	641.19	28.65

Appendix D. PAC Uptake and Elimination Rates

Table A.4. Uptake (k_1 and k_{tmax}) and elimination (k_2) rates determined for PACs measured in wood frog tadpoles during 3 exposure experiments. The k_2 values were determined using the slope of $\ln$ elimination concentrations for the sediment + aqueous (192 h) experiment, the aqueous (24 h) experiment, and the sediment + aqueous (24 h) experiment. For the sediment + aqueous (192 h) and the aqueous (24 h) exposure experiments, k_1 and k_{tmax} values were also determined; the initial uptake tadpole concentrations were used for calculating k_1 values, and Equation 3 (Section 2.2.6) for the k_{tmax} values. Log K_{ow} of the PACs (Irwin and Service, 1997) are also included.

Compound	Log K_{ow}	Sediment + aqueous (192 h) experiment			Aqueous (24 h) experiment			Sediment + aqueous (24 h) experiment
		k_2	k_1	k_{tmax}	k_2	k_1	k_{tmax}	k_2
Naphthalene	3.37	—	—	—	—	—	—	—
Acenaphthylene	4.07	—	—	—	—	—	—	—
Acenaphthene	3.92	—	—	—	—	—	—	—
Fluorene	4.18	0.13	—	—	0.06	0.65	0.01	—
Phenanthrene	4.57	0.09	—	—	0.03	0.53	0.01	—
Anthracene	4.54	0.06	—	—	—	—	—	—
Fluoranthene	5.22	—	—	—	—	—	—	0.02
Pyrene	5.18	0.01	—	0.08	0.06	0.74	0.01	0.12
Benz[a]anthracene	5.91	—	—	—	—	—	—	—
Chrysene	5.86	0.07	—	—	0.04	0.75	0.03	0.08
Benzo[b]fluoranthene	5.8	—	—	—	—	—	—	—
Benzo[k]fluoranthene	6	—	—	—	0.06	1.57	0.11	0.01
Benzo[a]pyrene	6.04	—	—	—	0.10	2.53	0.10	—
Indeno[1,2,3-cd]pyrene	7	—	—	—	—	—	—	—
Dibenz[a,h]anthracene	6.75	—	—	—	0.03	0.46	—	—
Benzo[g,h,i]perylene	6.5	—	—	—	—	—	—	—
Dibenzothiophene	4.49	0.12	—	0.08	0.13	—	0.04	—
2-Methylnaphthalene		—	—	—	—	—	—	—
1-Methylnaphthalene		—	—	—	—	—	—	—
3-Methylphenanthrene		—	—	—	—	—	—	—

Compound	Log K _{ow}	Sediment + aqueous (192 h) experiment			Aqueous (24 h) experiment			Sediment + aqueous (24 h) experiment
		k ₂	k ₁	k _{tmax}	k ₂	k ₁	k _{tmax}	k ₂
2-Methylphenanthrene		—	—	—	0.14	0.23	0.03	—
9/4-Methylphenanthrene		—	—	—	0.03	0.99	0.02	—
1-Methylphenanthrene		0.06	0.15	0.60	0.02	2.24	0.07	0.08
3,6-Dimethylphenanthrene		—	—	—	—	—	—	—
2,6-Dimethylphenanthrene		—	—	—	—	—	—	—
1,7-Dimethylphenanthrene		—	—	—	—	—	—	—
1,8-Dimethylphenanthrene		0.03	—	—	—	—	—	0.03
Retene		—	—	—	—	—	—	—
C2-Naphthalenes	4.37	—	—	—	—	—	—	—
C3-Naphthalenes	5	0.13	0.38	0.09	0.03	0.97	0.02	—
C4-Naphthalenes	5.55	0.01	4.19	0.07	0.10	4.99	0.10	0.06
C1-Fluorenes	4.97	—	—	—	0.13	7.46	0.04	—
C2-Fluorenes	5.2	0.15	4.89	0.39	0.02	3.50	—	—
C3-Fluorenes	5.5	—	—	—	0.05	4.28	0.03	0.10
C1-Phenanthrenes/Anthracenes	5.14	0.14	—	0.06	0.05	3.11	0.02	—
C2-Phenanthrene/Anthracenes	5.51	—	—	—	0.11	2.09	0.01	—
C3-Phenanthrene/Anthracenes	6	—	—	—	0.04	13.71	0.04	0.23
C4-Phenanthrene/Anthracenes	6.51	—	—	—	0.04	—	—	0.14
C1-Dibenzothiophenes	4.86	0.09	2.33	0.16	0.08	0.74	0.01	0.02
C2-Dibenzothiophenes	5.5	0.11	2.87	0.56	0.05	0.53	—	—
C3-Dibenzothiophenes	5.73	0.18	5.72	0.94	0.04	2.34	0.01	0.06
C4-Dibenzothiophenes		0.18	0.39	1.10	—	—	—	—
C1-Fluoranthenes/Pyrenes	5.72	—	—	—	0.05	0.02	—	0.07
C2-Fluoranthenes/Pyrenes		—	—	—	—	—	—	0.08
C3-Fluoranthenes/Pyrenes		—	—	—	—	—	—	0.07
C4-Fluoranthenes/Pyrenes		—	—	—	—	—	—	0.01

Compound	Log K _{ow}	Sediment + aqueous (192 h) experiment			Aqueous (24 h) experiment			Sediment + aqueous (24 h) experiment
		k ₂	k ₁	k _{tmax}	k ₂	k ₁	k _{tmax}	k ₂
C1-Benz[a]anthracenes/Chrysenes	6.42	—	—	—	—	—	—	0.10
C2-Benz[a]anthracenes/Chrysenes	6.88	0.02	—	—	0.07	1.01	0.02	0.10
C3-Benz[a]anthracenes/Chrysenes	7.44	—	—	—	0.08	0.25	0.02	—
C4-Benz[a]anthracenes/Chrysenes	8	0.09	—	—	0.13	4.46	0.01	—
C1- Benzofluoranthenes/Benzopyrenes		—	—	—	0.14	0.68	0.03	—
C2- Benzofluoranthenes/Benzopyrenes		—	—	—	0.15	0.36	0.04	—

Table A.5. Polycyclic aromatic compounds with the highest and lowest uptake (k_1 and k_{tmax}) and depuration (k_2) rates during the sediment + aqueous (192 h) experiment, the aqueous (24 h) experiment, and the sediment + aqueous (24 h) experiment. Out of the 52 PACs analyzed, the 3 compounds with the highest k values are listed in descending order of importance (1st being the highest k value and the 2nd being the second highest k value) and the 3 compounds with the lowest k values are also listed in descending order of importance (1st being the lowest k value and the 2nd being the second lowest k value).

	k _{tmax}	k _{tmax}	k ₁	k ₁	k ₂	k ₂	k ₂
	SedAq (192h)	Aq (24h)	SedAq (192h)	Aq (24h)	SedAq (192h)	Aq (24h)	SedAq (24h)
Highest	C4-DBT	BkF	C3-DBT	C3-PHE/ANT	C3-DBT	C2-BFLA/BPYR	C3-PHE/ANT
	C3-DBT	C4-NAPH	C2-FLU	C1-FLU	C4-DBT	C1-BFLA/BPYR	C4-PHE/ANT
	1-MPHEN	BaP	C4-NAPH	C4-NAPH	C2-FLU	2-MPHEN	PYR
Lowest	C1-PHE/ANT	C1-FLA/PYR	1-MPHEN	C1-FLA/PYR	C4-NAPH	1-MPHEN	C4-FLA/PYR
	C4-NAPH	C2-FLU	C3-NAPH	2-MPHEN	PYR	9/4-MPHEN	BkFLA
	PYR	C2-DBT	C4-DBT	C3-BaA/CRY	C2-BaA/CRY	C3-NAPH	FLA

Appendix E. Biota-Sediment Accumulation Factors and Bioconcentration Factors of PACs

Table A.6. Mean ($\pm$ SE) biota-sediment accumulation factors (BSAFs) of PACs measured in wood frog tadpoles after 24 hours of the sediment + aqueous (24 and 192 h) exposure experiments, as well as the log bioconcentration factors (BCF) determined after 24 hours of the aqueous (24 h) experiment.

Compound	Sediment + aqueous (192 h) experiment		Aqueous (24 h) experiment	Sediment + aqueous (24 h) experiment	
	BSAF	SE	Log BCF	BSAF	SE
Naphthalene	—	—	—	—	—
Acenaphthylene	—	—	—	—	—
Acenaphthene	—	—	2.00	—	—
Fluorene	0.36	0.30	2.70	—	—
Phenanthrene	—	—	4.12	0.17	0.04
Anthracene	1.46	0.73	—	—	—
Fluoranthene	—	—	—	1.77	0.52
Pyrene	0.40	0.11	2.71	0.25	0.10
Benz[a]anthracene	—	—	—	—	—
Chrysene	0.57	0.08	—	0.07	0.02
Benzo[b]fluoranthene	0.05	0.05	—	0.01	0.00
Benzo[k]fluoranthene	—	—	—	—	—
Benzo[a]pyrene	0.55	0.55	—	—	—
Indeno[1,2,3-cd]pyrene	—	—	—	—	—
Dibenz[a,h]anthracene	—	—	—	—	—
Benzo[g,h,i]perylene	—	—	—	—	—
Dibenzothiophene	0.15	0.10	3.49	—	—
2-Methylnaphthalene	—	—	—	—	—
1-Methylnaphthalene	—	—	—	—	—
3-Methylphenanthrene	0.13	0.09	2.70	0.02	0.02
2-Methylphenanthrene	—	—	3.27	—	—
9/4-Methylphenanthrene	0.03	0.03	3.40	0.01	0.01
1-Methylphenanthrene	0.16	0.08	3.61	0.03	0.02
3,6-Dimethylphenanthrene	0.10	0.02	1.15	—	—

Compound	Sediment + aqueous (192 h) experiment		Aqueous (24 h) experiment	Sediment + aqueous (24 h) experiment	
	BSAF	SE		BSAF	SE
2,6-Dimethylphenanthrene	—	—	—	—	—
1,7-Dimethylphenanthrene	—	—	—	—	—
1,8-Dimethylphenanthrene	0.08	0.04	—	0.03	0.00
Retene	1.06	0.72	—	0.02	0.01
C2-Naphthalenes	—	—	—	—	—
C3-Naphthalenes	—	—	—	—	—
C4-Naphthalenes	0.38	0.07	3.79	0.03	0.03
C1-Fluorenes	0.01	0.01	2.43	—	—
C2-Fluorenes	0.20	0.12	2.05	0.03	0.02
C3-Fluorenes	0.23	0.10	2.74	0.07	0.01
C1-Phenanthrenes/Anthracenes	0.13	0.07	2.95	—	—
C2-Phenanthrene/Anthracenes	0.03	0.03	2.12	—	—
C3-Phenanthrene/Anthracenes	0.06	0.02	2.87	—	—
C4-Phenanthrene/Anthracenes	0.02	0.00	1.61	—	—
C1-Dibenzothiophenes	0.10	0.06	3.46	0.02	0.01
C2-Dibenzothiophenes	0.10	0.02	2.66	0.02	0.00
C3-Dibenzothiophenes	0.09	0.02	2.71	0.01	0.00
C4-Dibenzothiophenes	0.05	0.03	—	0.01	0.00
C1-Fluoranthenes/Pyrenes	0.53	0.24	1.90	0.02	0.00
C2-Fluoranthenes/Pyrenes	0.16	0.06	—	0.01	0.00
C3-Fluoranthenes/Pyrenes	0.10	0.05	1.23	—	—
C4-Fluoranthenes/Pyrenes	0.02	0.02	—	—	—
C1-Benz[a]anthracenes/Chrysenes	0.08	0.03	0.30	—	—
C2-Benz[a]anthracenes/Chrysenes	0.03	0.03	2.72	0.01	0.00
C3-Benz[a]anthracenes/Chrysenes	0.09	0.09	2.93	—	—
C4-Benz[a]anthracenes/Chrysenes	—	—	3.48	—	—
C1-Benzofluoranthenes/Benzopyrenes	2.63	2.30	3.48	—	—
C2-Benzofluoranthenes/Benzopyrenes	0.05	0.05	3.82	—	—

Appendix F. Map of Study Area

Appendix G. General Characteristics of Wetlands Studied

Table A.7. General characteristics of the 5 wetlands in the Athabasca oil sands region included in this study. Data were provided by Environment and Climate Change Canada (Unpublished, 2016). The pH measurements were obtained by handheld meters in the field in 2014 and the conductivity and water hardness measurements were obtained by an accredited analytical laboratory in 2012-14. WF1 Pond was a new site, from which no data had been collected in 2014 (n.a.).

Study Site	Wetland characteristics	pH ¹	Conductivity ($\mu\text{S cm}^{-1}$) ²	Hardness (CaCO_3 , (mg L^{-1}) ³	Distance from nearest upgrader (km)
Gateway Hill	Opportunistic wetland on reclaimed overburden storage site. Clay bottom, surrounded by pine and spruce.	8.25 (0.19) ^{ab}	1300 (1100-1500) ^a	290 (220-590) ^a	5.3
Jenny Pond	Naturally occurring wetland with clay bottom less than 300 m from where the Muskeg River empties into the Athabasca River.	8.00 (0.07) ^{abcd}	430 (360-510) ^{ab}	220 (170-260) ^{ab}	10.1
WF1	Naturally occurring wetland within the Fort McKay community, surrounded by birch and spruce.	n.a.	n.a.	n.a.	16.6
Pat's Pond	Naturally occurring pond complex surrounded by jack pine stands.	7.64 (0.10) ^{bcde}	170 (160-200) ^{abcd}	97.5 (78-100) ^{abc}	28.9
Jetliner Pond	Borrow pit with clay bottom, 20+ years old. Abundant aquatic vegetation, surrounded by spruce and pine.	8.50 (0.21) ^a	230 (170-280) ^{abcd}	130 (72-150) ^{abc}	46.0

¹ pH data indicate mean pH (standard error). Different lower case letters indicate significant differences in water pH among sites, as determined by a one-way ANOVA followed by post hoc Tukey tests.

² Conductivity data indicate median value (min-max). Different lower case letters indicate significant differences in water conductivity among sites, as determined by a Kruskal-Wallis test followed by post hoc Dunn's test.

³ Water hardness data indicate median value (min-max). Different lower case letters indicate significant differences in water hardness among sites, as determined by a Kruskal-Wallis test followed by post hoc Dunn's test.

Appendix H. Linear Regressions of Wetland Sediment and Water PAC Concentrations

Figure A.1. Log concentrations of PACs in wood frog tadpoles in comparison to their concentrations in sediment (i) and water (ii). Tadpole, sediment, and water concentrations are the log-transformed average concentrations of duplicate or triplicate samples collected from 5 wetlands in the Athabasca oil sands region. All linear regressions were significant ($p < 0.05$).

a.i Gateway Hill Pond PAC concentrations in tadpoles vs. sediment

a.ii Gateway Hill PAC concentrations in tadpoles vs. water

b.i Jenny Pond PAC concentrations in tadpoles vs. sediment

b.ii Jenny Pond PAC concentrations in tadpoles vs. water

c.i WF1 Pond PAC concentrations in tadpoles vs. sediment

c.ii WF1 Pond PAC concentrations in tadpoles vs. water

d.i Pat's Pond PAC concentrations in tadpoles vs. sediment

d.ii Pat's Pond PAC concentrations in tadpoles vs. water

e.i Jetliner Pond PAC concentrations in tadpoles vs. sediment

e.ii Jetliner Pond PAC concentrations in tadpoles vs. water

Table A.8. Multiple linear regressions between log PAC concentrations in tadpoles, sediment, and water from 5 wetlands in the Athabasca oil sands region. SigmaPlot 12 was used for the equations and statistics. * indicates significant p-values and the independent variables from the linear combination (equation) with which the PAC concentrations in tadpoles can be predicted.

Wetland	Equation	R ²	# of Replicates	p-value Seds	p-value Water	Predictive Variables
Gateway Hill	$y = -1.67 + (0.39 * \text{Seds}) + (0.37 * \text{Water})$	0.60	19	<0.001*	0.008*	Both
Jenny Pond	$y = -1.03 + (0.19 * \text{Seds}) + (0.762 * \text{Water})$	0.56	5	0.472	0.001*	Water
WF1 Pond	$y = -2.17 + (0.91 * \text{Seds}) + (0.09 * \text{Water})$	0.48	7	<0.001*	0.422	Seds
Pat's Pond	$y = -3.03 + (1.02 * \text{Seds}) + (0.91 * \text{Water})$	0.59	3	0.001*	0.004*	Both
Jetliner Pond	$y = -0.53 + (0.40 * \text{Seds}) + (1.27 * \text{Water})$	0.76	7	0.047*	<0.001*	Both

Appendix I. Linear Regressions of Wetland Sediment and Tadpole Concentrations of Individual PACs

Figure A.2. Log concentration of individual PACs in field-collected wood frog tadpoles in comparison to their concentration in the sediment. Each point refers to the tadpole and sediment average concentration in one of the 5 wetlands studied in the Athabasca oil sands region. All equations and statistical details of the linear regressions are listed in Table A.9.

Table A.9. Linear regressions between PAC concentrations in wood frog tadpoles and sediment from wetlands in the Athabasca oil sands region. All equations, R^2 , p-values, n (sample size), and power of the regressions were determined using SigmaPlot 12. The compounds with p-values <0.05 (*) are considered to have significant correlations between the tadpoles and sediment.

Compound	Equation	R^2	p-value	n	Power
ANT	$y = -0.69 + (0.32 * \text{Seds})$	0.09	0.62	5	0.07
BaA	$y = -2.57 + (0.58 * \text{Seds})$	0.38	0.27	5	0.17
CRY	$y = -1.05 + (0.03 * \text{Seds})$	0.00	0.95	5	0.03
BbF	$y = -0.79 + (0.08 * \text{Seds})$	0.11	0.59	5	0.07
DBT	$y = -0.74 + (0.21 * \text{Seds})$	0.67	0.09	5	0.37
3-MP	$y = -0.98 + (0.37 * \text{Seds})$	0.23	0.41	5	0.11
2-MP	$y = -0.84 + (0.23 * \text{Seds})$	0.14	0.53	5	0.08
1-MP	$y = -0.08 + (0.29 * \text{Seds})$	0.17	0.50	5	0.09
2,6-DMP	$y = -1.07 + (0.07 * \text{Seds})$	0.15	0.53	5	0.08
1,7-DMP	$y = -1.23 + (0.43 * \text{Seds})$	0.45	0.22	5	0.21
C3-NAPH	$y = 0.92 + (0.02 * \text{Seds})$	0.03	0.78	5	0.04
C4-NAPH	$y = 1.14 + (0.01 * \text{Seds})$	0.00	0.93	5	0.03
C2-FLU	$y = 0.88 + (0.06 * \text{Seds})$	0.06	0.68	5	0.06
C3-FLU	$y = 0.39 + (0.32 * \text{Seds})$	0.96	<0.01*	5	0.90
C2-PHE/ANT	$y = 0.38 + (0.10 * \text{Seds})$	0.14	0.54	5	0.08
C4-PHE/ANT	$y = 0.34 + (0.12 * \text{Seds})$	0.10	0.61	5	0.07
C2-DBT	$y = 0.04 + (0.19 * \text{Seds})$	0.80	0.04*	5	0.53
C3-DBT	$y = 0.53 + (0.15 * \text{Seds})$	0.51	0.17	5	0.25
C4-DBT	$y = 0.96 + (0.02 * \text{Seds})$	0.01	0.87	5	0.04
C2-FLA/PYR	$y = -0.69 + (0.20 * \text{Seds})$	0.34	0.30	5	0.16
C3-FLA/PYR	$y = -1.10 + (0.22 * \text{Seds})$	0.31	0.33	5	0.14
C4-FLA/PYR	$y = -0.68 + (0.16 * \text{Seds})$	0.69	0.08	5	0.39
C2-BaA/CRY	$y = -1.33 + (0.35 * \text{Seds})$	0.20	0.45	5	0.10
C3-BaA/CRY	$y = -1.77 + (0.49 * \text{Seds})$	0.46	0.21	5	0.21

Appendix J. Biota-Sediment Accumulation Factors and Bioconcentration Factors of PACs

Table A.10. Biota-sediment accumulation factors (BSAF) and log bioaccumulation factors (BAFs) of PACs measured in wood frog tadpoles collected from 5 wetlands in the Athabasca oil sands region.

	Gateway Hill		Jenny Pond		WF1 Pond		Pat's Pond		Jetliner Pond	
Compound	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF
NAPH	—	1.75	0.59	1.90	—	2.27	0.56	2.32	—	—
ACY	—	—	—	—	—	—	—	—	—	—
ACE	—	—	—	—	0.31	—	—	—	—	4.94
FLU	0.06	1.49	0.99	1.16	0.16	1.94	0.79	1.77	—	2.51
PHE	0.04	1.74	0.31	1.32	0.10	2.90	—	2.70	—	2.35
ANT	0.27	2.41	3.77	1.90	4.16	—	0.54	—	0.54	3.30
FLA	—	0.76	—	0.78	—	0.74	—	0.74	—	—
PYR	0.04	3.65	0.07	2.59	0.24	2.34	—	2.06	0.21	3.36
BaA	0.02	1.46	0.12	1.09	0.02	1.93	0.04	1.68	0.03	—
CRY	0.05	2.91	0.11	2.25	0.01	2.59	0.04	2.00	4.90	—
BbF	—	1.15	0.15	0.82	0.26	2.39	0.09	2.13	0.24	2.55
BkF	0.03	0.41	0.13	0.52	0.20	2.55	—	2.40	—	2.35
BaP	0.01	—	—	—	—	—	—	—	—	—
IDP	—	—	0.03	—	0.41	—	—	—	0.07	—
DBA	—	—	—	—	—	—	—	—	—	—
BgP	—	—	0.02	—	0.10	—	—	—	—	—
DBT	0.02	1.87	0.35	1.45	0.23	2.16	1.84	1.84	1.24	2.21
2-MN	0.03	0.14	0.75	0.16	—	2.40	0.21	2.43	—	—
1-MN	—	—	0.68	—	—	1.98	—	2.16	—	—
3-MP	0.03	1.26	0.25	0.90	0.39	1.67	1.23	1.51	0.27	2.03
2-MP	0.02	1.99	0.42	1.52	0.53	2.73	0.80	3.11	0.29	2.40
9/4-MP	0.01	2.47	0.31	1.96	1.75	—	—	—	0.41	—

	Gateway Hill		Jenny Pond		WF1 Pond		Pat's Pond		Jetliner Pond	
Compound	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF
1-MP	0.21	2.76	2.34	2.16	3.30	—	3.03	—	1.42	3.79
3,6-DMP	0.02	2.06	0.12	1.46	0.14	—	—	—	0.49	—
2,6-DMP	—	—	0.10	—	0.09	—	0.33	—	0.14	3.69
1,7-DMP	0.05	—	1.18	—	0.25	—	0.24	—	0.33	—
1,8-DMP	0.01	—	—	—	0.21	—	—	—	0.06	1.92
RET	0.02	1.94	—	2.24	0.08	1.33	—	1.55	0.19	2.56
C2-NAPH	0.21	2.03	—	1.60	—	3.06	—	3.47	—	—
C3-NAPH	0.14	2.60	0.77	2.39	0.17	2.46	1.92	2.28	19.23	2.94
C4-NAPH	0.17	—	3.52	—	1.00	3.49	2.63	3.14	11.28	3.09
C1-FLU	0.08	1.82	0.01	1.45	1.49	1.12	0.64	0.88	—	2.06
C2-FLU	0.20	2.34	0.12	1.82	0.17	1.88	3.01	1.48	17.18	1.73
C3-FLU	0.27	2.49	0.89	1.59	0.93	2.95	6.20	2.49	7.12	1.70
C1-PHE/ANT	0.05	1.96	0.94	1.12	0.32	2.63	2.71	1.89	—	1.79
C2-PHE/ANT	0.03	1.67	0.10	0.90	0.85	1.36	1.81	1.35	1.04	1.54
C3-PHE/ANT	0.02	2.11	—	1.05	—	2.15	0.21	1.82	1.82	—
C4-PHE/ANT	0.01	1.90	0.12	1.03	0.53	1.71	1.85	1.46	1.15	1.71
C1-DBT	0.02	2.31	1.32	1.66	1.91	2.70	—	2.39	—	2.53
C2-DBT	0.02	2.08	0.52	1.30	0.86	2.72	5.32	2.11	1.37	2.23
C3-DBT	0.01	2.31	0.88	1.48	1.57	2.95	11.87	2.58	3.40	2.83
C4-DBT	0.01	2.18	0.38	1.69	0.76	2.66	0.68	2.24	5.27	2.48
C1-FLA/PYR	0.01	1.11	0.01	0.61	0.25	1.73	0.32	0.96	—	0.99
C2-FLA/PYR	0.01	1.41	0.06	0.98	0.15	1.97	0.14	1.62	1.32	1.50
C3-FLA/PYR	—	1.32	0.02	0.89	0.21	1.77	0.11	1.58	0.23	1.17
C4-FLA/PYR	—	1.27	0.10	0.45	0.09	1.83	0.24	1.55	0.23	1.16

	Gateway Hill		Jenny Pond		WF1 Pond		Pat's Pond		Jetliner Pond	
Compound	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF	BSAF	Log BAF
C1-BaA/CRY	0.01	1.38	0.04	0.77	0.04	1.93	0.04	1.38	—	1.08
C2-BaA/CRY	0.01	1.83	0.14	1.17	0.02	2.23	0.47	1.59	0.42	—
C3-BaA/CRY	0.01	1.82	0.18	1.54	0.14	2.36	0.03	2.22	0.10	1.89
C4-BaA/CRY	0.03	1.97	—	2.08	—	3.07	—	3.07	0.60	3.47
C1-BFLA/BPYR	0.05	1.80	—	1.80	—	—	—	—	—	3.33
C2-BFLA/BPYR	0.23	2.42	7.78	1.90	—	—	—	—	1.11	2.95

Table A.11. Statistical differences, using Mann-Whitney (M-W) or student t-tests (T-test), between the means or medians of BSAFs of the parent and alkyl PACs in *Lithobates sylvaticus* tadpoles from 5 wetlands in the Athabasca oil sands region. BSAFs from Gateway Hill Pond, Jenny Pond, WF1 Pond, Pat's Pond, and Jetliner Pond were normalized by log transformation. Shapiro-Wilk (S-W) normality tests were then performed. * indicates a significant difference. The mean log10 data were back-transformed to BSAF values.

	Gateway Hill	Jenny Pond	WF1 Pond	Pat's Pond	Jetliner Pond
	Median BSAF	Mean BSAF	Mean BSAF	Mean BSAF	Mean BSAF
Statistical Test	M-W	T-test	T-test	T-test	T-test
S-W Normality	p<0.05	p=0.83	p=0.95	p=0.30	p=0.32
Parent PACs	0.04 <i>n</i> =10	0.19 <i>n</i> =12	0.17 <i>n</i> =12	0.14* <i>n</i> =8	0.31 <i>n</i> =7
Alkyl PACs	0.02 <i>n</i> =34	0.23 <i>n</i> =30	0.34 <i>n</i> =28	0.70* <i>n</i> =25	0.87 <i>n</i> =26
p-value	0.11	0.74	0.14	0.03	0.14

Table A.12. Statistical differences, using Mann-Whitney (M-W) or student t-tests (T-test), between the means or medians of BSAFs of 2-3 ring or 4-6 ring PACs in *Lithobates sylvaticus* tadpoles from 5 wetlands in the Athabasca oil sands region. BSAFs from Gateway Hill Pond, Jenny Pond, WF1 Pond, Pat's Pond, and Jetliner Pond were normalized by log transformation. Shapiro-Wilk (S-W) normality tests were then performed. * indicates significant differences. The mean log10 data were back-transformed to BSAF values.

	Gateway Hill	Jenny Pond	WF1 Pond	Pat's Pond	Jetliner Pond
	Median BSAF	Mean BSAF	Mean BSAF	Mean BSAF	Median BSAF
Statistical Test	M-W	M-W	T-test	T-test	T-test
S-W Normality	p<0.05	p<0.05	p=0.54	p=0.47	p=0.64
2-3 ring PACs	0.03 <i>n</i> =28	0.51* <i>n</i> =27	0.47* <i>n</i> =26	1.24* <i>n</i> =22	1.12* <i>n</i> =21
4-6 ring PACs	0.02 <i>n</i> =16	0.10* <i>n</i> =15	0.10* <i>n</i> =14	0.07* <i>n</i> =11	0.89* <i>n</i> =12
p-value	0.15	0.002	<0.001	<0.001	0.03

Appendix K. Sediment and Water Quality Guidelines

Table A.13. Comparison between the PAC concentrations ($\pm$ SE) measured in the water of 5 wetlands from the Athabasca oil sands region ($\mu\text{g L}^{-1}$) and the current Canadian water quality guidelines based on the protection of freshwater life for 9 PACs ($\mu\text{g L}^{-1}$) (CCME, 2010). * indicates concentration exceeding water quality guidelines.

	Freshwater Life Guideline ($\mu\text{g L}^{-1}$)	Gateway Hill Pond ($\mu\text{g L}^{-1}$)	Jenny Pond ($\mu\text{g L}^{-1}$)	WF1 Pond ($\mu\text{g L}^{-1}$)	Pat's Pond ($\mu\text{g L}^{-1}$)	Jetliner Pond ($\mu\text{g L}^{-1}$)
NAPH	1.1	0.030 (0.019)	0.029 (0.020)	0.004 (0.004)	0.007 (0.005)	0.0025
ACY	5.8	-----	-----	-----	-----	-----
ACE	3.0	0.010 (0.008)	0.004 (0.004)	-----	0.011 (0.006)	0.0004
FLU	0.4	0.011 (0.010)	0.002 (0.002)	0.002 (0.001)	0.005 (0.002)	0.0005
PHE	0.012	0.022 (0.018)	0.002 (0.002)	0.010 (0.007)	0.010 (0.004)	0.0018
ANT	0.04	0.002 (0.002)	-----	0.002 (0.001)	0.001 (0.001)	0.0002
FLA	0.025	0.054 (0.051)*	0.005 (0.005)	0.002 (0.002)	0.012 (0.006)	0.0003
PYR	0.018	-----	0.001 (0.001)	-----	0.002 (0.001)	0.0003
BaA	0.015	0.002 (0.001)	0.001 (0.001)	-----	0.012 (0.006)	0.0001

Table A.14. Comparison between the PAC concentrations ($\pm$ SE) measured in the surface sediment of 5 wetlands from the Athabasca oil sands region ($\mu\text{g kg}^{-1}$ dw) and the current Canadian Interim sediment quality guidelines (ISQGs) and probable effect levels (PELs) for 13 PACs in freshwater sediments ($\mu\text{g kg}^{-1}$ dw) (CCME, 2010). * indicates concentration exceeding sediment quality guidelines.

	ISQL ($\mu\text{g kg}^{-1}$)	PEL ($\mu\text{g kg}^{-1}$)	Gateway Hill Pond ($\mu\text{g kg}^{-1}$)	Jenny Pond ($\mu\text{g kg}^{-1}$)	WF1 Pond ($\mu\text{g kg}^{-1}$)	Pat's Pond ($\mu\text{g kg}^{-1}$)	Jetliner Pond ($\mu\text{g kg}^{-1}$)
NAPH	34.6	391	-----	13.65 (6.83)	14.07 (1.64)	10.18	-----
ACY	5.87	128	1.18 (0.16)	0.22 (0.04)	-----	-----	1.35 (0.69)
ACE	6.71	88.9	34.52 (1.92)*	0.38 (0.20)	0.26 (0.01)	-----	-----
FLU	21.2	144	10.39 (0.66)	0.20 (0.11)	0.87 (0.59)	0.65	-----
PHE	41.9	515	54.56 (6.08)*	8.30 (0.90)	5.30 (2.78)	3.38	0.25 (0.04)
ANT	46.9	245	3.73 (0.14)	0.23 (0.11)	0.18 (0.10)	0.38	1.43 (0.41)
FLA	111	2355	-----	-----	0.45 (0.23)	1.25	0.12 (0.06)
PYR	53	875	23.04 (2.62)	3.02 (0.32)	0.80 (0.13)	1.43	1.14 (0.19)
BaA	31.7	385	5.54 (2.02)	0.64 (0.07)	0.13 (0.01)	0.42	0.79 (0.05)
CRY	57.1	862	16.87 (1.02)	2.23 (0.10)	0.31 (0.05)	1.29	0.07 (0.07)
BaP	31.9	782	70.42 (3.89)*	-----	-----	-----	0.69 (0.02)
DBA	6.22	135	2.39 (1.34)	0.23 (0.12)	0.04 (0.01)	0.01	2.48 (0.05)
2-MN	20.2	201	3.54 (0.89)	3.16 (1.01)	2.25 (0.04)	1.93	4.33 (0.42)