Evaluating the toxicity of Canadian Crudes on three species of fish

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Introduction

In Canada, there is an ever increasing demand for Canadian sourced crude oils, including diluted bitumen (dilbit). This has led to an increase in railway transport and proposals for several pipelines across the country. These pipelines would transport conventional crudes and dilbits from the Prairie provinces to the coasts, crossing a multitude of freshwater ecosystems along the way and increasing the risk of spills in these environments. Train derailments are also a non-negligible source of oil spills into freshwater environments.

However, little information is available regarding the toxicology of dilbits in freshwater systems. We have therefore undertaken to examine potential impacts of dilbits to various freshwater fish species. This knowledge will allow our partners to develop strategies adapted to the protection of freshwater environments across the country.

To maximize the knowledge gained, we are examining early life stages of three fish species that have different behaviours, reproductive strategies and thermal preferences. These are the fathead minnow (*Pimephales promelas)*, the rainbow trout (*Oncorhynchus mykiss*) and the Atlantic salmon (*Salmo salar*). They will be exposed to water contaminated by Canadian crudes, namely two dilbits (Bluesky and Clearwater McMurray) and a conventional heavy crude (Lloydminster).

25 °C

Fathead minnow

15 °C

Rainbow trout

7 °C

Atlantic salmon

Objectives

1. **Remedy our lack of knowledge around the toxicology of dilbits in freshwater systems.**
2. **Compare the toxicity of dilbits to a conventional crude of similar density**
3. **Determine critical concentration thresholds for freshwater fish**
4. **Evaluate the physiological status of freshwater fish exposed to crude oils**

Experimental design

1- Preparation of WAFs (*water accomodated fraction*)

The three crudes (Bluesky and Clearwater-McMurray dilbits; Concventional Lloydminster Heavy) were mixed at a de 1:9 ratio with reconstituted soft water. The mixture was stirred at a low speed so as not to create a vortex for 18 h and allowed to rest for 1 h before the aqueous fraction was recovered. The WAFs were then diluted to concentrations ranging from 0.32 to 75 % (adapted from Singer *et al.*, 2000).

2- Exposure to crude oils

The wide range of exposure concentrations will allow us to evaluate both acute and chronic toxicity endpoints, including survival, malformations, and growth. Fish exposed to WAFs will also be used to evaluate physiological and genetic changes resulting from exposures to crude oils.

	Pimephales promelas	***Oncorhynchus mykiss***	***Salmo salar***
Early life stages	Larvae (7 - 24 h post hatch) 7 day exposure 25 °C	Embryos Exposed until swim-up 15 °C	Embryos Exposed until swim-up 7 °C

3- Analysis of enzymatic and genetic responses

Oxidative stress:

- Antioxidant enzymes : Catalase (CAT; *cat*), Superoxide dismutase (SOD; *sod*) and Glutathione S-transferase (GST; *gst*)
- Oxidative damage and mutagenic potential: Lipid peroxidation (Malondialdehyde; MDA), DNA damage (8-OHdG) and *p53* expression

➢ Detoxification pathways:

- EROD and *cyp1a* activity

4- Analysis of the crude oils and WAFs

➢Characterization by the CEAEQ:

- BTEX, VOCc, C6-C10, C10-C50, PAHs, alkylated PAHs and metals

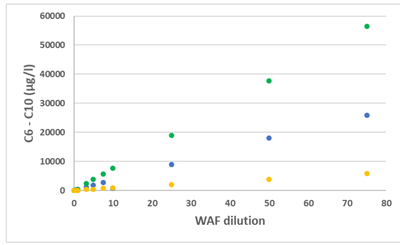
➢Characterization by NRCan:

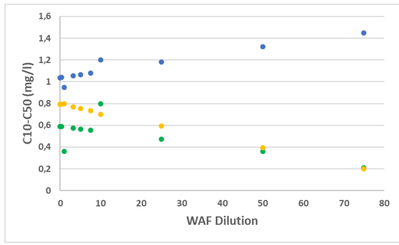
- C50 +

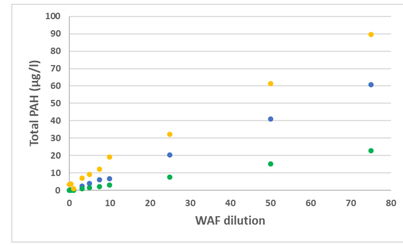
Protocols and references:

1. Singer et al., 2000. Standardisation of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Mar Pollut Bull,* Vol. 40, No. 11, 1007-1016.
2. Méthode d'essai biologique - Essai de croissance et de survie sur des larves de tête-de-boule. Environnement Canada, 2011.
3. Méthode d'essai biologique - Essai de létalité aigüe sur la truite arc-en-ciel. Environnement Canada, 1990, avec modifications de 1996 et 2017.
4. Méthode d'essai biologique - Essais toxicologiques sur des salmonidés (truite arc-en-ciel) aux premiers stades de leur cycle biologiques. Environnement Canada, Deuxième édition, 1998.
5. Madison *et al.*, 2015. Diluted bitumen causes deformities and molecular responses indicative of oxidative stress in Japanese medaka embryos. *Aquat Toxicol*, 165 : 222–230.

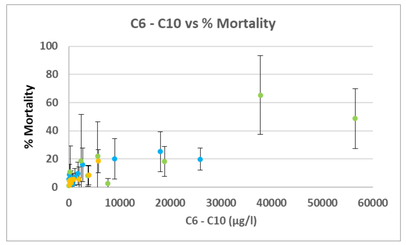
Results – Fathead minnow larval exposures

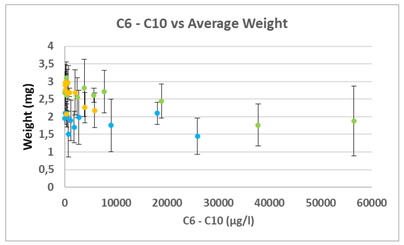


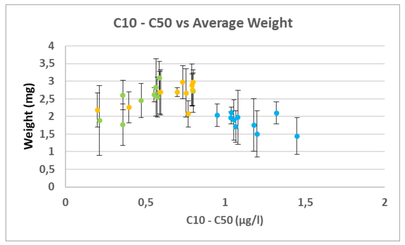




- Dilution of WAFs resulted in linear reductions of concentrations for all parameters except C10-C50.
- Clearwater dilbit (•) had highest concentrations of light ends, Lloydminster heavy crude (•) had highest [PAH], Bluesky dilbit (•) had high C10-C50







- Significantly increased mortality vs controls was observed from 50% WAF dilution for both dilbits, but 75% for conventional heavy.
- Positive correlations (0.77 to 0.86) were observed between percent mortality and C6-C10, VOC's and BTEX.
- No significant differences or relationships were observed between gross skeletal malformations and chemical composition.
- Significant linear correlations (-0.41 to -0,49) were observed between growth and C6-C10, VOC's and BTEX but an exponential relationship was observed with C10-C50 (r=0,71).

Conclusions and future work

Generally, acute thresholds (>50% mortality) were not reached and a significant negative effect on growth was only observed for one dilbit. Though no significant effects were observed for malformations, we only examined gross skeletal deformities and likely missed more subtle effects such as oedemas and cardiac anomalies typical of exposure to crude oils.

The Clearwater McMurray dilbit was more toxic than Bluesky or conventional heavy crude. Toxicity was most closely linked to light ends (C6-C10, COV, BTEX).

Upcoming biomarker analyses (enzymes, genetic responses, oxidative damage) will examine sublethal effects at the subcellular level.

Acknowledgments

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