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Biological effects and toxicity of diluted bitumen and its constituents in freshwater systems

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ABSTRACT: Approximately 50 billion cubic meters of bitumen resides within the oil sands region of Alberta, Canada. To facilitate the transport of bitumen from where it is extracted to where it is processed, the bitumen is diluted with natural gas condensate ('dilbit'), synthetic crude from hydrocracking bitumen ('synbit'), or a mixture of both ('dilsynbit'). A primary consideration for the effects of diluted bitumen products on freshwater organisms and ecosystems is whether it will float on the water surface or sink and interact with the stream or lake sediments. Evidence from a spill near Kalamazoo, MI, in 2010 and laboratory testing demonstrate that the nature of the spill and weathering of the dilbit, synbit or dilsynbit prior to and during contact with water will dictate whether the product floats or sinks. Subsequent toxicological data on the effects of dilbit and other diluted bitumen products on freshwater organisms and ecosystems are scarce. However, the current literature indicates that dilbit or bitumen can have significant effects on a wide variety of toxicological endpoints. This review synthesizes the currently available literature concerning the fate and effects of dilbit and synbit spilled into freshwater, and the effects of bitumen and bitumen products on aquatic organisms and ecosystems. Dilbit is likely to provide ecological impacts that are similar to and extend from those that follow from exposure to lighter crude oil, but the prospect of bitumen settling after binding to suspended sediments elevates the risk for benthic impacts in streams and lakes. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: bitumen; dilbit; dilsynbit; freshwater ecosystems; Kalamazoo River; oil spill; synbit

Introduction

The Canadian oil sands region of Alberta is estimated to contain up to 50 billion cubic meters (315 billion barrels) of bitumen, and represents the third largest oil reserve behind Saudi Arabia and Venezuela (National Energy Board, 2006; Environment Canada, 2013). Bitumen is an unconventional oil as it does not naturally flow and requires heating or dilution to be transported through pipelines (Centre for Energy, 2014). Given the high viscosity of extracted bitumen, transportation of the material for processing represents a major obstacle to utilizing the resource. To enable transport of bitumen through pipelines, a variety of bitumen-derived products have been developed, including diluted bitumen (dilbit), synthetic bitumen (synbit) and a combination of the two, dilsynbit. Dilbit is made by diluting bitumen with natural gas condensates (20–30% condensates, 70–80% bitumen), whereas synbit is a blend of synthetic crude oil from processed bitumen and bitumen in a 1: 1 ratio (Crosby *et al*., 2013). Pipelines traverse great distances and over a wide variety of eco-regions and aquatic ecosystems, creating the potential for spills to occur in a wide variety of ecologically sensitive regions. One of the most important issues concerning spills is whether the products or their components will sink when spilled into the water. Floating material can be skimmed off of the water surface before it has a major effect on an aquatic ecosystem, whereas sinking material is considerably harder to clean up before it leads to a detrimental effect. This review synthesizes the current available literature concerning the fate and effects of dilbit and synbit spilled into freshwater, and the effects of bitumen and bitumen products on aquatic ecosystems.

Composition of bitumen products

According to a recent report, there are seven different dilbit blends and seven different synbit blends originating from different regions in the Canadian oil sands patch (Environment Canada, 2013). In addition, there is one dilsynbit, Albian Synthetic Heavy, produced in the Canadian oil sands region (Hindin and Leis, 2012). The dilbit blends are produced by combining bitumen (70–80%) with natural gas condensate (20–30%) (Crosby *et al*., 2013). In comparison, synbit contains 50% bitumen and 50% synthetic light crude, most often made by coking or hydrolysis of bitumen, which removes larger molecules and decreases viscosity (Crosby *et al*., 2013). The condensate in dilbit contains primarily pentanes and heavier hydrocarbons, usually collected as a liquid by-product of natural gas extraction from field separators, scrubbers, or inlets of natural gas processing plants (National Energy Board, 2006). Dilsynbit contains bitumen, condensate and synthetic light crude (Oilsands Review, 2014).

Both dilbit and synbit contain a number of volatile organic compounds, the most notable of which are benzene, toluene, ethyl-

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benzene and xylene. Collectively, the four organic solvents are known as BTEX. The BTEX content of dilbits range from 0.8–1.2% by volume, whereas the BTEX content of synbits ranges from 0.4–1.1% by volume (Environment Canada, 2013). On average, there is approximately 21% more BTEX in dilbit than synbit. While the increased BTEX in dilbit is small in comparison to the makeup of the bitumen product (approximately 0.2% of the total volume of the product), during a spill more organic compounds would be expected to evaporate from dilbit as compared with synbit. There are approximately 22% more 'light ends', which are small organic compounds (3–10 carbon backbones) associated with oil, in dilbit relative to synbit. The increased amount of light ends in dilbit is as a result of the use of condensate (which contains primarily pentanes and heavier hydrocarbons) to dilute bitumen.

Dilbit, therefore, contains more organic solvents and a higher bitumen content than synbit, whereas synbit is entirely composed of oil products (bitumen and hydrolysed bitumen). Given these differences, it is possible that these products would react differently when introduced into water; unfortunately no studies have determined the fate of synbit in water, all have investigated the fate of dilbit in water.

Bitumen, an important constituent of dilbit, synbit and dilsynbit, is composed of a multitude of chemicals that can be broken down into four fractions via a saturate, aromatic, resin and asphaltene (SARA) extraction (Strausz *et al.*, 2010). A comparison of bitumen to heavy oils using SARA extraction demonstrated that bitumen has fewer saturates (16–17% vs. 23–30%), more resins (37% vs. 29–33%) and more asphaltenes (18–21% vs 10–16%) than heavy oil, whereas aromatics make up approximately 25–32% of both bitumen and heavy oils (Woods *et al.*, 2008). Therefore, while bitumen has similarities to heavy oils, there are significant differences between the two. An analysis of the aromatic fraction of bitumen demonstrated that there were just under 6000 different molecules within this one fraction alone (Strausz *et al.*, 2011). There are a number of groups of contaminants found within bitumen that have garnered significant attention, among these are naphthenic acids, polyaromatic hydrocarbons (PAHs) and metals. The toxicology of naphthenic acids is reviewed by Headley and McMartin (2004) and, the toxicity of PAHs is reviewed by Manzetti (2012) and Ball and Truskewycz (2013), and the effects of PAHs, metals and simple mixtures of the two is reviewed by Gauthier *et al.* (2014). While investigations into the effects of single contaminants and simple mixtures provide essential information concerning the toxicity of bitumen, they may not be fully representative of the effects of bitumen on an ecosystem as a result of a spill. It is for this reason that in this review we do not further discuss the effects of the components of bitumen, but instead review the known literature on the toxicological effect of dilbit as a whole on freshwater ecosystems.

Fate of dilbit/synbit in a freshwater environment

All 14 bitumen products listed in Environment Canada (2013) have a density less than that of freshwater, suggesting that if a spill were to occur in freshwater both dilbit and synbit would float. However, to date, all studies investigating the fate of spilled bitumen products in water have been done with dilbits, with no studies focusing on synbits or dilsynbit. The most important consideration for evaluating the risk of releasing dilbit or synbit to freshwater systems is whether or not they sink or float. Once released, light ends and BTEX would evaporate, leaving behind the heavier bitumen fraction. Given that dilbit contains more light ends, BTEX, and bitumen than synbit, dilbit is more likely to sink upon release to freshwater than synbit. Therefore, a dilbit spill could represent a more severe influence for some ecosystem components, with potentially less severe adverse impacts from synbit and dilsynbit. Three studies (Environment Canada, 2013, SL Ross Environmental Research Limited, 2013 and Yarranton *et al.*, 2014) investigated the effects of weathering, after or prior to a spill, on the density of dilbit, attempting to determine if dilibit would sink or float. Of these studies, two have assumed a spill of dilbit directly into a waterway, whereas the other assumes significant weathering of the dilbit prior to entry into a waterway.

Laboratory testing by SL Ross Environmental Research Ltd measured the density of Cold Lake Bitumen (CLB) after it was added to 15 °C freshwater under controlled wind conditions (1.5 m s^{-1}; 3 knots), water current (0.25 m s^{-1}; 0.5 knots) and (or) ultraviolet (UV) light for up to 11 days (SL Ross Limited, 2012). Samples of the resulting dilbit–water emulsion were taken throughout the test and the density of the emulsion was measured and compared with water at the same temperature. Over time, the density of the emulsion changed from 0.945 to 0.995 g ml^{-1} (no UV) or 0.998 g ml^{-1} (with UV). The UV-unexposed dilbit remained buoyant throughout the test, whereas the UV-exposed dilbit formed small oil droplets that dispersed throughout the water column. The formation of these droplets was attributed to the breakdown of the oil by UV. No dilbit was observed at the bottom of the tank in either of the UV-exposed or UV-unexposed treatments. At the end of the experiment, approximately 12–17% of the recovered oil was collected 10cm below the water level on the sides of the tank, whereas none was found on the bottom of the tank. The remainder of the recovered oil was collected from the top 10 cm of the water column. The droplets of oil observed in the water column, and the fact that 12–17% of the product was recovered below the surface of the water indicates that part of the dilbit went from being positively to neutrally buoyant. The amount of oil recovered at the end of the experiment was greater than the amount of dilbit added at the beginning of the experiment. The increase in volume was attributed to water being incorporated into the viscous material on the surface of the water. While this study does an excellent job in determining the changes in the density of dilbit added directly to water, it does not investigate the effect of suspended solids or organic matter in the water column, both of which have been shown to bind to dilbit (Environment Canada, 2013).

If suspended solids had been present in the water column during the testing by SL Ross Environmental Research Ltd, there is the potential that the neutrally buoyant oil droplets seen in the UV-exposed trials could have bound to suspended solids or organic matter in the water column and settled to the bottom of the tank. The effect of suspended solids on bitumen behaviour, after it is spilled into an aquatic environment, may be best exemplified by the aftermath of the Kalamazoo River dilbit spill. As of August 2013, 3 years after the spill, an estimated 20–30% of the oil remained bound to the river sediment (King *et al.*, 2014; USEPA, 2013). The influence of suspended solids on whether or not diblit sinks is detailed in a report by Environment Canada (2013), and is discussed in detail below. Extrapolation of the results of the SL Ross study to a real-world, ecological context, is difficult owing to the lack of sediment in the experimental design.

In a recent study by Yarranton *et al.* (2014), a dilbit formulation called Cold Lake Winter Blend (CLWB) was placed onto glass plates and allowed to weather for 30 days. The effect of weathering on the density, viscosity and a solvent content of CLWB was determined. In their study, the authors determined that after 30 days the density of the CLWB had reached 0.998 g ml^{-1}. As the test was designed to represent the weathering of dilbit spilled onto water, a number of samples were also weathered on the water. There was no difference in evaporation rates if the dilbit was weathered on glass or water. These results mirror the results by SL Ross Environmental Research Ltd, in that UV-exposed dilbit weathered in a large tank had the same density (0.998 mg l^{-1}) as dilbit placed onto glass plates in the Yarranton et al. (2014) study. It is likely that the CLWB allowed to undergo evaporation would have the same fate in water as was seen in the previous study, namely a small amount of the product would disperse into the water column.

A study by Environment Canada, the Department of Fisheries and Oceans, and Natural Resources Canada investigated the role of suspended solids on weathered dilbit buoyancy (Environment Canada, 2013). In their study, they used two dilbit products, the Access Western Blend (AWB) and the Cold Lake Blend (CLB). These blends were chosen because they are transported through pipelines more than any other bitumen product. Both dilbit samples were weathered via a rotary evaporator for up to 48 h. After weathering for 48 h at 15°C, the density of both CLB and AWB was greater than that of freshwater but less than that of saltwater. The authors also determined that the addition of small-particulate matter (kaolin or diatomaceous earth), but not sand, resulted in the dilbit binding to the suspended particles and settling to the bottom of the water column. These suspended solids experiments demonstrate that particle size is the most influential factor determining whether or not weathered dilbit will sink or float. The authors state that qualitatively the oil-particulate aggregates bear a striking resemblance to those found in the Kalamazoo River after a major dilbit spill in 2010 (Lee *et al*., 2012). The study also reported that the viscosity of both CLB and AWB increased by four orders of magnitude when comparing the samples with the greatest amount of weathering to the untreated controls. The report demonstrates that weathering of dilbit greatly affects physical properties such as density and viscosity, and while the weathered products float in saltwater, their density is such that weathered CLB and AWB would be expected to sink in freshwater. The study also demonstrated that mixing dilbit with fine particulates will result in sinking of both weathered and fresh dilbit.

These studies indicate that the fate (i.e. buoyancy) of dilbit in a spill depends on the nature of the spill. If a ruptured pipeline spills dilbit directly into water, it will float until the weathering process, including evaporation of volatile, light organic molecules, and oxidation and photodegradation of other components, causes small droplets of oil to become neutrally buoyant. Therefore, if there is an immediate response to the spill, there is the possibility a dilbit spill could be treated like a conventional oil spill, with booming to prevent the spread of oil and skimming for removal of the dilbit. If, on the other hand, the spill occurs on land and the dilbit travels over land, it may release volatiles and potentially become saturated with solid particles before entering into a water body. One might speculate that the weathering that occurred while the dilbit moved over land will increase the density of the dilbit to a point that some portion of the dilbit would immediately sink upon entry into water. Therefore, the location of the spill in relation to the water body would dictate effective mitigation strategies. In addition, the presence of suspended solids would also result in dilbit sinking, as it would bind to small particles in the water and sink. Therefore, the suspended solids in a waterway and the degree to which the suspended solids are mixed by the water would also have an effect on if and how much dilbit would be found at the bottom of the water column. It would be expected, then, that a spill into a fast-flowing or turbulent river might result in a greater amount of sinking dilbit as opposed to a spill into a calm lake or pond. The Kalamazoo River spill represents a worst-case scenario in that dilbit was spilled onto land, the dilbit weathered as it travelled overland, and entered into a fast-flowing river containing small particulates in the water column. As discussed in the next sections, the Kalamazoo River spill has resulted in detrimental effects to the aquatic ecosystem. The fates of dilbit in a freshwater ecosystem are summarized graphically in Fig. 1.

Spills of bitumen-containing products

There have been two major spills of bitumen-containing products in the last few years. The first spill was the Kinder Morgan spill in 2007 where 224 000 litres of Albian heavy synthetic crude was released (Stantec, 2012). Albian heavy synthetic crude is a dilsynbit containing a condensate as well as synthetic light crude, in addition to the bitumen (Environment Canada, 2013). Approximately 100 000 litres of oil entered into Burrard Inlet as well as Kask Creek (Stantec, 2012). An estimated 5636 litres was not recovered and released to the marine environment (Stantec, 2012). A report by Stantec details short- and long-term testing that was done after the spill, measuring a variety of organic compounds related to oil in water, sediment, algae and invertebrates (mussels and crabs). The classes of organic compounds that were measured included polyaromatic hydrocarbons (PAHs), BTEX, light extractable petroleum hydrocarbons (LEPHs), heavy extractable petroleum hydrocarbons (HEPHs), phytane and pristane. Two weeks post-release, surface water samples taken throughout Burrard Inlet contained some PAHs at concentrations above provincial guideline concentrations, whereas extractable petroleum hydrocarbon concentrations were below detection limits. Sediment samples taken after the spill in the Burrard Inlet showed total extractable petroleum hydrocarbon (TEPH) concentrations had decreased 1–2 months after the spill considerably. Of the 78 sediment samples taken, 20 had PAH concentrations higher than the marine sediment quality guidelines for protection of aquatic life. Interestingly, PAH fingerprinting demonstrated that only 10 of the 20 contaminated sites had elevated PAH concentrations owing to the release of the crude into the environment. The 10 sites with elevated PAH concentrations owing to the spill were close to the release site. The other 10 sites had elevated PAH concentrations unrelated to the spill; the source of these PAHs was not discussed in the report. In the intertidal zone, the oiling and subsequent remediation activities resulted in the death of brown algae (*Fucus* spp.), leading to mortalities in fauna (sea stars, barnacles, mussels and limpets) that use brown algae as a habitat. Mussels (*Mytilus* spp.) and red rock crabs (*Cancer productus*), but not Dungeness crabs (*Metacarcinus magister*), had elevated PAH tissue concentrations. There were 18 different birds (15 Canada geese, *Branta canadensis*, two gulls and one pelagic cormorant, *Phalacrocorax pelagicus*) captured because of oiling. All but two geese were cleaned and released; one goose went to a different facility and one had an eye injury and was euthanized. No effects on fish or marine mammals were noted. In terms of long-term effects, as of 2011 only one endpoint had not met guideline requirements, namely the concentration of PAH in blue mussels (*Mytilus edulis*). The report by Stantec on the spill partly credits favourable environmental conditions at the time that aided in preventing a significant dispersal of the oil (Stantec, 2012). These favourable conditions include the fact that it was

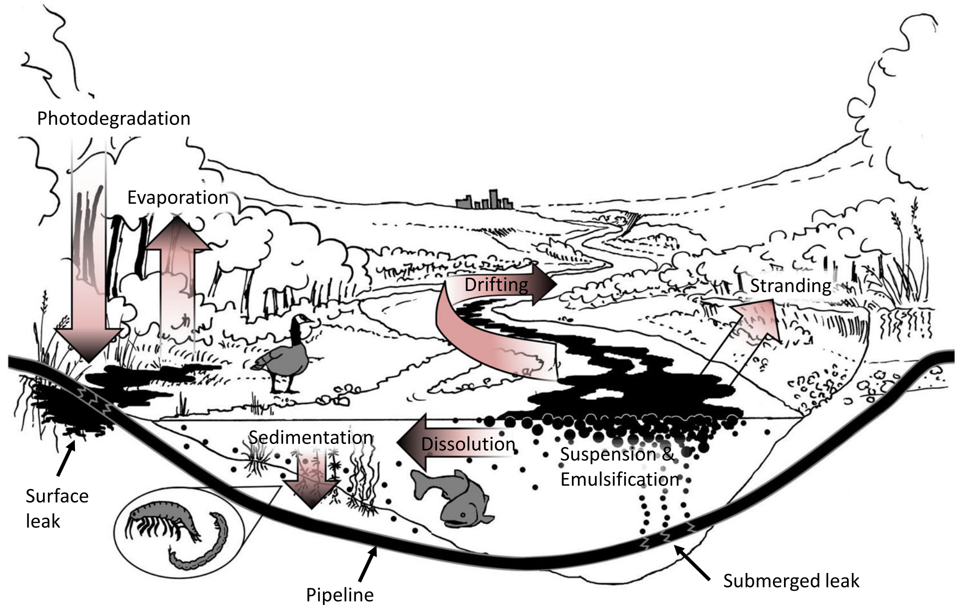

Figure 1. Effects of a dilbit spill into a freshwater ecosystem. Dilbit can enter into a freshwater ecosystem overland or via a leak in a submerged pipeline. In an overland spill, where there is weathering prior to entering a waterway, evaporation of diluents from dilbit will modify the product to a density greater than that of freshwater. Therefore, upon entering water some dilbit would be expected to sink immediately and mix with the sediment. With a submerged leak, the dilbit would be expected, at least initially, to float on the surface of the waterway. Over time, photodegradation would result in the diblit becoming neutrally buoyant, occurring up to 10 cm below the surface of the water. If the waterbody is slow-moving and does not have particulate matter, clean-up may be simple. However, any dilbit below the surface of the water can mix with particulates in the water column. Pipeline ruptures are more likely during high flow events that erode the channel thus exposing the pipe, and with overbank flows the released dilbit will filter through the riparian zone. With subsequent flow reduction, some material will be left on the floodplain, stranded in pools. Mixing of dilbit with particulate matter, and especially suspended sediments, results in the dilbit settling onto the bottom of the waterway and affecting sediment-dwelling organisms. Dissolution of bitumen into the waterway will have an effect on a variety of physiological endpoints in fish. The degree to which the spill spreads will also be dependent on the velocity of the waterway, a fast moving river will result in significant drifting that will cause a greater effect. Taken together, the fate and effects of dilbit following a freshwater spill depends on the type of spill, the amount of weathering of the dilbit, the velocity of the waterway, and the type and amount of particulate matter in the waterway.

not raining (rain would have hastened the movement of the oil into Burrard Inlet) and there was a slack tide (the incoming tide helped keep oil near the shore while booms were placed). In addition, the spill did not coincide with salmon migration periods and was outside the migration and over-wintering period for birds, preventing the spill from affecting these animals. Even although this study measured the effects of the spill in a wide variety of organisms, aside from the death of brown algae and related intertidal fauna and the oiling of a number of birds, it is unknown what effect, if any, the spill had on the aquatic ecosystem as only muscle tissue concentrations of organic compounds were measured in mussels and crabs. It is important to note that this spill occurred in a marine environment, which is outside the scope of this review, however, this spill and recovery from the spill demonstrates that when a bitumen-containing product is spilled into water and it floats, collection of the product is possible and minimizes the impact on aquatic ecosystems. In addition, there was still some component of the dilsynbit that sank, as there were elevated concentrations of organic compounds in the sediment. Taken together, this means that even in a best-case scenario where the material floats and the majority can be recaptured, some material will sink and be taken up into the ecosystem.

The most studied dilbit spill occurred in Marshall, MI, in 2010 resulting in extensive contamination of the Kalamazoo River. Up to 3.2 million litres (843 000 gallons) of material was released after a pipeline rupture (USEPA, 2013). The dilbit initially pooled in a marshy area before flowing 213 m (700 feet) into Talmadge Creek, eventually entering into the Kalamazoo River (National Transport Safety Board, 2010). Responders to the spill noted the presence of floating, submerged and sunken oil, which demonstrates that at least part of the dilbit immediately sank below the surface of the water (Crosby *et al*., 2013). The sinking of the dilbit may have been as a result of weathering that would have occurred as the dilbit flowed from the leak to the creek. In fact, there was a voluntary evacuation order for the area surrounding the spill owing to the presence of unsafe concentrations of benzene in the air, demonstrating that volatile organic compounds evaporated (CCPHD, 2010). Approximately 10–20% of the spilled oil mixed with sediment owing to the movement of water in the river and sank to the bottom of the river (USEPA, 2013). An analysis of the sunken particles demonstrated the presence of oil droplets encrusted with sediment (Lee *et al*., 2012). Both weathering of dilbit and binding to particulates (i.e. suspended solids) is what likely caused a significant portion of the dilbit to sink. In addition to affecting different groups of animals (detailed in the next section), the major ecological disturbance caused by the spill into the Kalamazoo River relates to the removal of bitumen from river sediments.

A number of novel techniques were employed to free the dilbit from sediment. These techniques included agitation using rakes, dragging chains through the sediments, spraying water into the sediments and driving a tracked vehicle back and forth over the sediments (Muller, 2012, Winter and Haddad, 2014). Any oil that was released was then collected with absorbent booms. These novel techniques were not entirely successful at removing bituminous materials from river sediments. Consequently, dredging of the affected section of the Kalamazoo River continues to the present day, resulting in the removal of over 1.2×10^5 m^3 (160 000 cubic yards) of dredge waste to date (USEPA, 2014). Future dredging will remove the remaining bitumen. However, it will also remove and damage the habitat used by a wide variety of species. Studies have demonstrated that dredging results in reduced abundance and diversity of benthic fish (Freedman *et al*., 2013), alters fish foraging ability (Abrahams and Kattenfeld, 1997), and decreases total invertebrate abundance and biomass (Milner and Piorkowski, 2004). A Net Environmental Benefits Analysis of the clean-up options by the USEPA identified numerous potential impacts of agitation and removal of the sediment including lethal effects on benthic invertebrates, eggs, larvae of bivalves and fish, and aquatic macrophytes (Winter and Haddad, 2014). The extensive and repetitive dredging might ultimately cause as much of an impact on the Kalamazoo River ecosystem as the original spill.

These two spills demonstrate that bitumen-containing products can sink or float when spilled into a waterway, depending on a number of factors including weathering of the product prior to entering a waterway and the presence of particulates within the water column. As these two case studies demonstrate, if the product floats it can be more easily removed, and the ecological impact can be minimized. However, if the product sinks, there can be profound and long-lasting effects on the aquatic ecosystem.

Effect of dilbit and bitumen on aquatic ecosystems

Although several reviews have been published on the toxicity of major constituents of dilbit/synbit, specifically naphthenic acids (Headley and McMartin, 2004), and Brown and Ulrich (2015), PAHs (Ball and Truskewycz, 2013; Manzetti, 2012), metals and their mixtures (Gauthier *et al*., 2014), to date, there are apparently no published studies to determine the effect of synbit or dilsynbit on aquatic ecosystems. When considering the effects of a dibit spill on a freshwater ecosystem, it is important to not only consider the effects of the product as a whole, but to consider the effects of the product's constituents as well. Condensate will evaporate after a spill, leaving behind a heavier fraction of diluted bitumen. For that reason, we discuss both the effects of bitumen-containing products and bitumen itself on aquatic ecosystems.

When interpreting the effects of dilbit or bitumen on aquatic ecosystems, it is essential to consider trophic cascades. A trophic cascade can occur when organisms from one trophic level are affected by a contaminant to a greater extent than other trophic levels in an ecosystem (Carpenter *et al*., 1985; Fleeger *et al*., 2003). There are two different models of trophic cascades, depending on what trophic level is affected. A top-down trophic cascade occurs when top predators or grazers are affected by a contaminant resulting in population-level effects in prey populations. A reduction in predator or grazer populations most often results in increases in populations of species occupying lower trophic levels (Fleeger *et al*., 2003). For example, if a grazer population is reduced by a contaminant then populations of primary producers are expected to increase. In a bottom-up cascade, if primary producer populations are affected, either by increased or decreased production, all trophic levels above the primary producer can be affected as well (Fleeger *et al*., 2003). For example, an increase in primary producers can increase primary and subsequently secondary consumers owing to a greater food supply whereas a reduction in primary producers will have the opposite effect. While death of organisms of a specific trophic level is often the cause of a trophic cascade, cascades are also seen when members of a specific tropic level are impaired in their ability to sense and/or obtain food (Fleeger *et al*., 2003).

Effects of dilbit on aquatic animals

To date, the majority of data describing the effects of dilbit on freshwater aquatic organisms were collected in relation to the Kalamazoo River spill. Currently, there is an on-going natural resource damage assessment of the spill by the National Ocean and Atmospheric Agency (NOAA) (J. Winter, Personal communication). Because the natural resource damage assessment of the Kalamazoo River spill is on-going, and as this is a legal process under the Oil Pollution Act in the United States, some of the results are confidential until a settlement is reached or litigation completed (J. Winter, Personal communication). However, some limited information concerning the effects of the Kalamazoo River spill has been released via two conference presentations from NOAA (Winter, 2013; Winter and Haddad, 2014), limited invertebrate testing with sediment from the Kalamazoo River and a report from the U.S. Geological Survey (USGS) (Papoulias *et al*., 2014). In addition, a recent, unpublished study has established the acute toxicity of dilbit to freshwater fish (P. Hodson and V. Langlois, Personal Communication).

Invertebrates

Toxicity testing with *Chironomus dilutus* and *Hyalella azteca* using sediment collected from affected areas of the Kalamazoo River post-spill demonstrated some sediment samples caused a significant increase in mortality (Great Lakes Environment Center, 2012). Results suggested that it was a combined effect of oil residues and other sediment characteristics that led to decreased *Chironomus dilutus* survival, whereas the mortality of *Hyalella azteca* was correlated to total extractable hydrocarbons (Fitzpatrick, 2012). These studies demonstrate that the dilbit that sank to the bottom of the river would, in fact, be acutely toxic to the invertebrates living in and on the sediment. There was also a change in the community structure of macroinvertebrates in the Kalamazoo River in 2010 and 2011 as a result of the spill, which was attributed to increased light owing to the loss of vegetative cover (Winter, 2013). It is unclear whether the shift in community structure was as a result of photodegradation of bitumen due to the increased light or simply an abiotic effect on the habitat. There was also evidence of mussel mortality in affected areas, but not in uncontaminated regions of the river (Winter, 2013). Therefore, more work is needed to determine if the effects on these invertebrate groups, which are primary consumers, have resulted in a bottom-up trophic cascade.

Vertebrates

After the Kalamazoo River spill, approximately 3000 turtles, 170 birds and 38 mammals were brought into rehabilitation centres with a survival rate of 97%, 84% and 68%, respectively (Winter, 2013). No fish kills were observed immediately after the spill. However, subsequent surveys noted a reduction in the fish community diversity and abundance in both Talmadge Creek and the Kalamazoo River (Winter, 2013).

A recent study from the US Geological Survey compared the expression of a wide variety of biomarkers in smallmouth bass (*Micropterus dolomieu*) and golden redhorse (*Moxostoma erthrurum*) collected from three oiled sites and a reference site 3 weeks after the Kalamazoo River spill (Papoulias *et al.*, 2014). The pattern of biomarker modulation indicated that fish from oiled sites had been affected by the spill and mirrored the pattern of the effect that is seen in laboratory exposures of fish to PAHs. Metrics of general health (condition factor, hepatosomatic index and health assessment index) all indicated that fish from oil-contaminated areas had a poorer health as compared with fish from the clean site. Macrophage aggregates in tissues, spleen lesions, gill lesions and mucocytes on gill lamellae were observed with increasing frequency on fish from contaminated sites as compared with the reference site. There was also a higher expression of CYP1A, a biomarker of exposure to PAHs, in fish from contaminated portions of the river as compared with fish from the clean site. No differences were seen in haematological measures or parasite load among fish from the clean and contaminated sites. Collectively, the results demonstrated that there was a measurable effect on fish health in two species of fish 3 weeks after the spill, and that the pattern of effects closely resembled those usually associated with exposure to PAHs.

In an unpublished laboratory study, medaka (*Oryzias latipes*) embryos were exposed to Access Western Blend, a dilbit, to determine how a dilbit affects fish embryos (P. Hodson and V. Langlois, Personal Communication,). Exposure of embryos to dilbit induced toxicity in a pattern consistent with other crude oils and many alkyl PAH compounds. The toxicity was lower than expected, but this was most likely a result of a low concentration of alkyl PAHs in the dilbit used. Research into the role of low-molecular-weight hydrocarbons in toxicity, specifically correlating molecular responses to exposure, is on-going.

Effects of bitumen on aquatic organisms

A number of studies have investigated the effect of bitumen on aquatic organisms, specifically microbes, fish and mammals. While some of the studies expose organisms to bitumen under controlled laboratory conditions, a number of these studies compare organisms in clean areas to those in areas with naturally occurring bitumen and areas that are being mined for bitumen. While the studies focus primarily on the effects of bitumen on fish, the effect of bitumen on microbes and mammals have also been investigated. These effects, as well as the ecological implications of the effects, are discussed below.

Microbes

In a laboratory study using a rotating biofilm bioreactor, exposure of biofilms to bituminous compounds from the Athabasca River and three tributaries resulted in less productive and lower biomass biofilms as compared with the controls (Yergeau *et al.*, 2013). These changes were attributed to a decrease in the activity and abundance of photosynthetic organisms. As discussed above, a decrease in primary producers (such as those found in the biofilms) can result in a bottom-up trophic cascade. In addition, it has been demonstrated that biofilms produce compounds, including amino acids, which give streams a scent detectable by fish. As fish rely on their sense of smell to navigate waterways and to follow migratory routes, affecting the smell of a river by affecting biofilm present in the river may result in fish not being able to follow migratory routes or find breeding grounds (Shoji *et al.*, 2000; Yamamoto and Ueda, 2009; Ishizawa *et al.*, 2010; Yamamoto *et al.*, 2013,).

Fish

The majority of studies on the effects of bitumen on aquatic animals have focused on fish. Studies include lethality testing, effects on reproduction and effects on early life stages.

Effects of weathered bitumen on fish

Small rainbow trout (*Oncorhynchus mykiss*), 2.7–6 cm fork length and 0.2–2 g live weight, were exposed in the laboratory to whole, weathered bitumen (i.e. bitumen collected from a bank of the Athabasca River) and fractions of weathered bitumen (Alberta Environmental Centre, 1986). The 96-h LC50s for rainbow trout exposed to the various bitumen fractions were all greater than 100 mg l^{-1}, demonstrating, as interpreted by the authors, a very low toxicity. Exposure to sublethal and lethal concentrations of the various fractions had no effect on histopathological or haematological endpoints.

Effects of transplantation to a bitumen-containing lake

Male yellow perch (*Perca flavescens*) that were transplanted from a reference reservoir into a lake lined with bitumen-containing sodic clays exhibited no differences in testosterone or 11-ketotestosterone concentrations as compared with fish from the reference reservoir (van den Heuvel *et al.*, 2012). In female perch transplanted to the bitumen-containing lake, there was no change in estradiol; however, there was a marked increase in testosterone compared with fish from the reference reservoir. These changes in hormone concentrations may affect reproduction in perch. Interestingly, even although it is not discussed in the study, there was a greater weight, length and condition factor in fish stocked into the bitumen-containing lake compared with fish held in the source lake. While this increase in morphological condition may be due to another factor in the lake, it is possible the increased growth is a response to the contamination. If the increased growth is as a result of the contamination, it may be that the contamination affects metabolic, hormonal or other physiological pathways of the perch, resulting in faster growth and earlier sexual maturation, prior to the onset of pathologies and mortality. Perch from metal-contaminated lakes have also shown an increased condition factor as compared with fish from clean lakes (Couture and Pyle, 2008). The presence of bitumen in the water may be causing populations of yellow perch to develop and mature faster, compared with populations in pristine systems, activating responses to ensure mating occurs before death.

Effects of bitumen-containing sediment

A number of studies have compared the effects of sediment collected from reference sites, sites containing naturally occurring bitumen deposits, and in some cases sites with active bitumen mining and processing from the Athabasca oil sands region (Colavecchia *et al.*, 2004, 2006, 2007; Tetreault *et al.*, 2003a, 2003b). Collectively, these studies have assessed the effect of bitumen exposure during the early life stages of a wide variety of fish. Fathead minnows (*Pimephales promelas*) showed an increased time to hatch, decreased hatching success, increased larval mortality, reduced size of hatched larvae and reduced steroid production when exposed to sediment from naturally occurring bitumen deposit sites as compared with reference sites (Colavecchia *et al.*, 2004). Early life stage experiments with white suckers (*Catostomus commersoni*) demonstrated increased mortality and teratogenesis, and decreased growth and weight in embryos exposed to sediment from naturally occurring bitumen deposit sites as opposed to reference sites (Colavecchia *et al*., 2006). In a study directly comparing the effect of sediment from bitumen deposit sites on white suckers and fathead minnows, there was increased mortality and eye damage as compared with fish exposed to reference site sediment (Colavecchia *et al*., 2007). A study with slimy sculpin (*Cottus cognatus*) and pearl dace (*Margariscus margarita*) exposed to sediment from reference sites, naturally occurring bitumen deposit sites, and active bitumen mining and processing sites demonstrated that induction of steroids in gonadal tissue was reduced in slimy sculpin, but not pearl dace (Tetreault *et al*., 2003a, b).

While young rainbow trout are unaffected by the presence of bitumen, it is apparent that the early life stages of a wide variety of fish species are. Over time, these effects may result in lower fitness (i.e. reproductive output) in individuals of the affected species, resulting in decreases in fish populations. Therefore, any bitumen left in sediments from a dilbit, synbit or dilsynbit spill can have detrimental effects on fish populations.

Mammals

The biggest concerns with mammals in a spill scenario are oiling of the animals and inhalation of fumes that evaporate from the spill. Rats exposed to bitumen fumes had increased gene expression in their lungs associated with a lung inflammatory response as well as genes associated with PAH mobilization and detoxification (Gate *et al*., 2006). Bitumen fumes have been demonstrated to be mutagenic in mammals and carcinogenic in humans (Binet *et al*., 2002). Contamination of skin with bitumen fume condensate resulted in uptake of the components of the condensate and metabolites into lymphocytes in the lungs (Binet *et al*., 2002). In the event of a spill, if dilbit travels overland before entering an aquatic environment, terrestrial animals may come into direct contact with the product and be affected. Even if terrestrial animals do not come into direct contact with the bitumen, there is the potential for inhalation of bitumen fumes.

Projected effects of dilbit and bitumen on aquatic vegetation

Apart from one study that reported that bitumen, but not water contaminated with bitumen, had a toxic effect on the micro algae *Pseudokirchneriella subcapitata* (Debenest *et al*., 2012), we were unable to find studies that investigated the effect of dilbit, synbit, dilsynbit, or bitumen on aquatic vegetation. In terms of the Kalamazoo River spill, in 2010 and 2011 there was a loss of aquatic vegetation, which most likely occurred owing to oiling of the plants (Winter, 2013). Our unpublished research indicates that dilbit generally has similar effects on riparian plant material that spills with other oils have. Therefore, the effects of dilbit spills on aquatic vegetation may be expected to be similar to the effects seen with other types of oil.

When considering the effects of dilbit on aquatic vegetation, it is important to consider a wide variety of plants including periphyton, aquatic macrophytes, floating plants, emergent plants and riparian plants. Periphyton is a form of biofilm consisting of a variety of organisms including photosynthetic algae and cyanobacteria. This biofilm is found in bed sediments in shallow, clear water, and would be relatively unaffected by floating dilbit, however, it would be smothered by sinking dilbit. Dilbit would have a similar impact on aquatic macrophytes, in that floating dilbit would not have an effect, whereas sinking or neutrally-buoyant dilbit could coat macrophytes resulting in an impairment of photosynthesis by blocking light. In contrast, floating plants would probably be more severely affected by floating dilbit as they are found on the surface of waterways. There may not be complete coverage of the photosynthetic area of floating plants, such as water lilies, as some floating plants have raised rims that may confer some protection. Emergent plants, such as cattails (*Typha latifolia*), rushes, sedges and reeds would be affected by both floating and sinking dilbit. Floating dilbit would coat bands around the emergent plant shoots, probably blocking gas exchange in these segments. While riparian plants are not in aquatic environments, they surround aquatic environments, and it would be expected that this group would be affected as well. As pipeline ruptures are more likely with flood events when the flowing water is over-bank, the floodplain would become inundated with flood-waters and floating or suspended dilbit resulting in coating of riparian shrubs and trees. Any coated leaves would have gas exchange blocked owing to the occlusion of stomata and this would prevent carbon dioxide uptake and photosynthesis, as well as the opposing release of transpirational water vapour. After the foliar suffocation, the coated leaves would subsequently senesce (yellow) and abscise (fall off), probably within a few weeks.

While there is relatively little information on how aquatic plants would be affected by exposure to dilbit, it is likely that the major effect would be as a result of coating of the plant material. The coating of leaves could result either in the death of the plant in the case of floating plants, aquatic macrophytes and periphyton or the loss of leaves in riparian plants. Given that plants will grow through asphalt, and also that bitumen has been used as a substrate binder to stabilize sand dunes, rocky mountain slopes, or other zones prior to reclamation plantings (Barr and McKenzie, 1976; Jordan and Sampson, 1967), it is unlikely that there are compounds released from bitumen or dilbit that would exert a direct toxic effect.

Knowledge gaps

There is a great deal of research to be done to determine how a dilbit, synbit or dilsynbit spill would affect freshwater ecosystems. The first major knowledge gap is to determine if weathered dilbit sinks or floats in freshwater. In the study by Environment Canada (2014), they reported that upon weathering the density of dilbit dropped to below that of freshwater. However, the weathered dilbit was placed into saltwater to determine if it floated or sank, not freshwater. It would be of great use to perform weathering on dilbit similar to what was done in the Environment Canada study and determine if the weathered dilbit sinks or floats when introduced to freshwater.

A systematic approach to establish species sensitivities based on the buoyancy of dilbit, synbit, or dilsynbit is necessary. For example, organisms occupying littoral zones and epilimnions will be more affected by floating fractions than neutrally buoyant or sinking fractions, pelagic species will be at risk from exposure to neutrally buoyant fractions and benthic species will be threatened by sinking fractions. These species groups (littoral/epilimnetic, pelagic and benthic) should then be tested for their relative sensitivities to floating, neutrally-buoyant or sinking fractions, respectively. By developing species sensitivity distributions on the basis of contaminant buoyancy ecological risk assessment can be greatly improved (Posthuma *et al.*, 2002).

These species sensitivity distributions should be developed for both acute and sublethal exposures. Acute exposures will allow for the establishment of acute toxicity threshold concentrations [such as the lowest observed effect concentration (LOEC) for different dilbit, synbit, or dilsynbit formulations]. Chronic endpoints should focus on parameters related to survival, growth and reproduction (i.e. the same criteria used by the Canadian Council for Ministers of the Environment to develop water-quality criteria for the protection of aquatic life) (CCME, 1999). These studies would contribute, for example, to understanding the factors that can either directly (e.g. acute toxicity) or indirectly affect growth (failure to meet nutritional demands), survival (failure to prevent predation or herbivory) and reproduction (failure to propagate genes to the next generation). Some examples of indirect effects on growth include the inability of plants to photosynthesize owing to their leaves being covered in viscous contaminant; or fish failing to find food either because of increased contaminant sensitivity of the food species or because of contaminant-impaired sensory input required to locate food (Tierney *et al.*, 2010). Similarly, indirect effects on survival include an inability of plants to access resources (bioaccessibility) or animals to find food or avoid predators. Examples of impaired reproduction could result from impaired reproductive physiology, failure to locate perennial spawning habitat or failure to locate or identify appropriate mates (e.g. impaired chemosensory function).

Another important consideration is to understand which environmental variables could affect dilbit, synbit or dilsynbit toxicity. Our review has identified a few confounding influences, such as photodegradation. However, a systematic approach should be adopted to understand how variables such as temperature, ionic water composition, dissolved the organic material, dissolved oxygen and pH could influence contaminant toxicity. This information will be important when attempting to estimate risk in different areas of the country. For example, photodegradation is probably more important in the open grasslands of the prairies than it is under dense forest cover of northern Québec. The complex water chemistry of central and western Canada will probably have a different influence on contaminant toxicity than the soft, sensitive waters typical of the Boreal Shield.

Developing an understanding of the basic mechanisms of biological uptake, accumulation, distribution, depuration, and toxicity is vital to both estimate and mitigate risk to sensitive species inhabiting contaminated habitats. This information could be useful for developing a basic kinetics model that can be used to make predictions about toxicity. Such an approach has been successfully applied for other contaminants to the point where the resulting model has been used for establishing environmental criteria (e.g. the biotic ligand model makes use of the same basic information) (Niyogi and Wood, 2004).

Conclusions

In conclusion, the effect of a dilbit, synbit or dilsynbit spilled into a freshwater ecosystem is not well understood. The most important consideration is the fate of the spilled material upon entry into a waterway, namely, will the material sink or float. If there was a spill directly into a freshwater environment containing little-suspended solids followed by a quick clean up, it would be expected that little material would be taken up into the ecosystem. However, if enough compounds volatilize to drop the density of the material to below that of freshwater, or if there is a significant amount of suspended solids in the waterway that will bind to the spilled material, the material will fall to the sediment and may cause a considerable amount of environmental damage. The extent to which dilbit, synbit or dilsynbit is taken up into an ecosystem thereby affecting various components of the ecosystem is mostly unknown, however, it is clear based on the currently available literature, that a spill with this material like other crude products has the potential to cause drastic effects across multiple trophic levels.

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Conflict of interest

The Authors did not report any conflict of interest.

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