Assessment of Florpyrauxifen-benzyl, the Active Ingredient in ProcellaCOR EC

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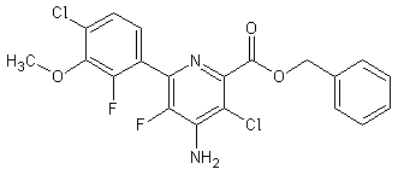
Recommendations

1) Since Shawnigan Lake is significant source of water for thousands of people, and since there has been no testing for potential toxicity of long-term intake of florpyrauxifen nor hydroxy-florpyrauxifen, it is recommended that the efficacy of florpyrauxifen-benzyl in killing Eurasian watermilfoil be done in a body of water that is not a source of drinking water.
2) Further, if such testing is done, then not only must the aquatic plants be monitored, so must the vertebrates and invertebrates that inhabit that body of water.
3) Further, the distribution of florpyrauxifen-benzyl and its metabolites should be measured over time, not only at the site of administration but also at the epilimnion throughout that body of water as well as in deeper sites, and in the sediment.
4) There needs to be testing for vertebrate toxicity of florpyrauxifen and, particularly, hydroxy-florpyrauxifen. The fact that repeated ingestion of florpyrauxifen-benzyl in mammals and birds showed no long-term toxic effect and even though florpyrauxifen and hydroxy-florpyrauxifen were found in liver and kidney in significant amounts does necessarily mean that these compounds when ingested have no toxic effects. If florpyrauxifen and hydroxy-florpyrauxifen were formed in the liver and kidney, then these compounds could be quickly inactivated through the actions of phase 3 enzymes and excreted through the bile and urine, respectively. I think there should be studies examining the long-term effect of chronically ingesting florpyrauxifen and hydroxy-florpyrauxifen in drinking water, with an emphasis on examining the gut epithelium and blood vessels that should compounds would initially encounter.

ProcellaCOR EC

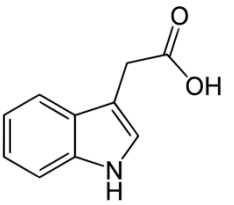
ProcellaCOR EC is being touted as a method to remove Eurasian watermilfoil from bodies of water. The active ingredient of ProcellaCOR EC is florpyrauxifen-benzyl. The EPA (2017), the Australian Pesticides and Veterinary Medicines Authority (2018), the European Food Safety Authority (2019), the Food Safety Commission of Japan (2019) and Health Canada (2023) have all found florpyrauxifen-benzyl to be safe in getting rid of unwanted weeds with no untoward effect on vertebrates and invertebrates.

The chemical formula of florpyrauxifen-benzyl, also known as XDE-848 BE, is shown on the right. The formal International Union of Pure and Applied Chemistry (IUPAC) name is: benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate.



florpyrauxifen-benzyl

The mechanism of action of florpyrauxifen-benzyl is that of an auxin, a plant hormone family that regulates plant growth. The first described, and most common, auxin is indole-3-acetic acid shown on the right. Auxins bind to a family of proteins known as TIR1/AFBs. Binding of auxins to TIR1/AFBs results in major changes in gene expression whose end result may include increases in growth rate and changes in responses to environmental stress.



Indole-2-acetic acid

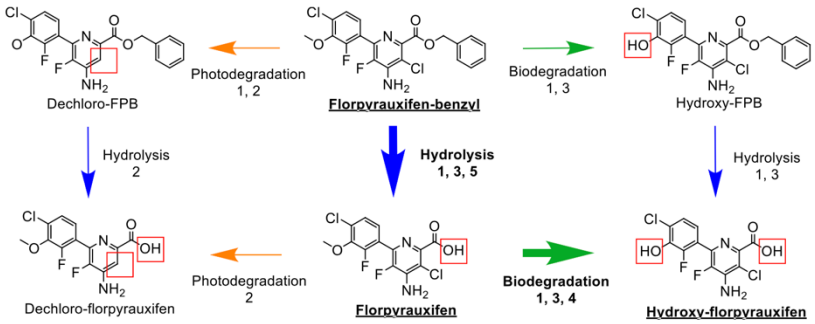
Florpyrauxifen-benzyl has a relatively low water solubility (15 ppb or 15 µg/L, i.e., 34 nM at 20° C: EPA, 2017). The octanol/water partition coefficient (K_{ow}) is high suggesting it can readily adsorb/absorb onto/into organic matter but fish studies have shown that it does not bioaccumulate, possibly due to either not being absorbed by gut tissue or it being rapidly metabolized (EPA, 2017). In contrast in many water plants there is accumulation in growing shoots to the level of 20 to 40 µg/g tissue (Haug et al. 2021).

Studies by Wang *et al.* (2021) have shown that florpyrauxifen-benzyl causes increased synthesis of abscisic acid and ethylene in *Echinochloa crus-galli*, a wild grass that is a major weed in rice fields Abscisic acid is a plant hormone produced in response to a variety of stresses resulting in inhibition of growth, closure of stomata, etc. Ethylene is also a plant hormone whose synthesis is increased following stress. One effect of ethylene is to promote maturation and senescence. Increased production of abscisic acid and ethylene likely are major factors that account for the toxicity of florpyrauxifen-benzyl to plants but does not explain the selectivity of the herbicide for certain plants. This selectivity may have to do with higher growth rates of the targeted plants since there appears to be preferential uptake in growing shoots (Haug et al. 2021). Another study has shown that with two other weeds in rice paddies, florpyrauxifen-benzyl had an order of magnitude greater inhibition on yerbadetajo (a dicotyledon) than on barnyard grass (a monocotyledon) and this correlated with greater induction of abscisic acid and the ethyelene precursor 1-aminocyclopropane-1-carboxylic acid in yerbadetajo compared to barnyard grass (Gao et al. 2022). Note that 1-aminocyclopropane-1-carboxylic acid is easier to measure than ethyelene. One cannot conclude from this study that monocotyledons in general are more resistant to florpyrauxifen-benzyl than dicotyledons since another study has shown (Haug *et al.* 2021) that some dicotyledons such as Crested Floating Heart can have low uptake of florpyrauxifen-benzyl while some monocotyledons such as the Common Hornwort have high uptake of florpyrauxifen-benzyl.

There have been several field studies in North America examining the efficacy of florpyrauxifen-benzyl in getting rid of Eurasian watermilfoil. In one study (Davidson, 2023), florpyrauxifen-benzyl was sprayed at the rate of 1.08 kg/acre that resulted in an order of magnitude or greater reduction of Eurasian watermilfoil three weeks after treatment. The nine species of native aquatic plants either had no change in abundance or had increased abundance. Similar findings were reported by Bloodsworth *et al.* (2022) in a Minnesota lake where the reduction of Eurasian watermilfoil was was one to two orders of magnitude, depending upon site, and little impact on native aquatic plants.

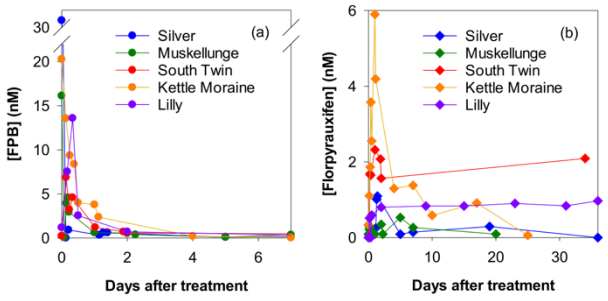
Metabolism of Florpyrauxifen-benzyl

The metabolism of florpyrauxifen-benzyl is shown on the right. This is Figure 4 in White *et al.* (2023). Additional information can be found in the supplemental material associated with the paper and in the MSc thesis of Sydney Van Frost (2023). The dominant pathway is an hydrolysis (de-esterfication) with the formation of benzoic acid and florpyrauxifen (XDE-848). The mechanism is mainly photolysis by light in the UV range and to some extent by biodegradation. A minor pathways is a photolytic dechlorination forming dechloro-florpyrauxifen-benzyl that in turn is photolytically de-esterfied forming dechloro-florpyrauxifen. A second minor pathway is an hydroxylation through biodegradation forming hydroxy-florpyrauxifen-benzyl that in turn is de-esterfied forming hydroxy-florpyrauxifen. The florpyrauxifen formed in the dominant degradation pathway has a minor photodegradation pathway forming dechloro-florpyrauxifen and a major biodegradation pathway forming hydroxy-florpyrauxifen.

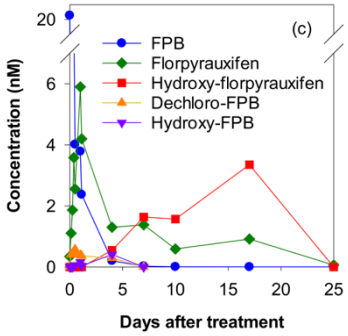


In laboratory experiments, in the absence of light, the half-life of florpyrauxifen-benzyl in lake epilimnion water is in the order of 25 days (Zhou et al. 2023). A more relevant study was done by White *et al.* (2023) who did florpyrauxifen-benzyl degradation studies from samples taken from field trials in five lakes in Wisconsin. Soon after application florpyrauxifen-benzyl reached a near saturation level of 32 nM in the epilimnion but this quickly decreased due to photolytic degradation and advection. The field trials were carried out during the summers of 2021 and 2022. Depending on the lake, anywhere from 5-12% of the lake surface area was sprayed. Within 4-6 hr there was an homogenous distribution throughout four of the five lakes. Note that these lakes ranged in surface area from 20% to 50% of that of Shawnigan Lake. The half-life of florpyrauxifen-benzyl ranged from 0.2 to 3 days in the five lakes.

An illustration of the dynamics in the epilimnion region of the treatment zone of the five lakes is given in the adjacent 'a' and 'b' components of Figure 1 from the White *et al.* paper. The 'a' part of the figure shows the rapid decrease in concentration of florpyrauxifen-benzyl. This decrease is due both to photolytic degradation and to advection. The 'b' part of the figure shows changes in concentration of the major metabolite, florpyrauxifen. Again the rapid decrease in concentration of florpyrauxifen can be mainly attributed to advection. Note that florpyrauxifen has a very long half-life since in some lakes the concentration was 1-2 nM even after 30 days post-treatment: although measured in the epilimnion region of the treatment zone, because of advection this must be the concentration throughout the lake.



The fate of florpyrauxifen formed is illustrated in the adjacent figure which forms the 'c' part of Figure 1 in the White *et al.* paper. The data are taken from the epilimnion region of the treatment zone of Kettle Moraine Lake, where 8.6% of the surface area was treated. Note that in this lake the dominant metabolite is hydroxy-florpyrauxifen one week after treatment and at this time the other metabolites, other than florpyrauxifen, are close to being undetectable. Hydroxy-florpyrauxifen becomes close to undetactable by 25 days after treatment in this lake. Kettle Morraine Lake is 84.6 hectares in area with a maximum depth of 9 metres. In comparison, Shawnigan lake is 553 hectares in area with a maximum depth of 50 metres and a mean depth of 12 metres.



In the absence of light, florpyrauxifen-benzyl that reaches the sediment is stable for much longer periods and was detected at levels of about 0.5 nmoles/Kg dried sediment up to 50 days after treatment.

Animal Toxicity Studies

The data presented by the proponent shows that administering florpyrauxifen-benzyl at high levels into a number of mammalian species as well as the chicken exhibited no toxicity nor carcinogenicity. There apparently is little toxicity towards fish or insects. Unfortunately, there are a paucity of data from independent laboratories.

Buczek et al. (2020) did acute toxicity testing of florpyrauxifen-benzyl with the juveniles of freshwater mussels *Lampsilis siliquoidea* and *Lampsilis radiata.* The juvenile mussels were tested over a 72-hr period in a laboratory setting with concentrations that ranged from 9 µg/L to supersaturated 2187 µg/L. No toxicity was observed with *L. siliquoidea* but at all concentrations tested, there was a 3% mortality rate for *L. radiata*, suggesting the mortality was not due to florpyrauxifen-benzyl or its metabolites. The problem is that it was not a long-term study.

Issues

1) I could find no long-term toxicity studies that were not performed by the proponent. However, all licencing agencies were satisfied that there were no acute nor long-term effects of administering florpyrauxifen-benzyl, even at doses orders of magnitude higher than used to eliminate unwanted aquatic plants.
2) I could find no long-term field studies that examined potential effects of florpyrauxifen application on vertebrates and invertebrates. In some lakes florpyrauxifen could be as high as 1-2 nM in the epilimnion throughout the lake more than a month after application. There have been no long-term studies to determine whether this has any effect on the vertebrate and invertebrate populations.
3) Concentrations of hydroxy-florpyrauxifen in the epilimnion of the lake ranged from 1-3 nM at 2-3 weeks after application of florpyrauxifen-benzyl. There have been no studies on the effect of hydroxy-florpyrauxifen on vertebrate and invertebrate populations.

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