# Review of non-target impacts of imidacloprid [notes prepared by Jon Sweeney, Natural Resources Canada, Canadian Forest Service, 1350 Regent Street, Fredericton, NB, E3C 2G6, June-July 2021]

As McCarty (2020) points out, in making decisions regarding the use of insecticides to protect hemlocks from HWA, we must weigh the potential negative effects of insecticide use on nontarget species of wildlife against the potential negative effects of not protecting the trees and letting them die.

## What are the consequences of not protecting hemlocks from HWA infestations?

Mature eastern hemlock forests support a unique assemblage of arthropods, birds, and other wildlife (Buck et al. 2005, Dilling et al. 2009, Ross et al. 2004; Snyder et al. 2002). Hemlock is important winter habitat for white-tailed deer (Reay et al. 1990) and porcupines (Greisemer et al. 198) and is nesting habitat for woodland hawks (Bosakowski et al. 1992). Deep shade provided by the dense canopy of hemlock stands produces a cooler, damper microclimate in the forest understory compared to other coniferous and deciduous forests (Rogers 1978; Hadley 2000). Once infested with HWA, hemlocks begin to deteriorate and usually die in 4–15 years depending on site conditions. As the canopy thins and hemlock die, species dependant on this unique habitat (such as the Acadian flycatcher, *Empidonax virescens,* blackburnian warbler, *Dendroica fusca,* and hermit thrush, *Catharsus guttatus*) disappear (Tingley et al. 2002). In the northeastern US, the numbers of hemlock-associated birds have declined with decline in habitat quality as a result of HWA infestation (Tingley et al. 2002). Canopy thinning by HWA also increases light levels at the forest floor, altering soil chemistry (Orwig et al., 2008), and increasing understory vegetation, including invasive plant species such as garlic mustard and tree-of-heaven (Eschtruth et al., 2006). Increased light levels also increase soil and water temperatures which may reduce the abundance of brook trout which so not spawn in water > 15°C (Jenkins and Burkhead 1993; Snyder et al. 2002).

**Although researchers in the US and Canada are testing and developing biological controls, silvicultural treatments, and breeding of genetic resistance in efforts to manage the HWA (Havill et al. 2016), the only method currently proven to prevent hemlock mortality is the application of chemical insecticides, like imidacloprid.**

## What are the potential negative effects of using imidacloprid on non-target organisms?

### Vertebrates

Imidacloprid binds to nicotinic acetylcholine receptors in the postsynaptic neuron, acting as false neurotransmitters, interfering with normal nerve function. **Imidacloprid has a greater affinity for- and binds more strongly to insect neuron receptors than to vertebrate neuron receptors. This results in much lower toxicity in vertebrates than in insects.**

The LD50 for mammals, birds and reptiles (lethal dose in mg/kg body weight required to kill 50% of test group) ranged from 425–475 for rats, 131–300 for mice, 283 for mallard ducks and 152 for bobwhite quail, all of which are classified as moderately toxic (51–500), to 41 (House sparrow), and 13.9 (Grey partridge) which are classified as highly toxic (LD50 of 10–50 or less). Note: 1 mg/kg = 1000 parts per billion.

The LC50 for fish and frogs (lethal concentration in mg/L required to kill 50% of test group) were mostly >100 and fell into the "practically non-toxic" category, except for rainbow trout fry (LC50=1.2, moderately toxic). See table below from Gibbons et al. 2015. N**ote: 1.2 mg/L = 1200 parts per billion.**

Imidacloprid can have sublethal effects on vertebrates such as reduced growth, reduced sperm production, reduced offspring weight, reduced hatching success (Gibbons et al. 2015). Note: in these tests (required for pesticide registration) the animals were given no other food choice but imidacloprid-dosed food. In a real forest situation where, for example, only a portion of hemlock trees would be treated (max of 1.89 L per ha per year, which translates to 40-60 trees per ha) only a fraction of insects fed upon by birds, mammals, fish and amphibians would have consumed foliage on imidacloprid-treated trees.

## Can people handle imidacloprid-treated hemlocks safely?

The highest average concentration of imidacloprid in hemlock trees that had been treated with soil drenches was 60.2 ppb in one study (Benton et al. 2016a) and ranged from 0 to 2371 ppb in another study (Benton et al 2015). The highest concentration of 2371 ppb is < 0.6% of the LC50 of rats (425 to 475 mg/kg = 425000 to 475000 ppb) so risk of acute toxicity is extremely low. Imidacloprid is not carcinogenic based on studies in rats and mice.

## What are the risks of breathing fumes from burning hemlocks that have been treated with imidacloprid?

[This question was asked following the presentation to the CMM Forest Advisory Group]. Safety data sheets for imidacloprid state: "On combustion, forms toxic gases" and "When heated to decomposition it emits toxic vapors of nitrogen oxides and chloride" (https://pubchem.ncbi.nlm.nih.gov/compound/135541675#section=Decomposition). Clark et al. (1998) analyzed the chemicals produced in the smoke of cigarettes made from imidacloprid-treated tobacco and found that the main component in the smoke was carbon dioxide, plus imidacloprid, a urea compound, and small amounts of carbon monoxide. Based on the very low concentrations of imidacloprid found in the tissues of imidacloprid-treated hemlocks (e.g., Benton et al. 2016a) the concentration of imidacloprid and its breakdown products in smoke from burning hemlock would be lower still and not likely to increase health risk compared to breathing smoke from untreated hemlocks. Note: there are health risks associated with breathing smoke from the burning of regular (i.e., untreated) wood. Many toxic gases are produced from wood smoke including benzene, formaldehyde, and polycyclic aromatic hydrocarbons such as fluorine and pyrene (https://www.epa.gov/burnwise/wood-smoke-and-your-health#health).

**Table 1. From Gibbons et al. (2015) review of acute toxicity of imidacloprid on vertebrates.**

**Table 1** Single (acute) dose $LD_{50}$ (for mammals birds and reptiles, mg/kg) and $LC_{50}$ (for fish and amphibia, mg/L) for imidacloprid, clothianidin and fipronil

| Taxon | Species | Imidacloprid | Clothianidin | Fipronil |
|---|---|---|---|---|
| Mammal | Rat, *Rattus norvegicus* | 425-475 (MT)[a] | 5,000 (PNT)[i] | 97 (MT)[l] |
| | Mouse, *Mus musculus* | 131-300 (MT)[a] | >389 (MT)[i] | 95 (MT)[m] |
| Bird | Mallard, *Anas platyrhynchos* | 283 (MT)[b] | >752 (ST)[j] | 2,150 (PNT)[l] |
| | Ring-necked pheasant, *Phasianus colchicus* | | | 31 (HT)[l] |
| | Grey partridge, *Perdix perdix* | 13.9 (HT)[c] | | |
| | Red-legged partridge, *Alectoris rufa* | | | 34 (HT)[l] |
| | Northern bobwhite quail, *Colinus virginianus* | 152 (MT)[a] | >2,000 (PNT)[k] | 11.3 (HT)[l] |
| | Japanese quail, *Coturnix japonica* | 31 (HT)[a] | 423 (MT)[k] | |
| | Feral pigeon, *Columba livia* | 25–50 (HT)[a] | | >2,000 (PNT)[l] |
| | House sparrow, *Passer domesticus* | 41 (HT)[a] | | |
| | Field sparrow, *Spizella pusilla* | | | 1,120 (ST)[l] |
| | Canary, *Serinus canaria* | 25–50 (HT)[a] | | |
| | Zebra finch, *Taeniopygia guttata* | | | 310 (MT)[n] |
| Fish | Bluegill sunfish, *Lepomis macrochirus* | 105 (PNT)[a] | >117 (PNT)[i] | 0.083 (VHT)[l] |
| | Japanese carp, *Cyprinus carpio* | | | 0.34 (HT)[l] |
| | Nile tilapia, *Oreochromis niloticus* | | | 0.042-0.147 (VHT-HT)[l] |
| | Rainbow trout, *Oncorhynchus mykiss* | >83–211 (ST-PNT)[a] | >105 (PNT)[i] | 0.246 (HT)[l] |
| | Rainbow trout (fry) | 1.2 (MT)[d] | | |
| | Sheepshead minnow, *Cyprinodon variegatus* | 161 (PNT)[a] | >93.6 (ST)[i] | 0.13 (HT)[l] |
| | Zebrafish, *Danio rerio* | 241 (PNT)[e] | | |
| Amphibia | Black-spotted pond frog, *Rana nigromaculata* | 129–219 (PNT)[a,f] | | |
| | Indian rice frog, *Rana limnocharis* | 82–366 (ST-PNT)[a,f,g] | | |
| | Western chorus frog, *Pseudacris triseriata* | 194 (PNT)[h] | | |
| | American toad, *Bufo americanus* | 234 (PNT)[h] | | |
| Reptile | Fringe-toed lizard, *Acanthodactylus dumerili* | | | 30 (HT)[o] |

Toxicity classification follows US EPA (2012): *PNT* practically non-toxic, *ST* slightly toxic, *MT* moderately toxic, *HT* highly toxic, *VHT* very highly toxic. For birds, mammals and reptiles: PNT >2,000, ST 501–2,000, MT 51–500, HT 10–50, VHT <10. For aquatic organisms, fish and amphibia: PNT >100, ST >10-100, MT >1-10, HT 0.1-1, VHT <0.1. Note that kg in the $LD_{50}$ units refers to body weight of the dosed animal. Source references denoted by superscripts are as follows: [a] SERA 2005, [b] Fossen 2006, [c] Grolleau 1991 in Anon 2012, [d] Cox 2001, [e] Tisler et al. 2009, [f] Feng et al. 2004, [g] Nian 2009, [h] Howard et al. 2003, [i] DeCant and Barrett 2010, [j] European Commission 2005, [k] Mineau and Palmer 2013, [l] Tingle et al. 2003, [m] Connelly 2011, [n] Kitulagodage et al. 2008 (NB : a formulation of fipronil containing the dispersant solvent diacetone alcohol was sevenfold more toxic than technical grade fipronil itself), [o] Peveling and Demba 2003 (NB: 42 %, rather than 50 %, mortality)

## Concentrations of imidacloprid measured in streams.

Imidacloprid photodegrades in water with a half-life of 1 hour to 3 days (Moza et al 1998). Environmental concentrations of imidacloprid in aquatic environments (measured mainly around agricultural areas) ranged from 0–0.22 ug/L, 0–3.3 ug/L, 1–14 ug/l. <15 ug/L, 17–36 ug/L and up to 49 ug/L. In a study in the Netherlands, 98% of 1,465 measurements ranged from 0–8.1 ug/L but 2% of measurements were as high as 320 ug/L. The LC50 for fish and amphibians range from 1200 ug/L (i.e., 1.2 mg/L for rainbow trout fry) to 366,000 ug/L. **Thus, except in the extreme cases, environmental concentrations are 2 to 7 orders of magnitude lower than LC50 measurements for fish and amphibians, so it is unlikely that the mortality rates of these taxa will be directly affected by imidacloprid under normal exposure** (Gibbons et al. 2015).

**Sub-lethal impacts on salamanders.** In a recently published study, Crayton et al. (2020) measured concentrations of imidacloprid in streams, aquatic invertebrates (mayflies, stoneflies, caddisflies), and salamanders in hemlock stands that had been treated with imidacloprid (mainly soil injection but also soil drench and stem injection), or had not been treated. They also measured corticosterone levels and "body condition index" (BCI) of the salamanders as measures of sublethal stress, predicting that corticosterone levels would increase and BCI would decrease with increasing imidacloprid concentration in streams. They found:

- **Imidacloprid was detected in streams adjacent to 23 of 27 treated hemlock stands at an average concentration of 32 picograms per mL** (5 – 303). [1 pg/ml = 1 part per trillion or 0.001 per billion so 32 pg/ml = 0.032 ppb. 32 pg/ml = 3200 pg/L = 3.2 ng/L = 0.0032 ug/L = 0.0000032 mg/L]. **The average concentration was < 0.001% of the lethal concentration required to kill 50% of the most susceptible vertebrates in the review article by Gibbons et al tested (fry of rainbow trout) and 3.2 x $10^6$ % of the concentration in water considered "practically non-toxic" by USEPA.**
- **The relationship between corticosterone levels in salamanders and imidacloprid concentration in water was positive but extremely weak – the imidacloprid concentration explained only 3% of variation in corticosterone levels (partial r2 = 0.03).**
- **Similarly, the relationship between body condition index of the salamanders and imidacloprid concentration in water was extremely weak – the latter explained only 0.6% of the variation in BCI (partial r2 = 0.006.**
- No olefin or imidacloprid-urea (imidacloprid breakdown products) were detected in water samples but both were found in some macroinvertebrate samples (1/15 samples for olefin and 13/15 samples for imidacloprid urea).
- **Imidacloprid concentration in water was positively related to imidacloprid concentration in benthic invertebrates ($r^2$ = 0.37) but there was no evidence that imidacloprid concentration in streams was related to imidacloprid concentrations in salamander tissues.**
- **there was no significant difference in imidacloprid concentrations in salamanders collected from streams in treated areas vs. streams in untreated areas**
- [they got strange results. 37% of salamanders collected from streams in which imidacloprid was detected, also had imidacloprid in their bodies at an average concentration of 7.3 ng/g (=7.3 ppb) but 70% of salamanders collected from streams in which imidacloprid was <u>not</u> detected had imidacloprid in their bodies and an average concentration of 15.7 ng/g. ]

## How much risk is there to birds in imidacloprid-treated hemlock stands?

Gibbons et al (2015) cite only one study on indirect effects of imidacloprid on insectivorous birds. **Falcone and DeWald (2010) found that imidacloprid soil drenches of hemlocks** (reduced population densities of foliage-feeding moth larvae and Hemiptera (true bugs) in the first year post application but) **did not reduce overall arthropod abundance or diversity and had no effect on populations of birds species sampled, e.g., black-throated green warblers, black-throated blue warblers or blue-headed vireos.** However, Falcone and DeWald (2010) speculated: "Although imidacloprid treatments may benefit hemlock-associated birds by maintaining vigorous hemlocks in the short term, repeated application of systemic imidacloprid, especially at higher rates, may cause compounding declines from which native herbivorous insects populations cannot recover from in the long-term, thus lowering habitat quality for hemlock-associated birds." Note: at the time of that paper (2010) I don't think it had yet been determined that soil drenches protected trees for 5-7 years, so in practice, only a portion of the hemlocks in any hectare of forest would be treated in a given year, leaving many trees in the stand untreated in that year.

## Effects of imidacloprid on non-target terrestrial arthropods

**Canopy arthropods (3 studies).**

1. **Falcone and DeWald (2010) found that imidacloprid soil drenches of hemlocks reduced population densities of foliage-feeding moth larvae and Hemiptera (true bugs) in the first year post application but did not reduce overall arthropod abundance or diversity and had no effect on populations of birds species sampled.**
2. **Dilling et al (2009) found the abundance of 33 of 293 insect species were significantly lower in imidacloprid-treated trees than in untreated trees. The insects were foliage feeding moth larvae (caterpillars) and bark lice (Dilling et al. 2009).** *Details*: Dilling et al. (2009) sampled insects in hemlock trees for 1 to 1.5 years after application of imidacloprid soil drenches, stem –injections (Mauget), horticultural oil, and untreated controls, using a variety of techniques (beat sheets, trunk vacuuming, handpicking, branch cuttings, visual observations, and Malaise traps). They found the insect communities differed among all treatments (controls, soil drenches, stem-injections, horticultural oil, and soil injection) according to NDMS ordination, and that both abundance and species richness was significantly reduced in soil drench trees vs. control trees. Abundance of total insects was significantly reduced in all treatments compared to untreated controls (including horticultural oils) but only soil drenches of imidacloprid reduced species richness. The most affected taxa were the Lepidoptera (herbivores) and Psocoptera (detrivores – bark lice that feed on dead organic material) – abundance of 33 of 293 insect species were significantly lower in soil drench treated trees (25 moth species, 8 bark lice species). All of the moth species that were affected pupate in the soil so it is unknown whether the effect of soil drenches was due to impact from imidacloprid residues in the foliage or the soil. Note – untreated trees later died due to HWA infestation so the canopy arthropod communities no longer had hemlock canopy habitat.
3. **Kung et al. (2015) found no difference in arthropod abundance, species richness or community composition between imidacloprid-treated vs untreated trees 3 years after treatment. Furthermore, 9 years after treatment, there were significantly more arthropod species in the treated trees than in the untreated trees.**

As McCarty (2020) states, "To put canopy arthropod risk in perspective, if the trees are not treated and die, which is common in the southern Appalachians, then canopy arthropods no longer have a habitat. Thus, for management decisions, potential shorter-term canopy arthropod impacts of insecticide use must be weighed against the impacts of HWA-induced hemlock canopy loss or mortality. Insecticide use impacts cannot be realistically contrasted with an ideal healthy untreated hemlock, because these no longer occur after a HWA infestation."

**Note: In Nova Scotia,** Cody Chapman, MSc candidate at Acadia University, is measuring abundance, diversity and species composition of terrestrial arthropods in imidacloprid-treated vs. untreated hemlock stands, focusing on ground beetles and bark and wood boring beetles.

**Pollinators**

There has been very little research on impact of imidacloprid use on pollinators in hemlock forests but much research has been done in agricultural settings where imidacloprid is applied to crop plants, bare soil, and frequently used as a seed coating to prevent insects from eating the seeds and to protect the plants as they grow. Bees may be exposed to imidacloprid that is taken up into the pollen and nectar of flowering plants. Meta-analysis found that field-realistic doses of imidacloprid in nectar and pollen had no lethal effects on honeybees but reduced colony performance by 6 to 20% (Cresswell 2011) Sub-lethal effects of imidacloprid and other neonicotinoids can impair sensory processing, learning and memory functions (Palmer et al., 2013) and result in impaired orientation and navigation (Fischer et al., 2014), nesting behavior (Crall et al., 2018) and foraging activity (Henry et al. 2012; Gill and Raine, 2014) and affect colony growth (Whitehorn et al. 2012). However, if pollinators avoid flowers that contain imidacloprid, then level of exposure may be lower than expected. Easton and Goulson (2013) found that Diptera, Coleoptera and spiders avoided yellow pan traps that contained imidacloprid at concentrations of 1 ug/L.

Imidacloprid underwent a thorough review by Health Canada in 2019 to address concerns about its toxicity to pollinators. Following the review, Health Canada cancelled some uses of imidacloprid [e.g., applications to foliage of pome fruits (e.g. apples, pears) and stone fruits (e.g., cherry, peach) is no longer permitted] and placed new restrictions on labels of products containing imidacloprid to minimize exposure to pollinators (e.g., foliar applications to potatoes and grapes are not permitted during bloom). Health Canada concluded that "*when imidacloprid is used in accordance with these new risk reduction measures, the reduced environmental exposure is considered adequate, and risks are acceptable*" (Health Canada 2019).

**Imidacloprid use in hemlock forests is expected to have little impact on pollinators at the stand or landscape level because: 1) hemlock is wind pollinated and hemlock pollen is not likely to be collected much by insect pollinators; and 2) exposure of pollinators would be limited to flowering plants growing within 50-100 cm of treated trees, because imidacloprid concentrations in the soil diminish significantly with distance from application[1]**. Thus only a small fraction of flowers that pollinators may encounter in a treated stand would potentially have imidacloprid in the nectar or pollen (McCarty 2020).

In lab studies, Fortuin et al. (2021) exposed a wild soil-nesting species of mason bee, *Osmia lignaria,* to imidacloprid-treated soil at various concentrations. They found no bee mortality at 20% soil moisture but >50% mortality when soil moisture was greater than 40%. At concentrations of 390 ppb, nesting activity was reduced by 42%; at concentrations of 780 ppb, nesting activity was reduced by 66%. These concentrations were comparable to what could be found within 50 cm of imidacloprid-treated trees but are not representative of concentrations found throughout the rest of a treated stand.

Luca Voscort (MSC candidate, Acadia University) has begun a project to determine the risks of imidacloprid basal bark spray to wild bee pollinators in hemlock forests. Luca is: 1) surveying hemlock stands to determine the species and abundance of flowering plants, and the portion of those plants that grow within 50 cm of hemlock trees. [The greater the density of flowering plants in hemlock stands and the greater the proportion that grow in close proximity to treated trees, the greater the risk of pollinator exposure to imidacloprid]; 2) comparing the abundance, diversity, and species composition of bee pollinators in treated and untreated stands, using blue vane traps and sweep nets to sample; and 3) measuring imidacloprid concentrations in pollen collected from flowers and wild bees in treated and untreated stands.

**Soil arthropods and other invertebrates**

Reynolds (2008) found that abundance and diversity of collembola (springtails) was lower in the immediate area of imidacloprid treated trees. However, Knoepp et al. (2012) found no difference in abundance of springtails, mites or total microarthopods in soil around treated vs. untreated trees.

Kreutzweiser et al. (2008a) measured significant weight loss in earthworms (*Dendrobaena octaedra*) at imidacloprid concentrations of 3 mg/kg of soil and 50% mortality at 5.7 mg/kg of soil – these soil concentrations were well within realistic concentrations for soil drenches. Soil injections generate imidacloprid concentrations of 20-25 mg/kg at depths of 0–15 cm and about 12 mg/kg at 15–30 cm soil depths. However, imidacloprid concentrations dropped to < 0.02 mg/kg in soil in samples collected >50 cm from injection points.

**Aquatic macroinvertebrates**

Insects and other stream invertebrates are very sensitive to imidacloprid – more so than most terrestrial insects, including bees (McCarty 2020). In lab microcosms, Kreutzweiser et al. (2008b) found no effects of imidacloprid on microbial decomposition of plant material, but found that the stonefly, *Pteronarcys dorsata,* died at concentrations of 48–96 ug/L and inhibited feeding by stonefly nymphs at concentrations as low as 12 ug/l. [Note: 12 ug/L = 12 parts per billion.] Imidacloprid concentrations in streams in hemlock forests range from 20 to 800 parts per trillion (Churchel et al. 2012; Benton et al. 2016b; Wiggins et al. 2018), much lower than those used in the Kreutzweiser et al. (2008b) microcosm study.

Benton et al (2017) found no difference in aquatic macroinvertebrate (Ephemeroptera, Plecoptera, Trichoptera) diversity and abundance measured upstream and downstream of hemlock stands that had been treated with imidacloprid soil drench (3 m buffer from streams, treated areas 14 to 42 ha, 1.8 to 112 kg applied, limit of 0.45 kg/ha/yr, 1 to 8 years before sampling) in nine different streams Great Smoky Mountains.

[1] Soil injections generated imidacloprid concentrations of 20-25 mg/kg of soil at depths of 0–15 cm and about 12 mg/kg at 15–30 cm soil depths but concentrations dropped to < 0.02 mg/kg in soil samples collected >50 cm from injection points (Kreutzweiser et al. 2008a).

Similarly, Churchel et al (2011) sampled streams in treated and untreated hemlock stands (soil injection) and found no significant effects on abundance (EPT metric) or species richness of aquatic invertebrates – they concluded that their results indicated soil injections of imidacloprid could be safely used to control HWA in the southern Appalachians.

**When used according to label hemlock forests can be protected without negative effects on water quality or aquatic insect fauna** (McCarty and Adesso 2019).

**The results of research on non-target impacts of imidacloprid are summarized in Table 2 (below).**

For more on sublethal effects of imidacloprid see: Desneux N, Decourtye A, Delpuech J (2007). The Sublethal Effects of Pesticides on Beneficial Arthropods. Annual Review of Entomology 52: 81–106.

Table 2. Summary of results of studies of the effect of imidacloprid applications, mostly applied as soil drench or soil injection, on non-target organisms. Checkmarks indicate number of studies.

| Group | Negative | Neutral | Positive |
|---|---|---|---|
| Aquatic invertebrates | | ✓✓ | |
| Canopy arthropods | ✓✓ | ✓✓ | ✓ |
| Soil arthropods[1] | ✓✓ | ✓ | |
| Pollinators[2] | ✓ | | |
| Birds | | ✓ | |

[1] In soil within 50 cm of treated hemlocks

[2] In lab tests at concentrations that would be found within 50 cm of treated hemlock but not representative of concentrations throughout a treated area

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