From:	Gary Rondeau
To:	ECY RE Shrimp Control Permit Comments; Rockett, Derek (ECY)
Subject:	Comment concerning using imidacloprid to control burrowing shrimp.
Date:	Sunday, January 26, 2014 11:06:45 AM
Attachments:	WashingtonShrimp.pdf

Dear Derek Rockett,

Attached please find my comments regarding the proposed use of imidacloprid to control burrowing shrimp in oyster beds. I have been motivated to seek what is harming our honeybees, so I have been investigating the time dependent toxicity of the neonicotionoid insecticides. As such, I have become quite concerned that this aspect of this class of insecticides is not well-appreciated. Please take a look at my analysis, and if you have further questions, I would be happy to discuss my understanding further with you or your staff.

Best regards,
Gary

To: Washington State Department of Ecology

Water Quality Program

Attn: Derek Rockett

Re: Proposed Individual Permit for the Control of Burrowing Shrimp using Imidacloprid on Commercial Shellfish Beds in Willapa Bay and Grays Harbor

From: Gary Rondeau, Ph.D.
1025 Elkay Drive, Eugene, OR 97404

The proposed permit to use imidacloprid on shellfish beds recently came to my attention. The fact that imidacloprid is being sought as an alternative to carbaryl particularly caught my interest because I have been studying the toxic profile of the various insecticide classes as they relate to ecotoxicity for pollinators. As it turns out, carbaryl and imidacloprid are on the opposite ends of time-dependent toxicity scaling spectrum. I would urge regulators to look closely at the time-dependent nature of the toxicity of residual concentrations for these two chemicals. With carbaryl we have one of the pesticides with the most "threshold-like" toxic action. Imidacloprid, on the other hand, has significant enhanced or delayed toxicity at residual concentrations.

Time-dependant toxicity of residual toxin concentrations is a poorly researched field, but pioneering work by Tennekes and Sanchez-Bayo[1] is a good place to begin this study. Sanchez-Bayo's (2009) work and my review[2] of honeybee, and termite studies of imidacloprid toxicity all indicate that imidacloprid exhibits delayed toxicity. Compounds that show and enhanced time-dependent toxic effect cannot simply be characterized by a 48 hr LD50. Rather, the duration of chronic exposure, or delayed effect from a single exposure, figure prominently into the amount of chemical that eventually produces a toxic effect.

The two insecticides under consideration here, imidacloprid and carbaryl, are both neurotoxins that disrupt the cholinergic neural pathway. Imidacloprid binds directly and strongly to nicotinic acetylcholine receptors (nAChR) on the post-synaptic terminal. The bound receptors cause the associated ion channels to open, which excites the post-synaptic neuron. Hence, imidacloprid acts as a direct nAChR agonist. In contrast, carbaryl is an acetyl cholinesterase (AChE) inhibitor. The compound binds reversibly to the AChE molecules which are responsible for clearing the synaptic junction of the natural occurring acetylcholine (ACh) neurotransmitter. When all of the AChE is inhibited by carbaryl, ACh will accumulate in the synaptic junction and continue to excessively stimulate the nAChR ion channels, which overexcites the post-synaptic neuron. We get the same result, an overexcited post-synaptic neuron with either chemical.

But now let's look at what happens as lower chronic doses. For carbaryl to function, a large fraction of the AChE molecules in the synaptic cleft must be bound with the toxin, since otherwise the remaining AChE moelecules will clear the junction of ACh and there will be no toxic effect. Furthermore, since carbarly binds reversibly to the AChE molecules, there must be a sufficient concentration of the carbarly molecules so that they can effectively out-compete the normal ACh reaction. These two mechanisms impose a minimum concentration of the insecticide required for a significant toxic effect. A small residual concentration of carbaryl molecules in the synaptic cleft would bind to a small fraction of the available AChE molecule, but since there are many other AChE molecules to clear the junction of ACh,

the residual concentration of carbaryl would have essentially no toxic effect. Reversible binding of the toxin additionally means that the toxin molecules will spend time un-bound where they are subject to metabolism and elimination by natural detoxification enzymes.

Imidacloprid is another story. The imidacloprid molecules bind directly to the nAChR ion channels. A single molecule of imidacloprid will permanently open an ion channel that in normal function only stays open for about a millisecond. Hence a few molecules of the toxin can cause major neural disruption. This is why the neonicotinoids are such potent toxins, requiring hundreds of times less active ingredient compared to AChE inhibitors such as carbaryl. Furthermore, imidacloprid binds strongly, essentially permanently, to the nAChR. This means that residual environmental concentrations will continue to add to the toxic load within the organism as additional molecules are encountered and permanently bound to the synapses. Finally, it appears that sub lethal levels of toxin still take their toll physiologically. The toxic time-dependent effect is more pronounced than a simple "accumulate to a threshold" scaling.

My work and that of Sanchez-Bayo suggest that imidacloprid has a toxicity that scales as exposure time squared (t^2) for a variety of arthropods. The one experiment that Sanchez-Bayo quotes for carbaryl with fish had a scaling exponent of 0.3 (instead of 2 for imidacloprid) suggesting a strong threshold effect, in agreement with our heuristic arguments above.

The big problem with a scaling exponent larger than one is that it very quickly becomes difficult to find a dose that will kill the pest but still not harm other species over their lifetime. This is best illustrated with an example. The table below lists the level of protection needed to avoid toxic effect to non-target organism with a natural life span that is 25 times longer than the kill-time for the target organism.

Time dependence	Description	Relative time-dependent toxicity	Include Safety Factor × 3
t^0	Threshold level only – doesn't depend on time	1/1	1/3
t^1	Accumulate to threshold with time – Haber's rule	1/25	1/75
t^2	Enhanced or delayed toxicity	1/625	1/1875

For the threshold-acting toxin, it need only decay away by a factor of three before we consider it safe, where as for the t^2scaling it has to decay away by almost 2000 times before we would consider it safe. If we were worried about an organism that normally lived 50 times longer than the kill time (say 200 days and 4 days respectively), that would require another factor of 4 less toxin or about 1/8000 the initial dose. These are difficult requirements and we are still not talking about creatures that are exposed for a full annual cycle. We have no idea if the scaling law will hold that long since our longest experiments only followed bees for about 60 days, but this represents the best guess we can make with the experimental data available.

The lifetime of these two chemicals in the environment is also worth comparing. In soil imidacloprid has a half-life of 0.5 to 3 years, depending on soil type, whereas carbaryl has a half-life of 4 to 30 days in soil.

The longer lifetime in the environment of imidacloprid will mean that low levels of exposure to non-target organisms would persist much longer, allowing accumulation of the this strongly bound toxin in non-target organism's nerve tissue.

Finally, what happens in the water? Carbaryl is rather insoluble in water where as imidacloprid is soluble. From reading the EIS report on the carbaryl application, I gather than this chemical is applied when tide is out and the oyster beds are nominally dry. The insecticide is applied in a granular or flake form with the intention that the toxins remain in the sediment when the beds are flooded by the tide. Such a plan will not work with imidacoprid because this chemical is highly mobile in water. Problems keeping the pesticide on-site will be exacerbated with a switch to imidacloprid.

From the above analysis, I would expect that non-target species, such as dungeness crab would be at a much greater risk using imidacloprid than with carbaryl. The imidacloprid has a longer environmental lifetime than carbaryl. This allows non-target organisms to be exposed to residual levels of the compound for longer periods of time. Because of the direct mode-of-action of imidacloprid, and its essentially permanent bonding at the active site on synapses, this toxin both accumulates and produces toxic effects from the first molecule that binds to a synapse. This is in contrast to carbaryl which binds to an intermediate molecule in the cholinergic pathway, and this binding is reversible. Very low concentrations of carbaryl will have essentially no toxic effect and the chemical will not accumulate in non-target organisms.

These arguments are equally valid when considering insecticides and non-target pollinators or other arthropods that can come into contact with such long-lived neonicotinoids. I encourage regulators to become familiar with this argument because it impacts not only this case, but the general question concerning the ecotoxicty of this class of pesticides.

1. Tennekes, H.A. and Sánchez-Bayo F. **The molecular basis of simple relationships between exposure concentration and toxic effects with time.** Toxicology 04/2013; 309:39-51. DOI:10.1016/j.tox.2013.04.007

2. Rondeau, G **Time-dependent Toxicity of Imidacloprid in Bees and Ants**