May 14, 2018

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Re: Comments on Supplemental Environmental Impact Statement for Control of Burrowing Shrimp using Imidacloprid on Commercial Oyster and Clam Beds in Willapa Bay and Grays Harbor, Washington

I write on behalf of Center for Food Safety and the Western Environmental Law Center to express our support of Washington Department of Ecology's decision to deny the application for a Clean Water Act National Pollutant Discharge Elimination System (NPDES) permit for to use imidacloprid on commercial oyster and clam beds in Willapa Bay and Grays Harbor, Washington. As Ecology notes, it must deny the permit application because the proposed discharge is not consistent with the requirements of the Sediment Management Standards and the permit cannot be conditioned such to meet the applicable standards. This conclusion is supported by the available science and is the best interest of all Washingtonians.

Imidacloprid is the oldest and most toxic of the neonicotinoid insecticides. Regulators around the world are finally waking up to the pollution of our soils and waterways with this class of insecticides and the extremely harmful consequences[1]—a second Silent Spring[2] according to some experts. Pesticides, more accurately described as "biocides" because they rarely only kill "pests," are designed to kill living things and as such their use in estuarine environments will have negative unintended effects. To continue the toxic legacy of carbaryl with another pesticide will only continue the pesticide treadmill. Not only is imidacloprid not the only option for restoring balance to the Bay, it is unlikely to be effective. More than 50 years of carbaryl use (a likely carcinogen) has not solved the shrimp problem identified by some shellfish growers, and there is no indication that imidacloprid will be any different. Indeed, the efficacy shown through field trials indicates that this plan is ready-made to breed resistant burrowing shrimp. While the poisoning of public waters may provide some limited short-term relief, it is not a long-term solution.

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[1] In response to alarming levels of aquatic contamination and impacts to pollinators, Canada's Pesticide Management Regulatory Agency (PMRA) is currently considering a ban on imidacloprid. *See* PRMA, *Update on the Neonicotinoid Pesticides*, https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/fact-sheets-other-resources/update-neonicotinoid-pesticides.html (*see also* CFS Comments to Health Canada PMRA on Proposed Re-evalution Decision PRVD2016-20, Imidacloprid, Exhibit A to CFS, WELC, CBD comments on the Imidacloprid SEIS, dated Nov. 11, 2017, incorporated herein by reference). In Europe, a temporary ban on major neonicotinoids will become a permanent ban. Damian Carrington, *EU agrees total ban on bee-harming pesticides*, The Guardian (Apr. 27, 2018). France has already imposed a full ban on neonicotinoids. *See France says ban on neonicotinoids will go ahead in 2018*, Farming UK (June 28, 2017).

[2] *Silent Spring*, a book by Rachel Carson published in 1962, detailed the detrimental effects of indiscriminate pesticide use—leading to the ban on DDT and inspiring the environmental movement and creation of the Environmental Protection Agency.

Ecology's own review of the latest science on imidacloprid and neonicotinoids shows that there is no scenario of use in the waters of Willapa Bay that is safe, and legal. Please continue to follow the science and ensure protection for Willapa Bay, its wildlife, and its people, and put an end to consideration of the use of neonicotinoids (or any pesticide) on shellfish beds once and for all.

This Process was Aimed at the Wrong Target

While as discussed below, Ecology's decision to deny this permit was properly grounded in both the science and the law, this permit was ill-conceived from the very beginning. Controlling (or extirpating) the native burrowing shrimp cannot be, or at least should not be, Ecology's purpose here. Rather, finding a solution that will allow Willapa Bay and Gray Harbor to continue to support viable shellfish operations while maintaining their ecological integrity and vitality should be the goal of this proposal. Here, Ecology has identified the objectives of the proposed action as "[p]reserve[ing] and maintain[ing] the viability of clams and oysters commercially grown in Willapa Bay and Grays Harbor by controlling populations of two species of burrowing shrimp on commercial shellfish beds," and "[p]reserve[ing] and restor[ing] selected commercial shellfish beds in Willapa Bay and Grays Harbor that are at risk of loss due to sediment destabilization caused by burrowing shrimp." SEIS at 2-1. By adopting the proponent's purpose and need statement for the proposed action, Ecology has unnecessarily limited the range of potential alternatives that could meet the true object—namely, ensuring the viability of clams and oysters commercially grown in Willapa Bay and Grays Harbor. While it is true that the impact of burrowing shrimp on the shellfish beds in the region are the focus of the proposed permit, limiting the scope of analysis to only solutions that will address that one piece of the puzzle is problematic. Indeed, as discussed below, to date, Ecology has failed to identify any "reasonable alternatives," WAC 197-11-440(b)(5), to the proposed action. This indicates that the purpose and need is too narrowly defined.

Controlling (or extirpating) the native burrowing shrimp cannot be, or at least should not be, Ecology's purpose here. Rather, finding a solution that will allow Willapa Bay and Gray Harbor to continue to support viable shellfish operations while maintaining their ecological integrity and vitality should be the goal of this proposal. Stepping back, now is the time for all the stakeholders to look critically at the *causes* of increased shrimp populations that have become imbalanced, and begin to develop viable, long-term solutions. Instead of focusing only on how to kill the shrimp, we should be looking at how to encourage the other elements in the Bay's complex ecology that would bring shrimp into balance.

First, if a loss of predators is part of the problem, than a solution that focuses on restoration of those species' habitat would go a long way to bringing back these needed pieces of the puzzle. Just as in gardening, if aphids are attacking you can spray the whole thing with biocides that will kill off most insects, or you can encourage beneficial insects, like ladybugs, to eat the aphids. The former may seem like a quick and easy solution, but it does not stop pests in the long term. This is the lesson from terrestrial agriculture: industrial farming has been relying on chemical pest control for decades, and still has major pest problems, whereas more and more evidence indicates that encouraging a diverse array of insects, many of which are beneficial, will keep pests in check.[3] Thus, an alternative that involves restoration of crucial shrimp-predator habitat could be both viable to control shrimp populations in the long-term and be more environmentally beneficial.

Second, Ecology failed to examine the interplay between eelgrass and shrimp in this SEIS, beyond noting that burrowing shrimp can inhibit eelgrass growth and density. *See e.g.* SEIS at 1-18. But this relationship runs both ways, as Ecology itself noted in its FEIS for the use of imazamox on Japanese eelgrass: research shows that "eelgrasses can reduce numbers of burrowing shrimp (ghost shrimp and mud shrimp) that are also problem species for shellfish growers. (Feldman et al. 2000; and Harrison 1987 as cited in Fisher Bradley and Patten 2011)."[4] This includes native *z. marina* and *z. japonica* eelgrasses, whose roots impede shrimp burrowing and inversely, that shrimp impede eelgrass. So this begs the question of whether the loss of eelgrass is contributing to increased shrimp numbers, and whether the intentional killing of eelgrass through chemical means is contributing. Ecology did not evaluate this interplay, but the same shellfish growers who now seek to use imidacloprid to kill shrimp have for years used imazamox to kill eelgrass, under the guise of the Japanese eelgrass being non-native and harmful to clam production. But Japanese eelgrass deters shrimp as well as native eelgrass. Is it possible that killing off eelgrass has allowed the shrimp to flourish? Growers used chemicals to kill off shrimp (carbaryl), possibly allowing Japanese eelgrass to flourish, then growers got a permit to kill the eelgrass through chemical means, and now shrimp numbers are increased, so the growers are back asking to spray different chemicals to kill off the shrimp. It is a never-ending pesticide treadmill, and because some shellfish growers have identified both eelgrass and burrowing shrimp as pests, they seek to use the easiest and cheapest solution to killing them both. But this is not how nature works—you cannot simply remove one element and assume that balance will be restored. Ecology needs to thoroughly evaluate how the removal of eelgrass may have contributed to an increase in shrimp, along with other causes of shrimp increase, before it can identify reasonable solutions. This may include comparing shrimp recruitment and eelgrass removal in the last few years (is there an overlap of acreage where eelgrass was sprayed and increased shrimp recruitment?).

Once the causes of shrimp imbalance are better understood, a solution that will actually be effective may be found. These include (or some combination of) the following:

- **Mechanical means (w/o pesticides)**: harrowing to expose shrimp and allow predators to consume them, *see* Comments of Erika Buck, FMO Aquaculture.

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[3] *See e.g.* David W. Crowder et al., *Organic Agriculture Promotes Evenness and Natural Pest Control*, 466 Nature 109 (2010) http://www.nature.com.lawpx.lclark.edu/nature/journal/v466/n7302/full/nature09183.html; Matthew J.W. Cock et al., *Trends in the Classical Biological Control of Insect Pests by Insects: An Update of the BIOCAT Database*, 61 BioControl 349 (2016), https://link.springer.com/article/10.1007%2Fs10526-016-9726-3; Matthias Tschumi et al., *Tailored Flower Strips Promote Natural Enemy Biodiversity and Pest Control in Potato Crops*, 53 J. Applied Ecology 1169 (2016). doi:10.1111/1365-2664.12653; Robin Drieu & Adrien Rusch, *Conserving Species-Rich Predator Assemblages Strengthens Natural Pest Control in a Climate Warming Context*, 19 Ag. Forest Entomology 52 (2016) 10.1111/afe.12180.

[4] Ecology, Final Environmental Impact Statement: Management of Zostera japonica on Commercial Clam Beds in Willapa Bay, Washington, 77-78 (2014).

- **Alternative culture (w/o pesticides)**: use of techniques that protect oysters from sinking into surface of substrate, while evaluating environmental impacts of these techniques (i.e. sediment retention, plastic introduction, etc).
- **Bay restoration**: restore the habitat that supports predators of burrowing shrimp, and all aspects of the food web. This would be useful in conjunction with any alternatives.

There is No Safe Way to Use Impidacloprid

Ecology is correct that would be impossible to issue a permit that complies with the law and protects the environment. Ecology is prohibited from issuing an NPDES permit if the discharge will harm "wildlife, birds, game, fish and other aquatic life . . ." and "[i]n no event shall the discharge of toxicants be allowed that would violate any water quality standard, including toxicant standards, sediment criteria, and dilution zone criteria." RCW 90.48.180 and 520. Ecology's exhaustive analysis demonstrates that the discharge, as proposed, will violate both of these criteria. Specifically, the "proposed discharge would create a sediment impact with an adverse effect on biological organisms above a minor adverse biological effects level within the sediment impact zone" and will "result in adverse effects outside of the sediment impact zone due to the movement of the pesticide in surface water." Ecology, Toxics Cleanup Program (TCP) Memo at 1.

Moreover, Ecology determined that there was no reasonable way to minimize the impacts of the proposal that would bring the "discharge into compliance" without "compromis[ing] the purpose for the discharge." Ecology, TCP Memo at 9. Specifically, Ecology determined that reducing the treatment area, the amount of active ingredient applied, or the location of those applications would not be sufficient to protect the bays and bring the discharges into compliance with the law. Ecology, TCP Memo at 9-10. Moreover, Ecology found that "[i]t is not physically possible to prevent imidacloprid from entering the water column or, once it dissolves in the water column, being transported throughout the estuary at acute and chronic toxicity levels." Ecology, TCP Memo at 9.

These conclusions are supported by Ecology's SEPA analysis. Under SEPA, Ecology is required to develop, consider, and compare "reasonable alternatives." WAC § 197–11–440(5)(b). Those alternative "*shall include* actions that could feasibly attain or approximate a proposal's objectives, but at a lower environmental cost or decreased level of environmental degradation." *Id.* (emphasis added). Here, Ecology offered four alternatives for consideration: 1) the no action alterative; 2) the continued use of carbaryl; 3) imidacloprid applications on up to 2,000 acres per year in Willapa Bay and Grays Harbor, and; 4) imidacloprid applications on up to 500 acres per year. Of these, the proposed action, Alternative 4, was considered the least environmentally harmful. Thus, Ecology—at least implicitly, if it was in fact complying with SEPA—acknowledged that there was no alternative approach that would have less environmental impact, and still meet the stated purpose. Yet, Ecology correctly determined that even these impacts were too much for Willapa Bay and Grays Harbor to bear.

Moreover, there are data gaps in terms of the long-term impacts of using imidacloprid in Willapa Bay. What we do know about imidacloprid is disturbing, but we applaud Ecology for recognizing that what we *do not* know is just as concerning. Given the impacts we are still uncovering on freshwater systems from neonic runoff, the precautionary principle is best used here to avoid those direct impacts on Willapa Bay and Grays Harbor from the intentional use of imidacloprid on aquatic resources.

The Case Against Imidacloprid Gets Stronger Every Day

Recognizing the devastating impacts of neonicotinoids (including imidacloprid) on honey bees, wild bees, and all insects, the European Union recently agreed to ban the use of several neonics from all fields (allowing them only in closed greenhouses).[5] The impacts of neonics are felt not just from direct contact, but through the widespread contamination of soil and water from their use on fields. *Id.* This must be compared to the intentional use of imidacloprid directly into an aquatic environment, as proposed by the WGHOGA.

Ecology examined the latest science on neonics and imidacloprid in its Supplemental EIS, but the body of science showing the harms of these neurotoxins grows everyday, leading support to Ecology's tentative denial. For example, these are just some of the studies on neonics and imidacloprid since November 2017:

Assessing combined impacts of agrochemicals: Aquatic macroinvertebrate population responses in outdoor mesocosms
https://www.sciencedirect.com/science/article/pii/S0048969718307769
Barmentlo, S. H., Schrama, M., Hunting, E. R., Heutink, R., van Bodegom, P. M., de Snoo G.R., & Vijver, M. G. (2018) Assessing combined impacts of agrochemicals: Aquatic macroinvertebrate population responses in outdoor mesocosms. *Science of the Total Environment, 631-632,* 341-347. https://doi.org/10.1016/j.scitotenv.2018.03.021

Agricultural ditches host a diverse community of species. These species often are unwarrantedly exposed to fertilizers and a wide-array of pesticides (hereafter: agrochemicals). Standardized ecotoxicological research provides valuable information to predict whether these pesticides possibly pose a threat to the organisms living within these ditches, in particular macro-invertebrates. However, knowledge on how mixtures of these agrochemicals affect macro-invertebrates under realistic abiotic conditions and with population and community complexity is mostly lacking. Therefore we examined here, using a full factorial design, the population responses of macroinvertebrate species assemblages exposed to environmentally relevant concentrations of three commonly used agrochemicals (for 35 days) in an outdoor experiment. The agrochemicals selected were an insecticide (imidacloprid), herbicide (terbuthylazine) and nutrients (NPK), all having a widespread usage and often detected together in watersheds. Effects on species abundance and body length caused by binary mixture combinations could be described from single substance exposure. However, when agrochemicals were applied as tertiary mixtures, as they are commonly found in agricultural waters, species' abundance often deviated from expectations made based on the three single treatments. This indicates that pesticide-mixture induced toxicity to population relevant endpoints are difficult to extrapolate to field conditions. As in agricultural ditches often a multitude (approx. up to 7) of agrochemicals residues are detected, we call other scientist to verify the ecological complexity of non-additive induced shifts in natural aquatic invertebrate populations and aquatic species assemblages.

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[5] Damian Carrington, *EU agrees total ban on bee-harming pesticides*, The Guardian (Apr. 27, 2018).

Calibration and validation of toxicokinetic-toxicodynamic models for three neonicotinoids and some aquatic macroinvertebrates.

https://link.springer.com/article/10.1007%2Fs10646-018-1940-6

Focks, A., Belgers, D., Boerwinkel, M., Buijse, L., Roessink, I., & Van den Brink, P. J. (2018) *Ecotoxicology.* https://doi.org/10.1007/s10646-018-1940-6

Exposure patterns in ecotoxicological experiments often do not match the exposure profiles for which a risk assessment needs to be performed. This limitation can be overcome by using toxicokinetic-toxicodynamic (TKTD) models for the prediction of effects under time-variable exposure. For the use of TKTD models in the environmental risk assessment of chemicals, it is required to calibrate and validate the model for specific compound–species combinations. In this study, the survival of macroinvertebrates after exposure to the neonicotinoid insecticide was modelled using TKTD models from the General Unified Threshold models of Survival (GUTS) framework. The models were calibrated on existing survival data from acute or chronic tests under static exposure regime. Validation experiments were performed for two sets of species-compound combinations: one set focused on multiple species sensitivity to a single compound: imidacloprid, and the other set on the effects of multiple compounds for a single species, i.e., the three neonicotinoid compounds imidacloprid, thiacloprid and thiamethoxam, on the survival of the mayfly *Cloeon dipterum*. The calibrated models were used to predict survival over time, including uncertainty ranges, for the different time-variable exposure profiles used in the validation experiments. From the comparison between observed and predicted survival, it appeared that the accuracy of the model predictions was acceptable for four of five tested species in the multiple species data set. For compounds such as neonicotinoids, which are known to have the potential to show increased toxicity under prolonged exposure, the calibration and validation of TKTD models for survival needs to be performed ideally by considering calibration data from both acute and chronic tests.

Toxicokinetics of the neonicotinoid insecticide imidacloprid in rainbow trout (Oncorhynchus mykiss).

https://www.ncbi.nlm.nih.gov/pubmed/29378254

Frew, J. A., Brown, J. T., Fitzsimmons, P. N., Hoffman, A. D., Sadilek, M., Grue, C. E., & Nichols, J. W. Toxicokinetics of the neonicotinoid insecticide imidacloprid in rainbow trout (Oncorhynchus mykiss). (2018) *Comparative Biochemistry and Physiology – Part C: Toxicology & Pharmacology, 205,* 34-42. https://doi.org/10.1016/j.cbpc.2018.01.002

Studies were conducted to determine the distribution and elimination of imidacloprid (IMI) in rainbow trout. Animals were injected with a low (47.6 μg/kg), medium (117.5 μg/kg) or high (232.7 μg/kg) dose directly into the bloodstream and allowed to depurate. The fish were then sampled to characterize the loss of IMI from plasma and its appearance in expired water (all dose groups) and urine (medium dose only). In vitro biotransformation of IMI was evaluated using trout liver S9 fractions. Mean total clearance (CL_T) values determined by non-compartmental analysis of plasma time-course data were 21.8, 27.0 and 19.5 mL/h/kg for the low, medium and high dose groups, respectively. Estimated half-lives for the same groups were 67.0, 68.4 and 68.1 h, while fitted values for the steady-state volume of distribution (V_{SS}) were 1.72, 2.23 and 1.81 L/kg. Branchial elimination rates were much lower than expected, suggesting that IMI is highly bound in blood. Renal clearance rates were greater than measured rates of branchial clearance (60% of CL_T in the medium dose group), possibly indicating a role for renal membrane transporters. There was no evidence for hepatic biotransformation of IMI. Collectively, these findings suggest that IMI would accumulate in trout in continuous waterborne exposures.

Changes of hematological and biochemical parameters revealed genotoxicity and immunotoxicity of neonicotinoids on Chinese rare minnows (*Gobiocypris rarus*)
https://www.sciencedirect.com/science/article/pii/S0269749117338496
Hong, X., Zhao, X., Tian, X., Li, J., & Zha, J. (2018) Changes of hematological and biochemical parameters revealed genotoxicity and immunotoxicity of neonicotinoids on Chinese rare minnows (*Gobiocypris rarus*). *Environmental Pollution, 233,* 862-871. https://doi.org/10.1016/j.envpol.2017.12.036

Adverse impacts of immunity in terrestrial non-target organisms exposed to neonicotinoid insecticides have been reported, but the causal link between insecticide exposure and possible immune alterations in fish remains limited. In the present study, the potential genotoxicity and immunotoxicity of three neonicotinoids (imidacloprid, nitenpyram, and dinotefuran) were assessed in Chinese rare minnows by using a 60-day chronic toxicity test. The hematological and biochemical parameters of juvenile Chinese rare minnows and changes in the transcription of six inflammation-related genes were determined after exposure to neonicotinoids at 0.1, 0.5, or 2.0 mg/L. A clear difference in the frequency of erythrocytes with micronuclei (MN) was observed after treatment with 2.0 mg/L imidacloprid ($p < .05$). Additionally, exposure to 0.5 or 2.0 mg/L imidacloprid significantly increased the binucleated (BN) erythrocytes and those with notched nuclei (NT) ($p < .05$). A serum protein electrophoresis (SPE) assay showed significant alterations in the serum protein in all treatments ($p < .05$), and further analysis indicated decreases in immunoglobulin (Ig) in treatments with 0.5 or 2.0 mg/L imidacloprid or dinotefuran or with 0.1 mg/L nitenpyram ($p < .05$). Moreover, a biochemical assay confirmed that immunoglobulin M (IgM) levels were indeed significantly decreased upon treatment with imidacloprid or dinotefuran at 0.5 or 2.0 mg/L ($p < .05$). In addition, the transcriptional levels of the inflammatory cytokines *IL-6*, *INF-a*, *TNF-a*, and *IL-1β* were markedly down-regulated after all imidacloprid treatments ($p < .05$), whereas the expression levels of only *TNF-a* and *IL-1β* were significantly down-regulated following the 0.5 and 2.0 mg/L dinotefuran treatments ($p < .05$). Taken together, our results clearly demonstrate that imidacloprid, rather than nitenpyram and dinotefuran, can induce genotoxicity. The responsiveness of these immune indicators provides new insight into and evidence of the adverse effects of neonicotinoids on aquatic non-target organisms.

The impacts of modern-use pesticides on shrimp aquaculture: An assessment for north eastern Australia
https://www.sciencedirect.com/science/article/pii/S0147651317307819?via%3Dihub
Hook, S.E., Doan, H., Gonzago, D., Musson, D., Du, J., Kookana, R., . . . Kumar, A. (2018). The impacts of modern-use pesticides on shrimp aquaculture: An assessment for north eastern Australia. *Ecotoxicology and Environmental Safety, 148*, 770-780. https://doi.org/10.1016/j.ecoenv.2017.11.028

The use of pyrethroid and neonicotinoid insecticides has increased in Australia over the last decade, and as a consequence, increased concentrations of the neonicotinoid insecticide imidacloprid have been measured in Australian rivers. Previous studies have shown that non-target crustaceans, including commercially important species, can be extremely sensitive to these pesticides. Most shrimp farms in Australia are predominantly located adjacent to estuaries so they can obtain their required saline water, which support multiple land uses upstream (e.g. sugar-cane farming, banana farming, beef cattle and urbanisation). Larval and post-larval shrimp may be most susceptible to the impacts of these pesticides because of their high surface area to volume ratio and rapid growth requirements. However, given the uncertainties in the levels of insecticides in farm intake water and regarding the impacts of insecticide exposure on shrimp larvae, the risks that the increased use of new classes of pesticide pose towards survival of post-larval phase shrimp cannot be adequately predicted. To assess the potential for risk, toxicity in 20day past hatch post-larval Black Tiger shrimp (Penaeus monodon) to modern use insecticides, imidacloprid, bifenthin, and fipronil was measured as decreased survival and feeding inhibition. Post-larval phase shrimp were sensitive to fipronil, bifenthrin, and imidacloprid, in that order, at concentrations that were comparable to those that cause mortality other crustaceans. Bifenthrin and imidacloprid exposure reduced the ability of post-larval shrimp to capture live prey at environmentally realistic concentrations. Concentrations of a broad suite of pesticides were also measured in shrimp farm intake waters. Some pesticides were detected in every sample. Most of the pesticides detected were measured below concentrations that are toxic to post-larval shrimp as used in this study, although pesticides exceed guideline values, suggesting the possibility of indirect or mixture-related impacts. However, at two study sites, the concentrations of insecticides were sufficient to cause toxicity in shrimp post larvae, based on the risk assessment undertaken in this study.

Chemical activity and distribution of emerging pollutants: Insights from a multi-compartment analysis of a freshwater system

https://www.sciencedirect.com/science/article/pii/S0269749117307315

Inostroza, P. A., Massei, R., Wild, R., Krauss, M., & Brack, W. (2017) Chemical activity and distribution of emerging pollutants: Insights from a multi-compartment analysis of a freshwater system. *Environmental Pollution, 231*(1), 339-347.

Emerging pollutants are ubiquitous in the aquatic system and may pose risks to aquatic ecosystems. The quantification and prediction of environmental partitioning of these chemicals in aquatic systems between water, sediment and biota is an important step in the comprehensive assessment of their sources and final fates in the environment. In this multi-compartment field study, we applied equilibrium partitioning theory and chemical activity estimates to investigate the predictability of concentrations in Gammarus pulex as a model invertebrate from water and sediment in a typical small central European river. Furthermore, KOW-based and LSER approaches were assessed for the calculation of sediment organic carbon-, lipid-, and protein-water partitioning coefficients and activity ratios between the different compartments. Gammarid-water activity ratios close to unity have been observed for many chemicals, while sediment-water and sediment-biota chemical activity ratios exceeded unity by up to six orders of magnitudes. Causes may be: disequilibrium due to slow desorption kinetics and/or an underestimation of partition coefficients due to the presence of strongly adsorbing phases in the sediments. Water concentrations, particularly when using LSER for prediction of partition coefficients were good predictors of internal concentrations in gammarids for most emerging pollutants. Some hydrophilic chemicals such as the neonicotinoid imidacloprid tend to accumulate more in G. pulex than expected from equilibrium partitioning. This conclusion holds both for KOW as well as for LSER-based predictions and suggests previously unidentified mechanisms of bio-accumulation which may include binding to specific protein structures.

Imidacloprid Causes DNA Damage in Fish: Clastogenesis as a Mechanism of Genotoxicity
https://link.springer.com/article/10.1007%2Fs00128-018-2338-0

Iturburu, F. G., Simoniello, M. F., Medici, S., Panzeri, A. M., & Menone, M. L. (2018) Imidacloprid Causes DNA Damage in Fish: Clastogenesis as a Mechanism of Genotoxicity. *Bulletin of Environmental Contamination and Toxicology*, 1-5. https://link.springer.com/article/10.1007%2Fs00128-018-2338-0

Neonicotinoids are one of the most widely used insecticides in the world. DNA damage is considered an early biological effect which could lead to reproductive and carcinogenic effects. The present study aimed to evaluate DNA damage and bases oxidation as a mechanism of genotoxicity, on the freshwater fish *Australoheros facetus* acutely exposed to imidacloprid (IMI). The Comet assay with the nuclease ENDO III enzyme was performed for detecting pyrimidine bases oxidation using blood samples. Micronucleus and other nuclear abnormalities frequencies were also quantified. A significant increase of damage index at 100 and 1000 μg/L IMI was detected; while ENDO III score increased from 1 to 1000 μg/L IMI; varying both in a linear concentration-response manner. MN frequency increased in fish exposed to 1000 μg/L IMI. These results show that short-term exposures to environmentally relevant concentrations of IMI could affect the genetic integrity of fishes through oxidative damage.

Can chronic exposure to imidacloprid, clothianidin, and thiamethoxam mixtures exert greater than additive toxicity in *Chironomus dilutus?*
https://www.sciencedirect.com/science/article/pii/S0147651318301830

Maloney, E. M., Morrissey, C. A., Headley, J. V., Peru, K. M., & Liber, K. (2018) Can chronic exposure to imidacloprid, clothianidin, and thiamethoxam mixtures exert greater than additive toxicity in *Chironomus dilutus?. Ecotoxicology and Environmental Safety, 156,* 354-365. https://doi.org/10.1016/j.ecoenv.2018.03.003

Widespread agricultural use of neonicotinoid insecticides has resulted in frequent detection of mixtures of these compounds in global surface waters. Recent evidence suggests that neonicotinoid mixtures can elicit synergistic toxicity in aquatic insects under acute exposure conditions, however this has not been validated for longer exposures more commonly encountered in the environment. Therefore, we aimed to characterize the chronic (28-day) toxicity of imidacloprid, clothianidin, and thiamethoxam mixtures under different doses and mixture ratios to determine if the assumption of synergistic toxicity would hold under more environmentally realistic exposure settings. The sensitive aquatic insect Chironomus dilutus was used as a representative test species, and successful emergence was used as a chronic endpoint. Applying the MIXTOX modeling approach, predictive parametric models were fitted using single-compound toxicity data and statistically compared to observed toxicity in subsequent mixture tests. Imidacloprid-clothianidin, clothianidin-thiamethoxam and imidacloprid-clothianidin-thiamethoxam mixtures did not significantly deviate from concentration-additive toxicity. However, the cumulative toxicity of the imidacloprid-thiamethoxam mixture deviated from the concentration-additive reference model, displaying dose-ratio dependent synergism and resulting in up to a 10% greater reduction in emergence from that predicted by concentration addition. Furthermore, exposure to select neonicotinoid mixtures above 1.0 toxic unit tended to shift sex-ratios toward more male-dominated populations. Results indicate that, similar to acute exposures, the general assumption of joint additivity cannot adequately describe chronic cumulative toxicity of all neonicotinoid mixtures. Indeed, our observations of weak synergism and sex-ratio shifts elicited by some mixture combinations should be considered in water quality guideline development and environmental risk assessment practices for neonicotinoid insecticides, and explored in further investigations of the effects of neonicotinoid mixtures on aquatic communities.

Cholinesterase activity in the cup oyster *Saccostrea* sp. exposed to chlorpyrifos, imidacloprid, cadmium and copper
https://www.sciencedirect.com/science/article/pii/S0147651317309016
Moncaleano-Niño, A., Luna-Acosta, A., Gómez-Cubillos, M. C., Villamil, L., & Ahrens, M. J. (2018). Cholinesterase activity in the cup oyster *Saccostrea* sp. exposed to chlorpyrifos, imidacloprid, cadmium and copper. *Ecotoxicology and Environmental Safety, 151,* 242-254. https://doi.org/10.1016/j.ecoenv.2017.12.057

In the present study, the sensitivity and concentration dependence of three functionally-defined components of cholinesterase activity (total: T-ChE; eserine-sensitive: Es-ChE; and eserine-resistant: Er-ChE) were quantified in the gill, digestive gland and adductor muscle of the tropical cup oyster *Saccostrea* sp., following acute (96 h) aqueous exposure to commercial formulations of the organophosphate (OP) insecticide chlorpyrifos and the neonicotinoid (NN) imidacloprid (concentration range: 0.1–100 mg/L), as well as to dissolved cadmium and copper (concentration range: 1–1000 µg/L). Oysters (1.5–5.0 cm shell length), field-collected from a boating marina in Santa Marta, Colombia (Caribbean Sea) were exposed in the laboratory to each substance at five concentrations. T-ChE, Es-ChE, and Er-ChE activity were quantified in the three tissues in pools of 5 individuals (3 replicates per concentration), before and after inhibition with the total cholinesterase inhibitor eserine (physostigmine, 100 µM). Oysters exposed to chlorpyrifos, imidacloprid and Cd showed reduced T-ChE and Es-ChE activity in gills at highest exposure concentrations, with Es-ChE activity being inhibited proportionally more so than T-ChE, whereas Er-ChE activity showed no significant concentration-response. Digestive gland also showed diminished T-ChE, Es-ChE and Er-ChE activity for highest chlorpyrifos and Cd concentrations relative to controls, but an increase of T-ChE and Er-ChE activity at the highest imidacloprid concentration (100 mg/L). For Cu, T-ChE, Es-ChE and Er-ChE activities in gills and digestive gland were elevated relative to controls in oysters exposed to Cu concentrations > 100 µg/L. In adductor muscle, T-ChE, Es-ChE and Er-ChE activity showed no apparent pattern for any of the four xenobiotics and concentration levels tested. Although this study confirms acute (96 h) concentration-dependent reduction of tissue T-ChE and Es-ChE activity in gills and digestive glands of *Saccostrea* sp. exposed to high concentrations of chlorpyrifos (100 mg/L), significant changes in T-ChE, Es-ChE and Er-ChE were also caused by exposure to Cd and Cu at concentrations > 100 µg/L and by exposure to imidacloprid (100 mg/L), indicating that cholinesterase activity is not a specific biomarker of organophosphate exposure in this species, but, rather, a biomarker of diverse xenobiotic exposure.

Pesticides from wastewater treatment plant effluents affect invertebrate communities
https://www.sciencedirect.com/science/article/pii/S0048969717305132
Münze, R., Hannemann, C., Orlinskiy, P., Gunold, R., Paschke, A., Foit, K., . . . Liess, M. (2017) *Science of the Total Environment, 599-600*, 387-399. https://doi.org/10.1016/j.scitotenv.2017.03.008

We quantified pesticide contamination and its ecological impact up- and downstream of seven wastewater treatment plants (WWTPs) in rural and suburban areas of central Germany. During two sampling campaigns, time-weighted average pesticide concentrations (cTWA) were obtained using Chemcatcher® passive samplers; pesticide peak concentrations were quantified with event-driven samplers. At downstream sites, receiving waters were additionally grab sampled for five selected pharmaceuticals. Ecological effects on macroinvertebrate structure and ecosystem function were assessed using the biological indicator system SPEARpesticides (SPEcies At Risk) and leaf litter breakdown rates, respectively. WWTP effluents substantially increased insecticide and fungicide concentrations in receiving waters; in many cases, treated wastewater was the exclusive source for the neonicotinoid insecticides acetamiprid and imidacloprid in the investigated streams. During the ten weeks of the investigation, five out of the seven WWTPs increased in-stream pesticide toxicity by a factor of three. As a consequence, at downstream sites, SPEAR values and leaf litter degradation rates were reduced by 40% and 53%, respectively. The reduced leaf litter breakdown was related to changes in the macroinvertebrate communities described by SPEARpesticides and not to altered microbial activity. Neonicotinoids showed the highest ecological relevance for the composition of invertebrate communities, occasionally exceeding the Regulatory Acceptable Concentrations (RACs). In general, considerable ecological effects of insecticides were observed above and below regulatory thresholds. Fungicides, herbicides and pharmaceuticals contributed only marginally to acute toxicity. We conclude that pesticide retention of WWTPs needs to be improved.

Complex mixtures of dissolved pesticides show potential aquatic toxicity in a synoptic study of Midwestern U.S. streams

https://www.sciencedirect.com/science/article/pii/S0048969717315735

Nowell, L. H., Moran, P. W., Schmidt, T. S., Norman, J. E., Nakagaki, N., Shoda, M. E., . . . Hladik, M. L. (2018) *Science of the Total Environment, 613-614,* 1469-1488. https://doi.org/10.1016/j.scitotenv.2017.06.156

Aquatic organisms in streams are exposed to pesticide mixtures that vary in composition over time in response to changes in flow conditions, pesticide inputs to the stream, and pesticide fate and degradation within the stream. To characterize mixtures of dissolved-phase pesticides and degradates in Midwestern streams, a synoptic study was conducted at 100 streams during May–August 2013. In weekly water samples, 94 pesticides and 89 degradates were detected, with a median of 25 compounds detected per sample and 54 detected per site. In a screening-level assessment using aquatic-life benchmarks and the Pesticide Toxicity Index (PTI), potential effects on fish were unlikely in most streams. For invertebrates, potential chronic toxicity was predicted in 53% of streams, punctuated in 12% of streams by acutely toxic exposures. For aquatic plants, acute but likely reversible effects on biomass were predicted in 75% of streams, with potential longer-term effects on plant communities in 9% of streams. Relatively few pesticides in water—atrazine, acetochlor, metolachlor, imidacloprid, fipronil, organophosphate insecticides, and carbendazim—were predicted to be major contributors to potential toxicity. Agricultural streams had the highest potential for effects on plants, especially in May–June, corresponding to high spring-flush herbicide concentrations. Urban streams had higher detection frequencies and concentrations of insecticides and most fungicides than in agricultural streams, and higher potential for invertebrate toxicity, which peaked during July–August. Toxicity-screening predictions for invertebrates were supported by quantile regressions showing significant associations for the Benthic Invertebrate-PTI and imidacloprid concentrations with invertebrate community metrics for MSQA streams, and by mesocosm toxicity testing with imidacloprid showing effects on invertebrate communities at environmentally relevant concentrations. This study documents the most complex pesticide mixtures yet reported in discrete water samples in the U.S. and, using multiple lines of evidence, predicts that pesticides were potentially toxic to nontarget aquatic life in about half of the sampled streams.

Imidacloprid exposure cause the histopathological changes, activation of TNF-α, iNOS, 8-OHdG biomarkers, and alteration of caspase 3, iNOS, CYP1A, MT1 gene expression levels in common carp (*Cyprinus carpio* L.)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5751999/

Özdemir, S., Altun, S., & Arslan, H. (2018). Imidacloprid exposure cause the histopathological changes, activation of TNF-α, iNOS, 8-OHdG biomarkers, and alteration of caspase 3, iNOS, CYP1A, MT1 gene expression levels in common carp (*Cyprinus carpio* L.). *Toxicology Reports*, *5*, 125–133. http://doi.org/10.1016/j.toxrep.2017.12.019

Imidacloprid (IMI) is a neonicotinoid that is widely used for the protection of crops and carnivores from insects and parasites, respectively. It is well known that imidacloprid exposure has a harmful effect on several organisms. However, there is little information about imidacloprid toxicity in aquatic animals, particularly fish. Thus, in the current study, we assessed the histopathological changes; activation of iNOS, 8-OHdG and TNF-α; and expression levels of caspase 3, iNOS, CYP1A and MT1 genes in the common carp exposed to imidacloprid. For this purpose, fish were exposed to either a low dose (140 mg/L) or a high dose (280 mg/L) of imidacloprid for 24 h, 48 h, 72 h and 96 h. After IMI exposure, we detected hyperplasia of secondary lamellar cells and mucous cell hyperplasia in the gills, as well as hydropic degeneration in hepatocytes and necrosis in the liver. Moreover, 8-OHdG, iNOS and TNF-α activation was found particularly in the gills and liver but also moderately in the brain. Transcriptional analysis showed that caspase 3 expression was altered low dose and high doses of IMI for 72 h and 96 h exposure ($p < 0.05$), iNOS expression was up-regulated with both low and high doses of IMI and in a time-dependent manner ($p < 0.05$, $p < 0.01$, $p < 0.001$), CYP1A expression was not significantly changed regardless of the dose of IMI and exposure time ($p > 0.05$) except with low and high doses of IMI for 96 h ($p < 0.05$), and lastly, MT1 gene expression was up-regulated only in the brain with low doses of IMI for 96 h and high doses of IMI for 48 h, 72 h and 96 h exposure ($p < 0.05$, $p < 0.01$). Our results indicated that acute IMI exposure moderately induce apoptosis in the brain but caused severe histopathological lesions, inflammation, and oxidative stress in the gills, liver, and brain of the common carp.

Neonicotinoid insecticides imidacloprid, guadipyr, and cycloxaprid induce acute oxidative stress in *Daphnia magna*

https://www.sciencedirect.com/science/article/pii/S0147651317307108

Qi, S., Wang, D., Zhu, L., Teng, M., Wang, C., Xue, X., & Wu, L. (2018). Neonicotinoid insecticides imidacloprid, guadipyr, and cycloxaprid induce acute oxidative stress in *Daphnia magna*. *Ecotoxicology and Environmental Safety, 148,* 352-358. https://doi.org/10.1016/j.ecoenv.2017.10.042

Cycloxaprid (CYC) and guadipyr (GUA) are two new and promising neonicotinoid insecticides whose effects on Daphnia magna are as yet unknown. In this study, the acute toxicities of CYC and GUA to D. magna, including immobilization and embryo-hatching inhibition, and their effects on antioxidant enzymes and related gene expression were determined after a 48-h exposure. Imidacloprid (IMI) was evaluated at the same time as a reference agent. The 48-h EC50 values of IMI, GUA, and CYC for neonate immobilization were 13.0–16.5 mg/L and for embryo hatching were 11.3–16.2 mg/L. The specific activity of the enzymes superoxide dismutase (SOD) and catalase (CAT) were interfered by IMI, but not by GUA and CYC, while the activity of acetylcholinesterase (AChE) was significantly increased by IMI, but inhibited by GUA and CYC. The relative expressions of the Sod-Cu/Zn, Sod-Mn, Cat, and Ache genes were usually inhibited by IMI, GUA, and CYC, except for Cat by CYC, Ache by GUA, and Sods by IMI. For vitellogenin genes with a SOD-like domain (Vtg1/2-sod), relative expression was increased by IMI and inhibited by GUA and CYC, indicating that IMI, GUA, and CYC have potential toxicity toward reproduction. CYC and GUA are highly active against IMI-resistant pests, and considering the similar toxicity of IMI to D. magna, CYC and GUA are suitable for use in future integrated pest management systems.

Acute Toxicity of Six Neonicotinoid Insecticides to Freshwater Invertebrates: Aquatic toxicity of neonicotinoid insecticides
https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.4088
Raby, M., Nowierski, M., Perlov, D., Zhao, X., Hao, C., Poirier, D. G., & Sibley, P. K. (2018) *Environmental Toxicology and Chemistry, 37*(5), 1430-1445. https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.4088

Neonicotinoids are a group of insecticides commonly used in agriculture. Due to their high water solubility, neonicotinoids can be transported to surface waters and have the potential to be toxic to aquatic life. The present study assessed and compared the acute (48 or 96 h) toxicity of 6 neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) to 21 laboratory-cultured and field-collected aquatic invertebrates spanning 10 aquatic arthropod orders. Test conditions mimicked species' habitat, with lentic taxa exposed under static conditions, and lotic taxa using recirculating exposure systems. Lethal (LC50) and effective (immobility, EC50) concentrations were calculated and used to construct separate lethal- and immobilization-derived species sensitivity distributions (SSDs) for each neonicotinoid, from which 5th percentile hazard concentrations (HC5s) were calculated. Results showed the most sensitive invertebrates were insects from the orders Ephemeroptera (e.g. Neocloeon triangulifer) and Diptera (Chironomus dilutus), while cladocerans (e.g. Daphnia magna, Ceriodaphnia dubia) were the least sensitive. HC5s were compared to neonicotinoid environmental concentrations from Ontario monitoring studies. For all neonicotinoids except imidacloprid, the resulting hazard quotients indicated little to no hazard in terms of acute toxicity to aquatic communities in Ontario freshwater streams. For the neonicotinoid imidacloprid, a moderate hazard was found when only invertebrate immobilization, and not lethality, data was considered.

Effects of imidacloprid on the ecology of sub-tropical freshwater microcosms
https://www.sciencedirect.com/science/article/pii/S0269749117345694?via%3Dihub
Sumon, K. A., Ritika, A. K., Peeters, E. T., Rashid, H., Bosma, R.H., Rahman, M. S., . . . Van den Brink, P.J. (2018). Effects of imidacloprid on the ecology of sub-tropical freshwater microcosms. *Environmental Pollution, 236*, 432-441. https://doi.org/10.1016/j.envpol.2018.01.102

The neonicotinoid insecticide imidacloprid is used in Bangladesh for a variety of crop protection purposes. Imidacloprid may contaminate aquatic ecosystems via spray drift, surface runoff and ground water leaching. The present study aimed at assessing the fate and effects of imidacloprid on structural (phytoplankton, zooplankton, macroinvertebrates and periphyton) and functional (organic matter decomposition) endpoints of freshwater, sub-tropical ecosystems in Bangladesh. Imidacloprid was applied weekly to 16 freshwater microcosms (PVC tanks containing 400 L de-chlorinated tap water) at nominal concentrations of 0, 30, 300, 3000 ng/L over a period of 4 weeks. Results indicated that imidacloprid concentrations from the microcosm water column declined rapidly. Univariate and multivariate analysis showed significant effects of imidacloprid on the zooplankton and macroinvertebrate community, some individual phytoplankton taxa, and water quality variables (i.e. DO, alkalinity, ammonia and nitrate), with *Cloeon* sp., *Diaptomus* sp. and *Keratella* sp. being the most affected species, i.e. showing lower abundance values in all treatments compared to the control. The observed high sensitivity of *Cloeon* sp. and *Diaptomus* sp. was confirmed by the results of single species tests. No significant effects were observed on the species composition of the phytoplankton, periphyton biomass and organic matter decomposition for any of the sampling days. Our study indicates that (sub-)tropical aquatic ecosystems can be much more sensitive to imidacloprid compared to temperate ones.

Joint toxic effects of triazophos and imidacloprid on zebrafish (*Danio rerio*)
https://www.sciencedirect.com/science/article/pii/S0269749117344597
Wu, S., Li, X., Liu, X., Yang, G., An, X., Wang, Q., & Wang, Y. (2018). Joint toxic effects of triazophos and imidacloprid on zebrafish (*Danio rerio*). *Environmental Pollution, 235*, 470-481. https://doi.org/10.1016/j.envpol.2017.12.120

Pesticide contamination is more often found as a mixture of different pesticides in water bodies rather than individual compounds. However, regulatory risk evaluation is mostly based on the effects of individual pesticides. In the present study, we aimed to investigate the individual and joint toxicities of triazophos (TRI) and imidacloprid (IMI) to the zebrafish (*Danio rerio*) using acute indices and various sublethal endpoints. Results from 96-h semi-static test indicated that the LC_{50} values of TRI to *D. rerio* at multiple life stages (embryonic, larval, juvenile and adult stages) ranged from 0.49 (0.36–0.71) to 4.99 (2.06–6.81) mg a.i. L^{-1}, which were higher than those of IMI ranging from 26.39 (19.04–38.01) to 128.9 (68.47–173.6) mg a.i. L^{-1}. Pesticide mixtures of TRI and IMI displayed synergistic response to zebrafish embryos. Activities of carboxylesterase (CarE) and catalase (CAT) were significantly changed in most of the individual and joint exposures of pesticides compared with the control group. The expressions of 26 genes related to oxidative stress, cellular apoptosis, immune system, hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal axis at the mRNA level revealed that zebrafish embryos were affected by the individual or joint pesticides, and greater changes in the expressions of six genes (*Mn-sod*, *CXCL-CIC*, *Dio1*, *Dio2*, *tsh* and *vtg1*) were observed when exposed to joint pesticides compared with their individual pesticides. Taken together, the synergistic effects indicated that it was highly important to incorporate joint toxicity studies, especially at low concentrations, when assessing the risk of pesticides.

Conclusion

Knowing what we now know about neonicotinoids, it is best to end consideration of any imidacloprid spraying into marine or estuarine waters, and instead to focus on habitat restoration, including eelgrass, and sustainable methods of restoring balance to the Bay.

Respectfully submitted,

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