The Impact of the Nation's Most Widely Used Insecticides on Birds

AMERICAN BIRD CONSERVANCY

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Dr. Pierre Mineau began his long and distinguished scientific career studying the effects of persistent organochlorine compounds, like DDT and PCBs, on fish-eating birds. He then became responsible for the Canadian assessment of new and existing pesticides to determine their adverse impacts on wildlife. In 1994 he transitioned from regulatory reviews to full-time research on the environmental impacts of pesticides, achieving the rank of Senior Research Scientist at Environment Canada. Working with international collaborators and graduate students, he works on assessing globally the environmental footprint of pesticides. He also studies how birds are exposed to pesticides and how bird populations respond to pesticide use and agricultural practices. His work includes defining the ecological values of birds in cropland as well as estimating the incidental take of birds from various other human activities. He has written more than 100 peer-reviewed publications and has authored some 200 presentations. He officially retired from public service in 2012 and now holds an Emeritus position with the Canadian government, serves as an adjunct research professor at Carleton University in Ottawa and at the University of Saskatchewan in Saskatoon, and consults privately.

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SYNOPSIS

First introduced in the 1990s in response to widespread pest resistance as well as health objections to older pesticides, the neonicotinoid insecticides quickly sailed to the top slot in global pesticide markets. Now the most widely-used insecticides in the world, it is difficult to find pest control commodities that do not contain one or several of the neonicotinoid insecticides. California alone has registered nearly 300 neonicotinoid products.

Neonicotinoids' toxicity to bees and other insects has brought them the most attention so far and has dominated recent concerns of regulatory institutions worldwide. In the United States, the Environmental Protection Agency's registration review of the neonicotinoids is focused on the threat to insect pollinators. The seriousness of this issue should not be underestimated, as one-third of the U.S. diet depends on these insect pollinators.

But much more is at stake. The environmental persistence of the neonicotinoids, their propensity for runoff and for groundwater infiltration, and their cumulative and largely irreversible mode of action in invertebrates raise environmental concerns that go well beyond bees.

This report reviews the effects on avian species and concludes that neonicotinoids are lethal to birds as well as to the aquatic systems on which they depend. A single corn kernel coated with a neonicotinoid can kill a songbird. Even a tiny grain of wheat or canola treated with the oldest neonicotinoid, imidacloprid, can poison a bird. As little as 1/10th of a corn seed per day during egg-laying season is all that is needed to affect reproduction with any of the neonicotinoids registered to date.

Birds depend heavily on the aquatic systems at the bottom of the food chain. But neonicotinoid contamination levels in surface and groundwater in the US and around the world are strikingly high, already beyond the threshold found to kill many aquatic invertebrates. EPA risk assessments have greatly underestimated this risk, using scientifically unsound, outdated methodology that has more to do with a game of chance than with a rigorous scientific process.

Major risk concerns raised by scientists both inside and outside the agency appear to have gone unheeded in agency registration decisions. The older insecticides that the neonicotinoids largely replaced – including organophosphates such as diazinon and chlorpyrifos, and carbamates such as carbofuran and methomyl – were highly damaging to people and wildlife. What is so disturbing is that in their rush to register alternatives, regulators have approved more and more neonicotinoid products for an

ever-growing number of uses without regard to the red flags raised by their experts concerning this persistent, cumulative, irreversibly-acting new class of pesticides.

Neonicotinoids are currently under registration review by EPA. The Agency's decision to approve, restrict, suspend, or cancel these powerful insecticides will have profound environmental and economic impact. We have a small window of opportunity in which to act; EPA's next review of this class of pesticides will not occur for at least 15 years, and the damage done in those intervening years will be irreversible.

The results of this study and others have led American Bird Conservancy and partners in the National Pesticide Reform Coalition to urge the EPA to take the following actions:

- Suspend all applications of neonicotinoids pending independent review of these products' effects on birds, terrestrial and aquatic invertebrates, and other wildlife.
- Expand its re-registration review of neonicotinoids beyond bees to include birds, aquatic invertebrates, and other wildlife.
- Ban the use of neonicotinoids as seed treatments.
- Require that registrants of acutely toxic pesticides develop the tools necessary to diagnose poisoned birds and other wildlife.

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EXECUTIVE SUMMARY

The neonicotinoids represent a relatively new group of insecticides. They were introduced in the early 1990s to counter widespread resistance in insect pests and increasing health and safety objections to the organophosphorous insecticides. Although of lower acute toxicity to vertebrates than the latter, the neonicotinoids' longer persistence, high water solubility, runoff and leaching potential as well as their very high toxicity to pollinators are placing them under increasing public and political scrutiny, especially now that they have become the most widely used pesticides in the world. Their toxicity to pollinators has brought them the most attention so far and has dominated the recent concerns of regulatory institutions worldwide.

The intent of this report is to review the risk that neonicotinoids pose to birds. Birds have borne more than their fair share of impacts from pesticides – from the early issues of eggshell thinning with DDT to the extensive mortality caused by the organophosphorous and carbamate insecticides that followed. Some researchers have suggested that birds may already be affected by neonicotinoids and that, at least in Europe, bird population declines can be blamed on these popular insecticides.

The main products reviewed here are acetamiprid, imidacloprid, thiacloprid, clothianidin and thiamethoxam. Minor compounds include dinotefuran, nitenpyram and nithiazine. For the sake of comparison, this report will discuss, where appropriate, a number of older insecticides that the neonicotinoids have replaced. This includes the organophosphorous insecticides diazinon, chlorpyrifos, malathion, terbufos and methamidophos, the carbamate insecticides carbofuran, methomyl, the pyrethroids tefluthrin and deltamethrin as well as the seed treatment insecticide carbathiin.

The report will emphasize US regulatory history although it will make reference to Canadian and EU regulatory reviews where relevant. For ease of consultation, summary points made here are detailed in the body of the report under the same section heading:

1. The history of neonicotinoid registrations highlights many of the critical failings of our current pesticide registration system. Regulatory agencies in both the US and Canada (and to some extent in Europe as well) exhibited a conflicted approach to the neonicotinoid class of compounds – on the one hand expressing serious concerns about the persistence, mobility and toxicity of the products – on the other hand, granting registrations in an ever-widening range of crops and non-agricultural use sites.

   There is evidence the neonicotinoids got a very 'soft ride' through registration. Based on the existing record, registration decisions concerning the neonicotinoid insecticides were overwhelmingly positive despite a consistent record of cautionary warnings from the scientists involved in the assessment process. Increased concerns in the scientific and popular literature over imidacloprid, clothianidin and other neonicotinoid insecticides did not deter pesticide manufacturers, who appeared to be in a race to register as many uses as possible. It looks as if the USEPA and other regulatory agencies consistently approved registrations despite their own scientists' repeated and ever-growing concerns. It is relevant to ask why we conduct scientific evaluations of products if those evaluations have little or no bearing on the registration decisions that are made, and when staff scientists warning of 'major risk concerns' appear to be ignored.

   Even though several early reviews of the first neonicotinoid, imidacloprid, correctly identified

issues of bird and mammal toxicity, persistence, runoff/leaching and aquatic toxicity, regulators failed to apply some of the lessons learned in the 1990s with imidacloprid to more recently developed compounds such as clothianidin and thiamethoxam. They also failed (and continue to fail) to consider the impact of combined neonicotinoid residues in the environment. Regulators have tended to place inordinate faith in precautionary labelling to mitigate very serious terrestrial and aquatic risks.

2. The acute toxicity of neonicotinoids to birds is lower than the acute toxicity of many of the insecticides they have replaced, notably the organophosphorous and carbamate insecticides. However, EPA and other regulatory agencies worldwide have underestimated the toxicity of these compounds to birds. This undervaluation is partly because the risk assessment methods fail to account sufficiently for interspecies variation in toxicity.

   Depending on the specific insecticide, we have found that EPA underestimates toxicity by 1.5 - 10 fold if the intent of the exercise is to protect most potentially exposed bird species, and not merely mallards and bobwhites, the two test species.

   In addition, there is some evidence that the neonicotinoid insecticides will debilitate birds at a much reduced fraction of a lethal dose compared to other pesticides and this debilitation will be longer-lasting. Small non-lethal doses are likely to cause partial paralysis and other sub-lethal effects in birds. These effects slip under the radar screen in regulatory assessments based entirely on lethal levels.

3. The chronic/reproductive toxicity of neonicotinoids to birds is high. This was recognised very early on in the regulatory reviews of the various active ingredients. Yet high reproductive toxicity in birds is typically ignored in the pesticide review process – whether for neonicotinoids or for other pesticides. Many pesticides fail the current reproduction screen, and many uncertainties exist surrounding the extrapolation of laboratory data to actual field conditions. These problems are not new but regulatory agencies have failed to address the situation. Because the neonicotinoids are systemic and persistent in soils, and because several are used as seed treatment chemicals, they are available to birds in a chronic fashion, making their potential to affect reproduction an even greater concern.

   The standard tests carried out by manufacturers place reproductive effects at dosing levels ranging from 2 to 13 mg/kg/day depending on the product. This level of exposure is easily achieved with seed treatment chemicals. However, very recent toxicological information from Japan suggests that testicular function in male birds as well as embryonic development in the offspring of exposed males is affected at levels much lower than indicated from these standard reproduction tests.

4. Of particular concern to birds are those compounds that are used as seed treatments, primarily imidacloprid, clothianidin, thiamethoxam and acetamiprid. Regardless of the exact label directions and requirements, seed-treatment chemicals are widely available to birds. Seeds are never fully covered with soil, making them easy to find by foraging birds. Spills are commonplace with current machinery. And many species have the ability to scrape and dig for planted seed. Seed treatments, by definition, will result in a high exposure situation for birds (as well as for small mammal species not discussed in this report). Both the EPA in the US and Pest

Management Regulatory Agency (PMRA) in Canada have failed at times to consider this high exposure potential in their assessments.

5. The amount of insecticide adhering to the average corn (maize) seed can result in acute intoxications in birds with all three registered products – imidacloprid, clothianidin and thiamethoxam. With imidacloprid, a single seed may prove lethal for an average-sized bird (e.g. blue jay-sized) likely to be picking up whole corn seed from seeded fields. A few seeds only are required in the case of clothianidin or thiamethoxam. Indeed, we believe that imidacloprid is too acutely toxic to be used as a seed treatment insecticide on any seed type based on our assessment of its use in cereals and oilseeds. Acute intoxications in wheat or canola are less likely with clothianidin or thiamethoxam because these neonicotinoids are less acutely toxic to birds. The birds would need to ingest a greater number of the treated seeds to receive a lethal dose.

   However, based on chronic/reproduction endpoints, all seed treatments are predicted to cause effects given the very small number of seeds (as low as 1/10 of a seed per day during egg laying season) needing to be ingested to push birds into a 'critical range' where reproductive effects are expected. The main uncertainty here is how long the seeds will be available to birds and how long dosing is necessary before the type of effects seen in the laboratory will be seen in the wild. There are huge uncertainties – for instance what types of effects might be seen in altricial species (those in which the newly-hatched young are born relatively helpless, such as most passerines, or perching birds) and how this differs from effects seen in precocial species (in which the newly hatched young are relatively mature, such as ducks and geese, grouse and pheasants).

   Based on our current understanding and risk assessment procedures in place, the neonicotinoids as a group have a high potential to affect avian reproduction. This is due in large part to the very high exposure potential that seed treatment chemicals represent and the persistent nature of the neonicotinoids.

   A publication currently in press advances the hypothesis that the neonicotinoids are a contributory factor to many wildlife diseases through immune suppression. The authors make this claim on the basis of geographic and temporal associations. The sheer scale and seriousness of the issue demands that this hypothesis be investigated more fully.

   Despite industry claims, the neonicotinoids are not repellant to birds. Any demonstrated avoidance can be explained by hesitation before a new food source or post-ingestion intoxication and illness. Neither is sufficient to spare birds from either acute or chronic effects. There are parallels with the cholinesterase-inhibiting insecticides where repellency was similarly thought to reduce in-field risks. For example, the organophosphorous insecticide diazinon is extremely well avoided in the laboratory. Yet, thousands of geese and other species have grazed their way to an early death on diazinon-treated turf.

6. The link between impacts on the insect food of birds and declines of bird species is difficult to establish unequivocally, save for the evidence linking the grey partridge to pesticide use in the UK. A review of the existing literature suggests that it is difficult to predict the relative importance of food supply during the breeding season (i.e. when an insect food base is critical) compared to other risks such as habitat loss, food supply during migration and during winter, predation or

even direct losses from poisoning or disturbances such as mowing or tillage. Each species responds to a different set of stressors and it is likely that many of the declines have multiple causes.

Nevertheless, it would be foolhardy to argue that dramatic losses of insect biomass from ecosystems is not going to have potential consequences on the integrity of those ecosystems and on the species that depend to varying degrees on the spring-summer flush of insect food. The impacts on terrestrial food chains from neonicotinoid (and other systemic) insecticides may be much longer-lived and pernicious than those we have seen with non-systemic products. Generally speaking, an over-efficient removal of insects in crop fields is seldom seen as a matter of serious concern by regulators – especially in North America. The indirect impacts of pesticides are not considered in registration reviews – whether in the US or anywhere else in the world.

In his book, Dutch toxicologist Henk Tennekes (2010) makes the case that the contamination of surface water by neonicotinoids is so widespread in the Netherlands (and possibly elsewhere in Europe), that loss of insect biomass on a continental scale is behind many of the widespread declines that are being seen, be they of marsh birds, heath or meadow birds or even coastal species. This suggests that we should be looking at possible links between neonicotinoid insecticides and birds, not on a farm scale, but in the context of whole watersheds and regions. Impacts from the neonicotinoids may very well be further afield than the arable area on which they are used, and many of those impacts may be mediated through the aquatic environment. Because aquatic impacts are considered during product registration reviews, it is reasonable to ask whether the potential impact of neonicotinoids to aquatic life has been assessed correctly.

7. Unfortunately, North American regulators have greatly underestimated the toxicity of imidacloprid and other neonicotinoids to aquatic invertebrates. Reference doses are set using outdated methodology which has more to do with a game of chance than with a rigorous scientific process. A complete disregard for the peer-reviewed literature is a constant factor throughout the history of neonicotinoid assessments.

   For imidacloprid, we believe that a scientifically defensible reference level (a water concentration at which undesirable effects are likely to be seen in reasonably sensitive species) for acute invertebrate effects (following short term exposure) is approximately 0.2 ug/l. European regulators acknowledge that acute effects are likely at levels exceeding 0.5 ug/l. In contrast, the EPA's regulatory and non-regulatory reference levels are set at 35 ug/l.

   Similarly, a reasonable reference level for effects following chronic exposure is at least an order of magnitude lower, or between 0.01 and 0.03 ug/l rather than the 0.5 ug/l used in the U.S.. EPA's approach to the assessment of aquatic risk is scientifically unsound and places aquatic environments at risk. In addition, there is evidence that risk managers at EPA have ignored aquatic risk ratios that exceeded the usual level of concern, notwithstanding the fact that those risk ratios were grossly underestimated in the first place.

   Based on the relative sensitivity of aquatic insects tested with several of the neonicotinoid insecticides, we suggest that these reference levels should also apply to the other neonicotinoid insecticides, notably acetamiprid, thiacloprid, clothianidin and thiamethoxam. In fact, because of their similarity in mode of action, the above reference levels should apply to the sum of all residues for all parent neonicotinoid compounds as well as some of the degradates.

Neonicotinoid insecticides may be totally unprecedented in the history of pesticide registration in that measured groundwater contamination levels have been high enough to cause aquatic impacts.

Data on surface water contamination from surveys to date, notably from California and from the Canadian Prairies, indicate that concentrations of several of the neonicotinoid insecticides are high enough to be causing impacts in aquatic food chains. Data from other jurisdictions (e.g. the Netherlands) show even higher levels of contamination.

It is clear that neonicotinoids have often replaced other insecticides of higher short-term toxicity to aquatic life – especially fish. However, the mode of action of neonicotinoids, which entails a cumulative irreversible action and delayed effects in invertebrates, as well as their persistence in the environment, makes them particularly worrisome. It is clear that we are witnessing contamination of the aquatic environment at levels that will affect aquatic food chains. This has a potential to affect consumers of those aquatic resources, be they birds, fish or amphibians.

In conclusion, policymakers and the public should be very concerned about the impact of neonicotinoid insecticides on birds and on the broader environment. Specifically, we should be concerned that:

- regulatory procedures are scientifically deficient and prone to the vagaries of chance
- risk managers appear to place minimal weight on concerns raised by environmental scientists who carry out the scientific evaluations of the products
- despite all the red flags, regulators are adding to the list of permissible uses
- neonicotinoids – the most heavily used insecticides in the world – are systemic products that are extremely persistent and very much prone to runoff and groundwater infiltration
- some neonicotinoids are capable of causing lethal intoxications and all are predicted to cause reproductive dysfunction in birds
- where we have looked, we have found broad-scale aquatic contamination at levels expected to cause impacts on aquatic food chains.
- any future re-evaluation of these products appears to focus solely on pollinator toxicity. The seriousness of pollinator losses should not be underestimated, but there is much more at stake.

A moratorium on any further use expansion is currently being discussed in the EU and Member States. Some countries have moved forward on limited cancellations. The North American regulatory system needs to act rather than continue to ignore evidence of widespread environmental damage. There is evidence that US regulators waited far too long to impose needed restrictions on the toxic insecticides responsible for millions of bird deaths per year (Mineau 2004) and that this is one of the more plausible reasons for the decline of grassland/farmland birds in North America (Mineau and Whiteside, 2013). The neonicotinoids have largely replaced that older generation of chemicals. We are urging regulators to take seriously the red flags raised by this persistent, cumulative, irreversibly-acting new class of pesticides.

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Soybean plants by Howard F. Schwartz, Colorado State University, Bugwood.org

DETAILED DISCUSSION

For ease of consultation, the numbering of the sections below is consistent with the main points made in the executive summary.

The neonicotinoid insecticides represent a relatively new group of insecticides. They were introduced in the early 1990s to counter widespread pest resistance and increasing health and environmental objections to the organophosphorous insecticides. Although of lower acute toxicity to vertebrates than the organophosphates, neonicotinoids' longer persistence, high water solubility and runoff potential as well as their very high toxicity to pollinators are bringing them increased scrutiny, especially now that they have become the world's most widely used insecticides. Their toxicity to pollinators has brought them the most attention so far and has dominated the recent concerns of regulatory bodies worldwide.

The intent of this report is to review the risk that neonicotinoids pose to birds. Birds have borne more than their fair share of impacts from pesticides – from the early issues of eggshell thinning with DDT to the extensive mortality caused by the organophosphorous and carbamate insecticides that followed.

The main products reviewed here are acetamiprid, imidacloprid, thiacloprid, clothianidin and thiamethoxam. Minor members of the neonicotinoid family include dinotefuran, nitenpyram and nithiazine.

For the sake of comparison, this report will discuss, where appropriate, a number of older insecticides that the neonicotinoids have replaced in key markets where they now dominate. This includes the organophosphorous insecticides diazinon, chlorpyrifos, malathion, terbufos and methamidophos, the carbamate insecticides carbofuran, methomyl, the pyrethroids tefluthrin and deltamethrin as well as the seed treatment insecticide carbathiin.

The report will emphasize US regulatory history although it will make reference to Canadian and EU documents where relevant.

1. The 'soft ride' of neonicotinoids through registration

A look at the regulatory history of the three main neonicotinoids (imidacloprid, clothianidin and thiamethoxam) shows the extent to which registration decisions have ignored repeated warnings about possible environmental impacts.

1.1. Imidacloprid

The oldest neonicotinoid, imidacloprid, was registered in 1994 in potatoes, cotton and apples. At the time, EPA[1] scientists cautioned that both the acute and the chronic aquatic risk triggers had been exceeded (USEPA 1994a, b) for both non-endangered and endangered species. A 200 ft. buffer around aquatic habitats frequented by endangered species was suggested in consultation with the

[1] The acronyms EPA, USEPA and US EPA are used interchangeably in this report. They all refer to the U.S. Environmental Protection Agency, the body responsible for federal pesticide regulation in the U.S.

registrant. Detailed geographical assessments for endangered species were to continue. In its initial review, EPA identified that imidacloprid was both persistent and mobile and was likely to give rise to groundwater contamination. The reviewers went as far as to say that there was no need to conduct long term field dissipation studies because: "…*the studies would probably only provide information that would confirm that (imidacloprid) is both persistent and mobile, which we already know*" (USEPA 1994b).

Jumping forward to more recent times, not much seems to have changed. The re-registration document of 2007 states that the ecological risk assessments to date have failed to properly assess the risks to endangered species. In this 2007 review, imidacloprid was said to carry '*an acute and chronic risk to both freshwater and estuarine/marine invertebrates*' in agreement with the 1994 conclusions, but it was also said to '*have the potential to cause chronic risk to avian species and small mammals*' as well. Under '**major risk concerns**' the EPA scientist stated: "*Regarding effects to nontarget terrestrial and aquatic organisms, the structure activity relationship between imidacloprid, a chloronicotinyl compound, and its analog nicotine, suggests a potential concern. Studies in the published literature show that nicotine can cause developmental toxicity, including functional deficits, in animals and/or humans that are exposed in utero*." This suggests a staff scientist who was concerned about the product but forced to follow an inadequate assessment paradigm when it came time to assess chronic or reproductive toxicity. **Unfortunately, there is no evidence that warnings of 'major risk concerns' such as this one have had any effect on registration decisions.**

In Canada, imidacloprid was first registered in 1995 for potatoes. Many other uses were registered in the years that followed (PMRA[2] 1997, 2001), including lettuce, turf, as well as seed treatments in canola and corn. Because of concerns over water contamination and pollinators, the PMRA stated in 2001 that only new uses with '*low environmental risk situations*' or '*critical uses in the context of sustainable pest management programs where mitigative measures can be incorporated into product labelling*' would be considered. Seed dressings were considered to represent use patterns with low environmental risk – despite an acknowledgment that imidacloprid was 'persistent' with soil DT50[3] values in the range of 1-2 years. Such slow breakdown means that the pesticide has the potential to gradually increase in concentration in the soils if used on a repeated basis. Also, the compound is extremely water soluble and therefore mobile. In September of 2001, the PMRA acknowledged that imidacloprid had the potential to contaminate ground water and that once contaminated, no practical remedial action was possible. This admission came close on the heel of the registration of imidacloprid as a seed treatment in field corn (May 2001). This raises obvious questions about the wisdom of the use expansion to corn when serious concerns about the product were known.

1.2. Clothianidin

Clothianidin, like thiamethoxam, is used principally as a seed treatment though several other uses have been registered also. This active ingredient may provide the best example of the apparent disconnect between registration decisions and the scientific review of the data.

[2] Pest Management Regulatory Agency. Under the responsibility of Health Canada, the agency responsible for pesticide regulation in Canada.
[3] DT50 is the time required for half of the parent material to break down. Units are typically days.

In 2003, EPA first reviewed the ecological risk from clothianidin – initially for corn and canola[4] seed treatments, two major uses on a continental level (USEPA 2003a, b). The Agency concluded that exposure to treated seed through ingestion might result in chronic risk to birds and mammals, especially mammals where consumption of 1-2 seeds only could push them to an exposure level at which reproductive effects are expected.

The detailed assessment of clothianidin's reproductive toxicity in mammals certainly provides cause for concern:

*"These chronic effects in mammals can include decreased body weight gains and delayed sexual maturation (males only); decreased absolute thymus weight in F1 pups (both sexes), and increased stillbirths (F1 and F2 litters). Reproductive effects were noted for adult rats that included decreased sperm motility and increased number of sperm with detached heads. These effects could especially result in toxic risk to those species that have a limited reproductive capacity (e.g., few litters or broods, those animals that reproduce only once per year, and Endangered Species). Although effects on sperm mobility may not effect (*sic*) the number of offspring in some cases, there can be an impact on the ratio of gender composition (e.g., more males produced as opposed to females) which can result in population reductions. Over time, developmental effects were noted in rabbits at 75 mglkglday, and included premature deliveries, decreased gravid uterine weights, and increased litter incidence of missing lung lobe in the fetus. The possibility of chronic risk suggests a Restricted Use compound, Endocrine Disruption candidate, as well as Endangered Species concern for mammals, birds, and invertebrates."*

The EPA made a critical error, however, in stating that "…*the prescribed agricultural practice of drilling seeds at planting should reduce exposure to these animals*." A quick review of the scientific literature (see section 2) would have shown that this was a naïve and incorrect assumption.

In the same 2003 assessment, EPA described the chemical as persistent and mobile, with '*potential to leach to ground water as well as runoff to surface waters*' (USEPA 2003a).

The high toxicity to bees was identified as well, but this is outside the scope of the current review.

With this level of concern, one might have expected regulators to move slowly on new uses, especially major ones. However, a plethora of registered uses for clothianidin followed in quick succession: Tobacco, turf, apples, pears and ornamentals (USEPA 2004); potatoes, grapes, sorghum and cotton (USEPA 2005); sugar beets (USEPA 2006, 2007); tuberous, corm and bulb vegetables, leafy greens, cole crops, cucurbits and other miscellaneous vegetables, cranberry and other low growing berries, tree nuts, cereals, figs, pomegranates, more cotton, soybean, peaches, and more potato uses (USEPA 2009); increased application rates to vegetables and tree trunk spraying (USEPA 2010); mustard and cotton seed (USEPA 2011a). By 2005, EPA scientists had significant concerns about pollinators; they had also increased concerns about both direct and indirect effects on terrestrial ecosystems. Now that more data had been gathered on acute and chronic aquatic toxicity, they had also started raising concerns about possible aquatic impacts.

[4] Canola is the term developed in Canada for specific varietals of oilseed rape. These are varietals of rape modified to have a low glucosinolate and erucic acid content and therefore fir for human consumption (e.g. 00 oilseed rape in Europe).

As is the case with many other hazardous products, the manufacturers and regulatory authorities appear to be satisfied with product labelling that shifts the responsibility of protecting the environment to the end user. A label statement on a clothianidin product such as '*Do not apply where runoff is likely to occur*' (Arena 50 WDG Reg. 59639-152) may seem reasonable enough at first blush. However, have the regulatory authorities assessed what proportion of their country's agricultural area can be treated without risk of runoff? If this is deemed to be a small proportion of the total, is it still reasonable to place this product in the hands of all growers? The same label goes on to specify: '*The properties of this chemical suggest it may leach into ground water if used in areas where soils are permeable and where the water table is very shallow.*' This statement is said to apply specifically to the State of Florida. Apparently, users elsewhere need not worry about groundwater contamination!

1.3. Thiamethoxam

Thiamethoxam's first registration dates back to 1999. Based on EPA's 2011 re-registration review document (USEPA 2011b), thiamethoxam is registered for several agricultural and non-agricultural commodities as well as for turf, ornamentals and as an antimicrobial on wood. The most recent ecological reviews are from 2010 when the Agency approved the uses in alfalfa, onions, peanuts, corn and leafy vegetables. Yet, as early as 2008, the Agency had stated, in their risk assessment for citrus fruits and tree nuts, that thiamethoxam posed a potential for '*direct adverse effects on freshwater invertebrates, birds and mammals*' (USEPA 2008b). They had also predicted '*structural and functional changes of both the aquatic and terrestrial ecosystems.*' As was the case with clothianidin, it would appear that these warnings from EPA scientists went unheeded.

Also, as of 2011, the Agency was still missing key pieces of data in order to support current uses of thiamethoxam. This included soil metabolism studies, terrestrial field dissipation studies as well as various aquatic toxicity requirements and new studies made necessary by emerging data on pollinator toxicity (USEPA 2011b). Thiamethoxam is considered by the Agency to be slightly toxic to birds on an acute and sub-acute basis. No mention is made of its reproductive toxicity in the 2011 re-registration summary document**. Interestingly, the same 2011 re-registration document stated that: *"… the Agency does not have data to indicate that thiomethoxam shares a common mechanism of toxicity with other chemical substances and therefore does not see a need for a cumulative risk assessment*." This is a strange statement indeed, if only because the major degradation product for thiamethoxam is clothianidin. Furthermore, all neonicotinoid insecticides registered to date are considered to have the same mode of action for resistance development purposes (nAChR agonists, Group 4A)** (Jeschke et al. 2011). The Agency proposes to complete reregistration review by 2018.

In 2001, Canada's PMRA registered thiamethoxam for use as a seed treatment in canola, replacing the standard lindane seed treatment or a lindane/granular terbufos combination. Despite its demonstrated water solubility and soil persistence, the agency did not assess any environmental toxicology except potential impacts on birds. It seems to have assumed that any possibility for impacts on aquatic systems was negligible. The ecological review of thiamethoxam came in 2007 (PMRA 2007) in order to register the product for use on potatoes and on a number of tree fruit crops. The Agency concluded that, other than requiring labeling for pollinators and buffer zones around aquatic bodies, the compound '*presents a negligible risk to wild mammals, birds, earthworms, fish, crustaceans, amphibians, algae and aquatic plants.*' We believe that the evidence reviewed below shows otherwise.

Increasing concerns raised in the scientific and popular literature in the last decade over imidacloprid, clothianidin and other neonicotinoid insecticides did not seem to deter pesticide manufacturers, who appeared to be in a race to register as many uses as possible in complete disregard of any environmental consequences. It looks as if the USEPA and other regulatory bodies were rushing to oblige despite their own scientists' repeated and ever-growing concerns, raised in internal reviews.

In California alone, the California Department of Pesticide Regulation, as of July 2012, stated that the re-evaluation of neonicotinoids of concern *viz*. pollinator exposure (excluding pet uses and the like) would involve 293 pesticide products from 57 registrants and that as more products became registered (suggesting that registrations are on-going), DPR would '*roll them into the re-evaluation*' (CADPR 2012). It is now to the point that it has become difficult to find commodities that do not have a registration of one or several of the neonicotinoid insecticides. Based on information from Bayer, one of the main registrants (Jeschke et al. 2011), neonicotinoids have overtaken all other insecticide classes world-wide.

2. How acutely toxic to birds are the neonicotinoids?

The standard way in which the toxicity of pesticides to birds is measured is through an acute study. Birds are given the product by gavage (forced feeding) in varying amounts and the quantity of pesticide causing the death of half of the test birds is recorded and expressed as a proportion of bodyweight (i.e. the LD50 expressed as mg of pesticide per kg of bodyweight). One of the serious failings of current risk assessment is the underestimation of interspecies variation in pesticide susceptibility. Typically, one or two species only are tested (Mallard and Bobwhite are the usual mandated species) and the risk assessment is carried out with the more sensitive of the two. Even with the application of the customary safety factors, it has been shown that sensitive bird species are under-protected (Luttik et al. 2011). When more species are tested (as happens through academic or government research with older products) inconsistencies develop among regulators as to how these data should be used. Sometimes, regulators will use the data if the supplementary species are shown to be more sensitive than the usual ones; at other times the data are ignored, especially if they were obtained in ways that differ from the usual test protocols. **The end result is often arbitrary and the toxicity of different pesticides ranked based on 'luck of the draw.' This prevents any rigorous comparison of different chemical options.**

Species sensitivity distributions were designed in part to provide an unbiased comparison among chemicals. They will be used here. The method uses all available toxicity data and fits those data to a frequency distribution – often a log normal distribution. This process has been modified (in the case of birds) by incorporating body weight scaling (Mineau et al. 2001a). This is because it has been shown that small birds tend to be more sensitive to acutely toxic pesticides (Mineau et al. 1996a), most likely because they succumb more easily to the rigours of debilitation and the resulting starvation. Scaling for body weight in birds has been accepted in principle by the US EPA in their risk assessment process (*e.g.* their internal TREX software). However, the use of species sensitivity distributions or the use of small sample methods that approximate these distributions are not yet commonplace in regulatory circles.

The main acute toxicity endpoint presented here is a value called the HD5 ('Hazardous Dose at the 5% tail of the species distribution'). It represents the amount of pesticide in mg of chemical per kg of body weight estimated to lead to 50% mortality in a species more sensitive than 95% of all bird species, calculated with a 50 percent probability of over- or underestimation. The HD5 can be calculated from a fitted distribution where several toxicity values exist, or approximations can be used for smaller data sets. The 5% threshold is totally arbitrary although it has gained a great deal of use in the published literature. It does seem to fulfill the criterion that, for reasons of ecological integrity, we might not wish to see more than 5% of species being seriously affected in any system. The 5% tail will therefore be used throughout this report with the view that this should protect 'most' exposed species.

The first approach used here is the ETX 2.0 program (Van Vlaardingen et al. 2004) developed by the Dutch Government to calculate the hazardous concentrations and fraction of species affected by given exposure levels. It assumes log-normally distributed toxicity data. Distribution-fitting was carried out for all datasets with more than 5 data points – in this case, imidacloprid only. For the other compounds with only one or two toxicity values, a 'small sample method' was used (Aldenberg and Luttik 2002). This consists of estimating the HD5 on the basis of a mean LD50 and a pooled variance estimate calculated for a large group of pesticides at large (Luttik and Aldenberg 1997). The median estimate of the HD5 is calculated here in order not to bias for data availability. We recognise that this does not guarantee that any of the estimated values achieve the 95% level of protection.

The second approach estimates a body weight scaled value following Mineau et al. (2001). The approach ensures that species at one or the other end of a weight range spanning 10 to 1000g are adequately covered. The available data are tabulated in table 2.1. The derived HD5 values are given in table 2.2**.**

In the case of neonicotinoids, the exact method does not matter very much; similar values were estimated by both methods. However, it is clear that regulatory risk quotients use much less protective values as their point of departure. Depending on the specific insecticide, EPA underestimates toxicity by 1.5 to 10 fold if the intent of the exercise is to protect most species, not merely mallards and bobwhites. This will result in non-conservative (i.e. non-protective) assessments, especially since the endpoint is lethality to half of the tested population.

Table 2.1. Acute toxicity of the main neonicotinyl insecticides to birds based primarily on industry studies tabulated by regulatory authorities (principally EPA & EU sources). Original references obtained through the US Freedom of Information Act and consulted for this report are indicated with an asterix.

Active ingredient	Species	LD50 (mg/kg bw)	Probit slope[5] when	Dose vehicle	Reference to original industry study	Notes

[5] The LD50 is measured using a statistical construct called the probit. The probit is a normalised proportion of birds dying from the dose administered. The slope of the probit is an indication of the relationship between dose and mortality and is used to predict the dose at which certain proportions of birds (e.g. 1% or 5% rather than 50% as in the LD50) are expected to die. The higher the slope, the faster the proportion of birds at risk will increase with slight increases in exposure.

			provided		when available	
acetamiprid	bobwhite	180			European Commission 2004	
	mallard	98	6.0	sodium carboxymethyl cellulose	Johnson 1994*	Serious clinical signs seen at lowest dose level of 52 mg/kg.
	zebra finch	5.7	8.6	water	Hubbard 2011*	NOEL for clinical signs of 1.8 mg/kg. Onset of serious debilitation between 2.5 and 3.6 mg/kg.
clothianidin	bobwhite	>2000		corn oil	Johnson 1998*	NOEL for clinical signs of 500 mg/kg. Serious clinical signs and 20% mortality at 1000 mg/kg
	Japanese quail	430		corn oil	Gallagher and Beavers 2000*	NOEL for clinical signs of 12.5 mg/kg. Light signs at 25 mg/kg. More serious incapacitation at 100 mg/kg.
	mallard	>752			European Commission 2005	
thiacloprid	bobwhite	2716	2.4	Gelatin capsules	Grau 1995*	Clinical signs NOEL of 152. Severe signs onset at 551 mg/kg.
thiamethoxam	bobwhite	1552	8.5	methyl cellulose	Johnson 1996*	NOEL for clinical signs of 500 mg/kg.
	mallard	576	8.2	methyl cellulose	Johnson 1996*	Emesis observed at all dose levels. NOEL for clinical signs of 137 mg/kg.
imidacloprid	bobwhite	152	2.7	Gelatin capsules	Toll 1990*	EFSA (2008) gives value as 503 which is in error. NOEL for clinical signs of 25 mg/kg. Onset of serious incapacitation between 50 and 100 mg/kg.
	canary	35 (25-50)		Cremophor EL in water	Grau 1986*	Serious incapacitation at lowest dose of 10 mg/kg.
	gray partridge	15			Grolleau 1990 in EC database	
	Japanese quail	31	2.4	Gum Arabic in water	Grau 1988*	Severe clinical signs at 5 mg/kg. NOEL for clinical signs at 3.1 mg/kg (2.5 mg/kg nominal).
	mallard	283	6.6	Gelatin capsules	Hancock* 1996	Severe signs at lowest dose tested – 25 mg/kg; mortalities up to 8 days post dose.
	Rock dove	25**		Gelatin capsule	Grau 1987*	Severe signs at lowest dose tested – 12.5 mg/kg
	House	41			Stafford 1991	

	sparrow				in CCME 2007	

** Female value. Male approximate LD50: 25-50.

Table 2.2. Derived HD5 values for the major neonicotinoid insecticides. This is the amount of pesticide in mg of chemical per kg of body weight estimated to lead to 50% mortality in a species more sensitive than 95% of all bird species, calculated with a 50 percent probability of over- or underestimation. The value used by the USEPA to generate risk quotients is given for comparison.

Active ingredient	Unscaled HD5 (ETx software)	Scaled HD5 (based on Mineau et al. 2001)	Acute value used by USEPA in risk assessment to which 0.5 factor has been applied to reflect current 'Levels of Concern' application factor	Notes
acetamiprid	8.0	20.9*	49	
clothianidin	149	115	211**	
thiacloprid	467	315	1358	
imidacloprid	8.5	8.4	76 (20.5***)	Dietary LC50 values were favored initially for calculating risk quotients****. These were 1536 ppm for bobwhite/mallard; 143 ppm for songbirds based on the house sparrow.
thiamethoxam	162	98	288	

* The higher value reflects the lack of a small sample extrapolation factor for the zebra finch in Mineau et al. 2001.
** Corresponds to the lower value of 430 for Japanese quail. The USEPA routinely reruns probit analyses and reports slightly different values from the cited studies.
*** This lower value is acknowledged and used in the assessment of a granular product.
**** Toxicity endpoints have changed over the years. Dietary toxicity was initially favored for risk assessment by the USEPA but attention has now shifted to acute toxicity as a more reliable measure. The dietary toxicity test has several problems associated with it that can make interpretation difficult (Mineau et al. 1994).

The neonicotinoid insecticides have replaced a number of insecticides of high acute toxicity to birds – notably organophosphorous and carbamate compounds. Table 2.3 provides scaled HD5 values for several of those alternatives in order to compare with the neonicotinoids.

Table 2.3. Comparison of avian toxicity of neonicotinoid insecticides (in bold) and several of the insecticides they have replaced, ordered from most to least toxic to birds based on avian scaled HD5 values. (Data from multiple sources – Table 3.2 above and see Mineau et al. 2001).

Active ingredient	Avian HD5 [mg/kg bw] (Most are	No. species tested

	scaled values)	
terbufos	0.16	5
carbofuran	0.21	18
diazinon	0.59	14
methamidophos	1.70	3
Carbathiin (carboxin)	3.44	5
chlorpyrifos	3.76	18
dimethoate	5.78	10
acetamiprid	8.0*	3
imidacloprid	8.43	7
methomyl	8.46	13
deltamethrin	97	5
thiamethoxam	98	2
clothianidin	115	3
malathion	139	8
tefluthrin	179	3
thiacloprid	315	1

* unscaled HD5

The toxicity of imidacloprid and acetamiprid, the most acutely toxic of the neonicotinoids, compares with the carbamate methomyl. However, the toxicity of thiamethoxam and clothianidin, both extensively used as seed treatments, is much lower and comparable to the least toxic organophosphorous insecticides such as malathion or the synthetic pyrethroids.

We do need to keep in mind that these data only refer to lethality. Different families of pesticides elicit sub-lethal effects at different fractions of the lethal dose. Callaghan and Mineau's (2008) review of 166 studies in birds found that very few compounds (< 5%) cause observable sub-lethal effects at doses as low as 1/10 of the lethal dose. **But in the case of the neonicotinoids, as seen in table 2.1 above, severe signs of debilitation (e.g. ataxia) were observed with imidacloprid a full order of magnitude below lethal doses.** Thiacloprid may behave similarly and, based on a similar mode of action, other neonicotinoids may elicit similar effects as well. There is also some indication that these symptoms persist for a long time post dose, at least relative to cholinesterase-inhibiting insecticides.

3. What is the chronic or reproductive toxicity of neonicotinoids to birds?

As mentioned in section 1, the US EPA has often commented on possible risks to avian reproduction. Only one measure of chronic risk is available for birds – a reproductive test that is typically conducted on either the bobwhite or the mallard. It is a truncated test which consists of feeding a constant concentration of the pesticide and then collecting the eggs and incubating them artificially. There is therefore no test of the ability of the birds to incubate, hatch or raise their young. The test is a hybrid between chronic toxicity and true reproductive effects and has been the subject of criticism over the years (Mineau et al. 1994, 1996, Mineau 2005). One of those criticisms is that, because of the long duration of the test, and the occasional pair that fails to 'get along,' spurious variance is introduced in a number of parameters which decreases the power of the test to see reproductive deficits. On the other hand, because the birds are offered contaminated diet only with no other food choice, the test may overestimate likely exposure. However, it remains the only test available with which to model non-acute risk.

Chronic toxicity endpoints are provided in table 3.1.

Table 3.1. Chronic toxicity endpoints for the main neonicotinoid insecticides. The values are given in ppm in mash given to the birds for the duration of the test. All studies obtained from US EPA FOIA process and reviewed for this report. Conclusions may differ from reported information in regulatory summaries.

Active ingredient	Species	NOEL[6] (ppm)	LOEL (ppm)	Dose levels	Reference	Effect
acetamiprid	bobwhite	250	500	250, 500, 1000	Taliaferro et al. 1997	Difficult study to interpret. Outlier pair should have been removed from controls. Chick survival per hen shows clear dose-response. Eggs laid also.
	bobwhite	400	800	100, 200, 400, 800	Temple et al. 2005	Chick survival, eggs laid
	mallard	125	250	62.5, 125, 250, 500	Taliaferro and Miller 1999	Tentative. Difficult to interpret because of poor dose response with worst performance in 62.5 ppm dose group.
	mallard	125	250	62.5, 125, 250, 500	Stafford 2004	Eggs laid, fertility
clothianidin	bobwhite	525 (500 nominal)	NA	0, 80, 200, 500	Gallagher et al. 2000a	
	mallard	250	525 (500 nominal)	0, 80, 200, 500	Gallagher et al. 2000b	Several small non-sig deficits in many parameters
imidacloprid	bobwhite	120	240	0, 30, 60, 120, 240	Toll 1991a	Difficult to interpret. Variable results, aberrant controls. Effect on male weight only. No true reproductive effects.
	mallard	120	240	0, 60, 120, 240	Toll 1991b	Study continued longer than normal leading to high variability. Hatching, egg laying clearly affected at higher dose
thiacloprid	bobwhite	466		53, 153, 466	Schmuck 1997	Agree with author that slight decrease in feeding rate at all concentrations is not biologically relevant
	mallard	NA	48	48, 140, 418	Hancock 1997	Parental effects, fertility or early embryonic death at high dose
	mallard	28	55	14, 28, 55	Hancock 1998	Early embryonic death, non-sig egg breakage
thiamethoxam	bobwhite	300	900	100, 300, 900	Taliaferro and Miller 1998	Non sig. but large diff. in eggs laid
	mallard	300	900	100, 300, 900	Brewer et al. 1998	Parental effects, non-sig effects in several parameters.

As with acute toxicity testing, assessing risk to all potentially exposed bird species from the lower of two bird species tested is not very realistic. This approach seriously underestimates the likely difference in sensitivity between species and the possibility that bird species other than mallards or

[6] NOEL (No Observable Effect Level) is highest dose level at which no effects were seen. It is more properly termed NOAEL to specify 'Adverse' effects. The LOEL (or LOAEL) is the lowest level producing effects in the study.

bobwhites will be affected at much lower levels of exposure. It has been argued that chronic toxicity is no less variable among species than acute toxicity and that the variance in inter-species chronic toxicity endpoints could (and should) be used as a proxy for the variance in reproductive toxicity (Mineau et al. 2001b, Luttik et al. 2005). This may give a more reliable estimate of the dose at which chronic toxicity effects are expected in those species that happen to be more sensitive to the pesticide than the standard bobwhite or mallard. Yet, EPA allows for a ratio between exposure and effect of 1 in their risk assessment before they consider that their 'Level of Concern' has been exceeded. This means that birds could be exposed to a level as high as the lower NOEL of either bobwhite or mallard without exceeding their 'level of concern'. Given that EPA's reproductive LOCs for birds are routinely exceeded with the neonicotinoids (section 1), this means that even insensitive species are likely to be affected reproductively.

3.1. A proposal for a more rigorous consideration of endpoints from the avian reproductive study.

Following a major international review hosted by the British Government in 2004, recommendations were made by the panel of assembled scientists to improve the assessment of long term or reproductive toxicity in birds. As a first step, it was recommended that the various endpoints in the studies be separated rather than basing a risk assessment on a single NOEL (Bennett et al. 2005, Shore et al. 2005). This followed on analyses (Mineau et al. 1994) showing that three major effects were at play in the current avian reproduction studies: parental effects, eggshell effects and effects on the reproductive & embryonic development process proper. Mineau and colleagues (1994) suggested that we should be most interested in those 'true' reproductive effects that are manifest at concentrations lower than those that elicit parental toxicity because these more 'pernicious' effects will be harder to detect in simple feeding studies.

Unfortunately, this proposal has not been endorsed by regulators although the US EPA is currently investigating risk models that would help with a sounder interpretation of reproductive test results (R. Bennett, pers. comm.). In the meantime, we offer the following solution, developed by Mineau and colleagues (2006) for the Canadian Government. It still uses a single NOEC (No Observable Effect Concentration) or LOEC value (as do all regulatory bodies currently) but does apply an extrapolation factor to account for inter-species differences in susceptibility as recommended previously.

For all the neonicotinoid insecticides being reviewed here (table 3.1) NOEC values were determined. Where this is not the case (i.e. when the lowest dose tested produced an effect; this occurred in some of the other seed treatment chemicals to which we compared the neonicotinoids below) Mineau et al. (2006) compiled available NOECs and LOECs from the USEPA one liner database (B. Montague pers. comm.) and calculated that the median spacing between the log NOEC and log LOEC was 1.23 based on a sample of 272 studies. This ratio was therefore used to obtain a NOEC where the lowest level tested produced an effect.

The NOEC (or more correctly stated, NOAEC – No Observable Adverse Effect Concentration) has been criticised as a toxicological endpoint because of very valid reasons having to do with statistical power, especially in the context of aquatic toxicity testing, and we fully agree with this criticism. However, it is currently not feasible to extract an ECx type of value (the concentration producing a pre-defined level of reproductive deficit) from the current avian reproduction test designs.

Manipulations of the test endpoints are carried out as follows in order to arrive at a reasonable estimate of the dose of a pesticide a bird would need to ingest daily to adversely affect reproduction.

- In the usual reproduction study, bobwhites (weight 210 g; unpublished industry studies) have a peak food consumption of approximately 10% of their bodyweight in food per day; measured food intakes for the mallard (approx. 1000g) are highly variable and peak above 20% of bodyweight (unpublished industry studies). This is counter to expected allometric relationships where, the smaller the bird, the larger its proportional food intake. Mallards in the laboratory tend to spill a lot of food and it is therefore difficult to estimate their true consumption. As verification, the allometric equation of Nagy (1987) for non-passerine birds was used to estimate food consumption even though it is recognised that Nagy's algorithms apply to birds in the wild. One expects wild birds to have higher maintenance requirements than birds kept in the laboratory. On the other hand, the birds in the laboratory are induced to lay an unreasonable clutch size which is likely to increase their food intake compared to an equivalent bird laying a normal clutch in the wild.

- Dry food intake = $0.302 * bw(g)^{0.751}$

- Laboratory diet was estimated to have 11% moisture content based on a personal communication from Joann Beavers with Wildlife International, one of the major testing laboratories.

- Therefore, for the bobwhite intake of lab diet (actual weight) should be:

  Intake = $(0.302 * 210^{0.751})/0.89$ (propn. dry wt.) = ~ 19 g

  …. which is approximately 90% of the observed 10% of bodyweight.

- For the mallard, the same formula returns a value of 61 g/day or a little over 6% of its bodyweight per day rather than the observed 20%. Because of the spillage problem mentioned previously, and assuming the figure of 21 g per day (10% of bodyweight) in the bobwhite to be correct, we adjusted the result of the Nagy calculation by the same proportionate amount – raising the approximate food intake in the Mallard to 67 g/day.

- The estimated food intakes of 21 g/day or 67 g/day for the bobwhite and mallard respectively were used to convert all NOAEC values to NOAELs (critical pesticide intake levels) expressed as mg a.i. of pesticide / kg bird / day. We believe this correction to be adequate where there is no large demonstrated effect of the chemical on food intake. This is the case for the neonicotinoids reviewed here.

- Therefore :

  $NOAEL_{mallard}$ (mg a.i./kg bw/day) = ($NOAEC_{mallard}$ (mg/kg food) * 0.067 kg food/day) / 1 kg bw

  $NOAEL_{bobwhite}$ (mg a.i./kg bw/day) = ($NOAEC_{bobwhite}$ (mg/kg food) * 0.021 kg food/day) / 0.210 kg bw

- A geometric mean of $NOAEL_{mallard}$ and $NOAEL_{bobwhite}$ was calculated as the best available average for all bird species.

- In order to use the compound-specific interspecies variation in acute toxicity, we derived standard deviations (SDs) for acute data in the following way:
    - A single geometric mean log LD50 value was obtained for each species-pesticide combination as outlined in Mineau et al. (2001).
    - Where the number of species tested was 4 or more, we derived a standard deviation. This was possible here for the active ingredient imidacloprid. For all other active ingredients, a pooled SD of 0.465 (after Aldenberg and Luttik 2002) was used.

- The extrapolation factor (a factor to be applied multiplicatively to the mean untransformed NOAEL) was defined as follows after Aldenberg and Luttik 2002):

  $EF^{median} = (10^{\sigma})^{Kp}$

  … where Kp is the z score of 1.64 in the case of the 5% tail of a normally-distributed species sensitivity distribution. This is equivalent to:

  $EF^{median} = 44.14^{\sigma}$ … or to an extrapolation factor of 5.8 for the pooled variance estimate of bird acute data.

- The median extrapolation factor (EF) was then applied to the geometric mean NOAEL in order to obtain the critical toxic effect level for a sensitive bird at 5% of the putative distribution of reproductive toxicities.

The resulting value (the calculated critical dose for a bird at the 5% tail of sensitivity) is tabulated in table 3.2 for the main neonicotinoids insecticides and some other common seed treatment pesticides (insecticides or fungicides). For the neonicotinoid insecticides, a critical intake value based on study LOAELs is also given. Assuming the current avian reproduction studies can be relied upon, effects from chronic intake should start occurring at some exposure levels between those two values.

Table 3.2. Calculated critical chronic dose intakes (NOAEL and LOAEL) for a sensitive bird species (at the 5% tail of sensitivity) for the main neonicotinoids insecticides and some other common seed treatment pesticides.

Active ingredient	Avian Chronic - Critical intake level (NOAEL - mg/kg/day) for a sensitive bird at 5% tail of acute sensitivity distribution	Avian Chronic - Critical intake level (LOAEL - mg/kg/day) for a sensitive bird at 5% tail of acute sensitivity distribution
Acetamiprid	2.49	4.97
Clothianidin	5.10	7.38
Imidacloprid	1.41	2.82
Thiacloprid	1.61	2.10
Thiamethoxam	4.22	12.66

Captan	4.95	
Carboxin	1.30	
Diazinon	0.12	
Difenoconazole	1.76	
Fludioxonil	4.16	
Iprodione	4.22	
Metalaxyl	2.44	
Tebuconazole	2.01	
Triadimenol	1.15	
Triticonazole	1.70	

4. A short primer on seed availability and bird exposure after seeding

Of particular concern to birds are those neonicotinoid compounds that are used as seed treatments. Historically, seed treatments have been associated with extensive exposure to and impacts on birds. These were well documented for mercury-based seed treatments and for coatings with the cyclodiene insecticides aldrin and dieldrin. Any contamination of avian food items from spray applications to field crops as well as non-dietary exposure is likely small in comparison to the potential for very heavy exposure from seed treatments[7]. The main point to be made here is that, as far as birds are concerned, seeds are available ad libitum on fields.

The type of machinery and planting techniques dramatically influence incorporation of treated seeds. All seed drills use the same basic principles. Seeds are dropped from a row of individual dispensing units behind the tractor. In front of each dispenser a furrow is made by soil openers (disks, hoes or knives). The seed is dropped, either by gravity, by a train-driven auger system or through a pneumatic system (air seeder). Air seeders provide better control of application rate. Side-firming discs (closure wheels) push soil laterally against the seed. Alternatively, “press wheels” roll over the whole furrow area to compress the soil against the seeds. In North America, the term “planter” is usually reserved for crops that need wide spacing between rows (*i.e.* maize/corn) and the seed dispensing units are placed far apart; the term “drill” (as in hoe drill or press drill) is used for crops that can be planted at higher densities (*i.e.* cereals) so the dispensing units are close together. In Europe, the term “seed drill” is used for all crop types.

Additional implements may be added to aid incorporation of the seeds, such as harrows[8] placed in front of the seed dispenser to help clear debris (also known as trash) for more successful seed incorporation. Many variations on seeding exist depending on the specifics of the machinery, and also on how the drill is configured. Specific implements and machinery are recommended for each type of crop; however, this is usually left to the preference of each individual grower. Growers may not always be using the “best” equipment for any given crop because the same drill is often used for several crops in rotation. A good example of this is the use of air seeders to dispense canola on the soil surface after which it is crudely harrowed in. Air seeders are designed for precision seeding of cereals (usually 4-5cm in depth). However, growers find it difficult to get good results when air

[7] For this reason, this report will not conduct an assessment of avian toxicity resulting from the contamination of avian foods such as insects or weed seeds from spray applications. It is not that this risk is necessarily negligible, but it pales in comparison to the risk from treated seed.

[8] A harrow is an agricultural implement that loosens and levels the surface of the field. Harrowing is typically carried out before or during planting operations.

seeding at very shallow depths as with canola (1-3cm). They can get around this problem by running the furrow openers above the soil surface and crudely harrowing in the seeds.

The following factors have been tested experimentally and also significantly affect the proportion of planted seed that are actually buried under the surface (i.e. placed where they will have the best chance of germinating):

- Soil condition measured as clump weight of the soil (Leeuw *et al.*, 1995).
- Field trash which impedes the action of the incorporation. Surface counts of seeds were significantly lower in summer fallow fields than in stubble fields (Maze *et al.,* 1991).
- Rain which makes incorporation during planting difficult (Davis, 1974; Leeuw *et al.,*1995)
- Seed size and planting depth: larger seeds are typically planted deeper and at lower densities (Tamis *et al.,* 1994). Conversely, small seeds such as canola are planted very shallow with a much higher risk of large numbers left on the soil surface. Deeper plantings typically result in fewer surface seeds (Pascual et al. 1999a)
- Travel speed during planting: the insertion of seeds at intended depths using drills is ensured only at certain speeds and farmers will change speed during planting. This has been shown with granular insecticides (Ellis, 1982) which are subject to the same limitations as seeds.

The soil disturbance associated with planting typically increases avian hazard by exposing soil invertebrates on the field surface which attracts invertebrate-eating birds with a higher probability that treated seed will also be ingested.

Some crops are seeded from an aircraft (e.g. rice). This may be a very high risk factor given that rice grains are likely to be widely distributed on levees and other dry areas in and around paddies.

High densities of treated seed occur on certain areas of the field, namely at row ends where the planters are lifted out of the furrows to permit turns (termed headlands in the European literature) (Pascual et al. 2009b), at irregularities in field contour resulting in the planter shoe rising out of the soil, and where the farmer loads the machine with the treated seed. These areas may increase risk to avian species because spills are more visible; they present a profitable feeding opportunity (Leeuw *et al.*, 1995). High densities can also occur midfield by means of erratic application from dispensing units resulting from incorrect calibration, clogging or obstructions such as stones.

While environmental conditions can cause high avian risk in localized parts of the field, growers have a dramatic influence on the overall number of treated seeds left on the soil surface after planting. Therefore, densities available to avian species are highly unpredictable, as illustrated by actual field counts. Furthermore, exposure can still occur if seeds are fully incorporated in the soil. Geese dig for seed in upper surface layers of soil (Lorenzen & Madsen, 1986). Western meadowlarks and many blackbirds will probe for seed by pushing their bill into the ground or beneath an object and then the buried food items are made accessible by spreading their mandibles wide (gaping) (Lanyon, 1994). In Europe, skylarks will bring grain to the surface by uprooting seedlings (Green, 1978), a technique favored by cranes and geese in North America. This will expose them to systemic residues, residues still carried on the seed or granules caught in the root hairs of the seedlings. Mourning doves will move light ground litter using their bill to find food (Mirarchi & Baskett, 1994). In light of these feeding patterns and the inefficiency of all currently used incorporating devices, we can conclude that avian exposure to high numbers of treated seeds cannot be prevented even if the product is applied at recommended rates using proper equipment.

Birds also have preferences for certain seed types. This is species dependent. For example, it has been suggested that some species dislike canola seed. Pawlina and Proulx (1996) showed that the daily consumption of canola by house sparrows was far less than that of millet or sunflower seeds even when no other food source was available. However, closely-related mustard seeds have long been used in the pet bird trade, and canola was found to be a preferred seed for house finches and was used successfully as a lethal bait (laced with strychnine) (Palmer, 1972). A mixture containing rapeseed as well as millet and canary seed is given as standard diet for Japanese quail (Barfknecht 1998a). Smith (2006) looked at the attractiveness of wheat, corn, barley, oat and soybean to a subset of common farmland species. He found that soybean was the least preferred. However, Smith's review did not include some of the larger farmland species more likely to be attracted to a larger seed: waterfowl, grouse and pheasants, turkeys etc.

In conclusion, given the high variability and lack of control regulators and manufacturers have on the methods of application at planting (field conditions, equipment, calibration, etc.), it should be assumed that unlimited quantities of treated seed will be available to foraging birds. **Therefore, we believe that regulators are clearly mistaken in believing that exposure to treated seed can be minimized by label statements or adherence to good agricultural practice.** The only factors that should be considered in avian risk assessments of seed treatment chemicals are: 1) the maximum consumption rates (preferably under food stress realistic of field conditions) of farmland foraging birds, and 2) the amount of active ingredient per seed. For the purpose of this report, we will express risk as the number of seeds needing to be ingested for a given biological effect. An initial assumption will be that seeds contain the labeled amount of active ingredient. It has been shown however, that actual concentrations of active ingredients are lower at planting than the initial 'in the bag' concentration (McKay et al. 1999). This loss of active ingredient may be to the advantage of foraging birds but has proven to be a problem with the neonicotinoids, resulting in large kills of bees from dust exhausted from pneumatic seeders.

5. What is the acute and chronic risk to birds from the ingestion of treated seed?

Working out the typical loading of active ingredient per seed is not a simple task given the many labels, formulations and inconsistent ways of reporting treatment concentrations. Only a few representative examples are given here for the main field crop seeds likely to be consumed by birds – wheat (as a representative of cereal crops), corn and canola.

Representative seed weights were obtained from a compilation of the PMRA based on a number of different sources (Chris Fraser, PMRA, *pers. comm*.) as well as EPA review documents. Seed weight values used here are as follows: canola = 2.9 mg, cereals = 35 mg and corn = 377 mg.

For imidacloprid, we consulted the Gaucho 75ST label (EPA Reg. No. 264-959). This is one of the labels for commercial seed treatments. Wheat is labeled at 2 oz. product per hundredweight (Cwt - 100 lb of seed) which works out to 0.033 mg/seed. A direct figure of 1.34 mg a.i./kernel is given for corn. The highest treatment rate for canola is 21.3 oz of product per hundredweight. This works out to 0.03 mg of imidacloprid per seed.

For clothianidin (USEPA 2003; as well as the following label: Poncho™ 600; Reg. No. 264-789; updated 22 April 2010), a loading of 1.25 mg a.i./kernel is given directly for corn. The maximum rate

on canola/rapeseed is 10.23 oz. product per Cwt which works out to approximately 0.012 mg/kg seed. For wheat and other cereals not included on this particular label, USEPA (2010) gives 70 g a.i/100 kg seed. For wheat specifically, this would work out to an approximate loading of 0.025 mg/seed.

For thiamethoxam, we looked at various Cruiser labels (e.g. EPA Reg. No. 100-941, 100-1365, 100-1369). The following direct loading rates were given: 0.8 mg/corn kernel, 0.03 mg/rice seed, 0.375 mg/cotton seed, 0.25 and 0.29 mg/kg for sunflower and peanut respectively. The higher rates for wheat and canola work out to 0.018 and 0.012 mg/seed.

Based on a US EPA 2002 memorandum, acetamiprid was registered for canola treatment at 0.25 lb a.i./100 lbs of seed. However, the specific label with those use instructions (Adjust 70WP – Reg. No. 8033-27) is currently inactive and we do not know whether it has been superseded yet by a new label with the same uses (e.g. Vault™ insecticide registered in Canada). That concentration of acetamiprid would work out to 0.0072 mg/seed.

We are not aware of a seed treatment use for thiacloprid.

The chosen risk measure for the current assessment is the number of seeds that a 15 g bird could ingest before reaching either a median lethal dose (Table 5.1) or the estimated reproductive NOAEL or LOAEL (Table 5.2). At this point, we assume no active avoidance of any of the seed by birds (see discussion below) and a concentration on the seeds reflecting the usual label rate. A consistent bird weight of 15g was chosen in line with previous calculations by Mineau and colleagues (2006). It is acknowledged however that sparrow-sized birds may be a little small to ingest whole corn seed in any quantity.

Table 5.1. Estimated no. of seeds needing to be ingested by a 15g bird to achieve a 50% chance of lethality given sensitivity at the 5% tail of the bird distribution.

Active ingredient	Seed type	mg/seed	Critical endpoint	Endpoint value (mg/kg)	No. seeds to endpoint
imidacloprid	Corn	1.34	HD5*	8.5	0.1
	canola/rapeseed	0.029	HD5*	8.5	4.4
	Wheat	0.033	HD5*	8.5	3.9
clothianidin	Corn	1.25	HD5*	149	1.8
	canola/rapeseed	0.012	HD5*	149	186.3
	Wheat	0.025	HD5*	149	89.4
thiamethoxam	Corn	0.8	HD5*	162	3.0
	canola/rapeseed	0.012	HD5*	162	202.5
	Wheat	0.018	HD5*	162	135.0
acetamiprid	canola/rapeseed	0.0072	HD5*	8	16.7

* Unscaled LD50 for birds at the 5% tail of species sensitivity

Table 5.2. Estimated no. of seeds needing to be ingested by a 15g bird to achieve estimated reproductive effects.

Active ingredient	Seed type	mg/seed	Critical endpoint	Endpoint value (mg/kg)	No. seeds to endpoint
imidacloprid	Corn	1.34	LOAEL	2.82	0.03
	canola/rapeseed	0.029	LOAEL	2.82	1.46
	Wheat	0.033	LOAEL	2.82	1.28
clothianidin	Corn	1.25	LOAEL	7.38	0.09
	canola/rapeseed	0.012	LOAEL	7.38	9.23
	Wheat	0.025	LOAEL	7.38	4.43
thiamethoxam	Corn	0.8	LOAEL	12.66	0.24
	canola/rapeseed	0.012	LOAEL	12.66	15.83
	Wheat	0.018	LOAEL	12.66	10.55
acetamiprid	canola/rapeseed	0.0072	LOAEL	4.97	10.35

It is clear that the loading of all neonicotinoid insecticides on corn is such that acute intoxications are possible with the three registered products. With imidacloprid, the number of seeds needing to be consumed is less than 1 even for a larger (e.g. blue jay-sized) bird more likely to be picking up whole corn seed from seeded fields. Acute intoxications in wheat or canola are not likely with clothianidin or thiamethoxam because of the number of seeds needing to be ingested.

However, based on chronic/reproduction endpoints, all seed treatments are predicted to cause effects given the very small number of seeds needing to be ingested to push birds into 'critical range.' Indeed, recent work on clothianidin in Japanese quail (Tokumoto et al. 2013 – see below) suggests that effects on bird fertility and embryonic development are occurring at dose levels lower than the critical levels indicated by the standard reproduction study. The main uncertainty here is how long the seeds will be available to birds and how long dosing is necessary before the type of effects observed in the laboratory will be seen in the wild. There are huge uncertainties such as what types of effects might be seen in altricial[9] species. However, based on our current understanding and risk assessment procedures in place, the neonicotinoids as a group have a high potential to affect avian reproduction. This is due in large part to the very high exposure potential that seed treatment chemicals represent.

Recently, Lopez-Antia and colleagues (2012) tested the effect of imidacloprid seed treatments on captive red partridges. They applied the material to wheat at the intended labeled rate of 0.7 g a.i./g of seed and twice that rate[10]. Actual measured concentrations were 74% and 62% of nominal for the

[9] Altricial species are those where the young are born naked and blind and need a prolonged period of parental care to fledge. Mallard and bobwhite, the two test species, are both precocial species with young being mobile and able to feed themselves within a few hours of hatching.

[10] This works out to 0.025 and 0.050 mg/seed. The maximum allowed in the US falls between those two concentrations. Given the reported measured concentrations, the high rate used in the experiment is almost exactly the high rate allowed in the US.

low and high dose rates respectively, meaning that the high rate was only slightly higher than labeled rate. Exposure lasted for 10 days. Exposure started 15 March and, based on a personal communication from one of the co-authors (Rafael Mateo, pers. comm.) egg-laying began 16 April and was concluded 28 May. Several of the birds died during treatment, reducing the number of pairs available for breeding. Unfortunately, food consumption was not measured, preventing an assessment of any avoidance. However, birds in both imidacloprid groups showed reduced body condition suggesting a reduction in food intake. (This was confirmed in the personal communication described above. However, in a parallel trial, it was found that birds ate a normal amount of seed when treated and untreated seed were mixed.) Cellular immune function is reported to have declined in males at the high dose rate. Both males and females showed reduced eye ring pigmentation – an interesting finding but one of unknown significance at this point. Data on reproductive success are difficult to interpret. Because of the small number of birds that survived, data were analysed using each egg laid as an independent statistical unit. This ignores that eggs within a clutch are not statistically independent and therefore limits the inferences that can be made from the research. The experiment does not add critical reproduction information beyond what is known from the regulatory studies. It does raise some questions as to new and unexpected effects not typically measured in the standard studies.

Tokumoto and colleagues (2013), gave male Japanese quail daily doses of formulated clothianidin (Dantotsu™ – a 16% formulation of clothianidin manufactured by the Sumitomo Chemical co.) at rates of 0.02, 1 and 50 mg/kg over a 30 day period. These males, along with the usual control individuals, were then mated to unexposed females and the eggs collected and checked for fertility and embryonic development. Testes, livers and spleens were collected for examination. Their working hypothesis was that sperm production would be affected though oxidative stress as seen in mammalian systems. They were most concerned about the welfare of the crested Ibis (*Nipponia nippon*), a critically endangered species being released into the wild from captive breeding facilities and known to frequent rice fields and other areas where neonicotinoid insecticides are being used. The 50 mg/kg dose in quail caused one mortality and signs of toxicity in several individuals. Interestingly, dosing caused an increase in bodyweight which the authors attributed to impaired liver function. (The authors reviewed similar reports of impaired liver function with imidacloprid and thiacloprid in chickens.) There was a dose-related increase in testicular abnormalities and DNA breakage in germ cells even though eventual fertility was not affected. Embryo length was reduced in a dose-dependent fashion with significant effects seen in the 1mg/kg group. Embryo weight was also affected. Some of the dosed embryos stopped developing altogether but sample sizes are too small to assess whether this was a significant issue.

Finally, a rather sweeping proposal was recently made by Mason and colleagues (2013). They postulate that many of the severe epizootic diseases that seem to arise with alarming frequency (chytrid fungus in amphibians, white nose syndrome in bats, mycoplasmal and other recently discovered pathogens in finches and other bird species in North America and Europe) may be the result of immune suppression resulting from low level exposure to neonicotinoids. They base their hypothesis on reports of immune suppression in bees, fish and rats following neonicotinoid exposure as well as on time and place correlations between neonicotinoid uses and disease outbreaks. Additional research is needed, given the serious potential consequences of this hypothesis.

It is relevant to ask how the neonicotinoid insecticides compare to other registered seed treatments. It is beyond our scope to conduct a complete review of all US-registered seed treatments. However,

a review of seed treatments was carried out in Canada by Mineau and colleagues (2006) and has been modified for this report. The comparison of acute lethal toxicity (Table 5.3) uses scaled HD5 values with the exception of acetamiprid where the new information available for the zebra finch is considered a critical piece of information. Application rates were adjusted for the neonicotinoids in order to reflect US conditions described in this report. This could not be done for all other active ingredients (primarily fungicides) but differences between seed treatment rates in Canada and in the US are thought to be slight. As discussed above, a 15g bird is not likely to be feeding on whole corn seed; avian body weight was increased to 50g for that seed type – a weight somewhere between that of a large sparrow and a blue jay.

Table 5.3. A comparison of the acute lethal toxicity of seeds treated with neonicotinoid insecticides (in bold) and other common seed treatment chemicals. Seed treatment rates for neonicotinoids are as labeled in the US; those for other active ingredients are as labeled in Canada.

AI Accepted Name	Type of seed treated	Average weight of individual seed (g)	Scaled HD5	Maximum rate AI per particle (mg/seed)	bird weight (g)	Risk as no. seed needed to reach HD5
Imidacloprid	Canola	0.003	8.40	0.0290	15	4.3
Acetamiprid	Canola	0.003	8.00	0.0072	15	17
Thiram	Canola	0.003	36.81	0.0197	15	28
Carbathiin	Canola	0.003	10.68	0.0030	15	53
Thiamethoxam	Canola	0.003	98.00	0.0121	15	121
Clothianidin	Canola	0.003	115.00	0.0120	15	144
Iprodione	Canola	0.003	158.40	0.0089	15	267
Metalaxyl	Canola	0.003	89.09	0.0011	15	1205
Metalaxyl-m (mefenoxam)	Canola	0.003	137.00	0.0004	15	4852
Difenoconazole	Canola	0.003	207.13	0.0006	15	5021
Fludioxonil	Canola	0.003	208.12	0.0002	15	20351
Carbathiin	Cereal	0.035	10.68	0.1504	15	1.1
Imidacloprid	Cereal	0.035	8.40	0.0330	15	3.8
Thiram	Cereal	0.035	36.81	0.0243	15	23
Clothianidin	Cereal	0.035	115.00	0.0250	15	69
Maneb	Cereal	0.035	345.34	0.0743	15	70
Thiamethoxam	Cereal	0.035	98.00	0.0180	15	82
Tebuconazole	Cereal	0.035	347.30	0.0588	15	89
Metalaxyl	Cereal	0.035	89.09	0.0129	15	103
Difenoconazole	Cereal	0.035	207.13	0.0084	15	368
Metalaxyl-m (mefenoxam)	Cereal	0.035	137.00	0.0049	15	416
Triadimenol	Cereal	0.035	965.25	0.0118	15	1231
Fludioxonil	Cereal	0.035	208.12	0.0018	15	1744
Triticonazole	Cereal	0.035	232.29	0.0019	15	1861
Diazinon	Corn	0.38	0.59	0.1421	50	0.21
Imidacloprid	Corn	0.38	8.40	1.3400	50	0.31
Captan	Corn	0.38	25.32	2.2230	50	0.57
Carbathiin	Corn	0.38	10.68	0.4723	50	1.1

Thiram	Corn	0.38	36.81	0.6866	50	2.7
Clothianidin	Corn	0.38	115.00	1.2500	50	4.6
Metalaxyl	Corn	0.38	89.09	0.7917	50	5.6
Thiamethoxam	Corn	0.38	98.00	0.8000	50	6.1
Mancozeb	Corn	0.38	710.95	0.6688	50	53
Thiophanate-methyl	Corn	0.38	482.63	0.2660	50	91
Difenoconazole	Corn	0.38	207.13	0.0916	50	113
Metalaxyl-m (mefenoxam)	Corn	0.38	137.00	0.0537	50	128
Fludioxonil	Corn	0.38	208.12	0.0212	50	490

Again, it is clear that imidacloprid seed treatments carry a higher risk of lethal intoxication than most other seed treatments. Two exceptions are diazinon on corn seed and carbathiin on cereal, both of which have been replaced by – or in the case of wheat, is in the process of being replaced by – the neonicotinoids.

For the comparison of chronic toxicity (Table 5.4), endpoints were modified to reflect the review of reproductive endpoints carried out in this report (Table 3.1). For other active ingredients, NOAEL values were obtained from EPA summary data (One liner database - B. Montague, pers. comm.).

Table 5.4. Comparison of the chronic/reproductive toxicity of neonicotinoids and other registered seed treatment chemicals.

Active ingredient	Type of seed treated	Bobwhite NOAEL (ppm)	Mallard NOAEL (ppm)	Critical intake level (mg/kg/day) for sensitive bird at 5% tail	Particle load (mg/seed)	Risk (no. seeds to critical intake)*
Imidacloprid	Canola	120.0	120.0	1.4	0.029	0.7
Thiram	Canola	500.0	9.6	1.2	0.020	0.9
Acetamiprid	Canola	250.0	125.0	2.5	0.007	5.2
Thiamethoxam	Canola	300.0	300.0	4.2	0.012	5.2
Clothianidin	Canola	525.0	250.0	5.1**	0.012	6.4
Carbathiin	Canola	1000.0	70.0	1.3	0.003	6.5
Iprodione	Canola	300.0	300.0	4.2	0.009	7.1
Metalaxyl	Canola	300.0	100.0	2.4	0.001	33.0
Difenoconazole	Canola	125.0	125.0	1.8	0.001	42.6
Fludioxonil	Canola	125.0	700.0	4.2	0.000	406.9
Carbathiin	Cereal	1000.0	70.0	1.3	0.150	0.1
Maneb	Cereal	500.0	20.0	1.4	0.074	0.3
Tebuconazole	Cereal	73.0	75.8	2.0	0.059	0.5
Imidacloprid	Cereal	120.0	120.0	1.4	0.033	0.6
Thiram	Cereal	500.0	9.6	1.2	0.024	0.7
Triadimenol	Cereal		100.0	1.2	0.012	1.5
Metalaxyl	Cereal	300.0	100.0	2.4	0.013	2.8
Clothianidin	cereal	525.0	250.0	5.1	0.025	3.1

Difenoconazole	Cereal	125.0	125.0	1.8	0.008	3.1
Thiamethoxam	Cereal	300.0	300.0	4.2	0.018	3.5
Triticonazole	Cereal	99.3	236.0	2.2	0.002	17.3
Fludioxonil	Cereal	125.0	700.0	4.2	0.002	34.9
Diazinon	Corn	32.0	6.0	0.1	0.142	0.04
Imidacloprid	Corn	120.0	120.0	1.4	1.340	0.1
Thiram	Corn	500.0	9.6	1.2	0.687	0.1
Captan	Corn	1000.0	1000.0	5.0	2.223	0.1
Mancozeb	Corn	125.0	125.0	1.8	0.669	0.1
Carbathiin	Corn	1000.0	70.0	1.3	0.472	0.1
Metalaxyl	Corn	300.0	100.0	2.4	0.792	0.2
Clothianidin	Corn	525.0	250.0	5.1	1.250	0.2
Thiamethoxam	Corn	300.0	300.0	4.2	0.800	0.3
Thiophanate-methyl	Corn	150.0	103.0	1.7	0.266	0.3
Difenoconazole	Corn	125.0	125.0	1.8	0.092	1.0
Fludioxonil	Corn	125.0	700.0	4.2	0.021	9.8

* For a 15g bird in the case of cereals or canola; 50g bird for corn.
** It is noteworthy that Tokumoto and colleagues (2013) found that doses as low as 1mg/kg clothianidin daily caused testicular anomalies and increased DNA breaks in males as well as reductions of embryonic length when those males were mated to undosed females. These endpoints have not been specifically studied with other pesticides and they are therefore not used here.

This comparison highlights one of the current problems in pesticide risk assessment. Several active ingredients currently registered have the potential to cause reproductive effects – at least based on the available laboratory studies. The need to verify some of these problems in the field was brought up almost two decades ago (Mineau et al. 1994) but persists to this day. Indeed, extrapolation from the contrived laboratory study with two precocial gamebird species to the intricacies of reproductive behavior in the field takes a veritable leap of faith. The lack of realism of the current study protocol as well as the existing difference between the two tested species (making extrapolation to a third species even more tenuous) has been well documented (Mineau et al. 1994, Mineau 2005, Luttik et al. 2005, Fernandez-Perea et al. 2009). What is missing is a solution to this conundrum. Fernandez-Perea et al. (2009) believe that the solution lies in the application of large safety factors in the risk assessment process. However, this is not the case now nor is it likely to happen given that a large number of pesticides currently fail the test even before safety factors are applied.

Nevertheless, on a comparative basis, the use of imidacloprid on corn or canola appears to be ill-advised given that consumption of 1 seed per day is expected to bring birds over the limit where reproductive effects might be seen. Even if part of the seed coating is lost at seeding or some of the pesticide is discarded when the seeds are hulled by the birds, critical dosing is likely to be exceeded.

5.1. Incidents

The monitoring and reporting of bird kills in the US has been very limited in recent years due to 1997 amendments to federal pesticide laws[11]. There have been relatively few reports involving neonicotinoids. This is in part because the acute toxicity of these insecticides is lower than that of the organophosphorous and carbamate insecticides that they replaced. Moreover, methods for diagnosing kills either do not exist, or are not widely used. There is no easy biomarker as there is for cholinesterase inhibitors, although we believe that one should be developed. **There is a strong case to be made that, where acute intoxications are possible, registrants should be compelled to develop the diagnostic tools necessary. In the case of neonicotinoids, it should be relatively easy to work out a binding assay for the neural receptor which is affected by this class of insecticides.**

Acute intoxications with seed treatments have been seen in France (Berny et al. 1999). In its 2008 re-assessment of imidacloprid, the USEPA reported an incident where grubs surfacing after a lawn treatment appear to have poisoned young robins (USEPA 2008a). More details are available from the American Bird Conservancy's AIMS database of kills (unfortunately, government funding for this database ended in 2006). A total of seven birds were found dead or dying in this incident, which occurred on an area of residential turf in Pennsylvania in 1998. In another case, 4 Canada geese were found dead or dying on a New Jersey golf course in June 2001. A mixture of chlorpyrifos and imidacloprid had been used on the course but the lack of cholinesterase inhibition suggested imidacloprid might have been responsible. On a South Carolina residential property treated with imidacloprid in 2002, 6 mallards were found dead or dying but no further information was provided. In the spring of 2012, a large number of bee deaths were reported in SW Ontario following the seeding of corn fields with clothianidin. In one such bee kill, a dead robin was reported amidst dead and dying bees thirteen days after seeding in Hensall, Ontario. About a week later, a flycatcher was also found in the same yard. The incident is currently being investigated by the PMRA.

5.2. The issue of repellency

5.2.1. Imidacloprid

Based on early research with imidacloprid-treated rice seed (Avery et al. 1993a and a follow-up study, 1993b/1994) the registrants of imidacloprid have tried to make the case that the active ingredient repels birds and, therefore, is less of a risk to birds than calculated.

Some of the tests reported employed a 'two cup' design (e.g. Avery et al. 1993b). This design makes it easy for birds to recognise and avoid treated seed when untreated seed is fed alongside. In addition, exposure to the treated seed was brief and the birds received their normal ration before and immediately after exposure to the treated seed and untreated alternative. The birds were therefore not subjected to any form of food stress. Birds given treated seed only in a single cup exhibited a marked reduction in feeding at all dosing levels. Work on seed treatments (e.g. CSL 2002) has shown that, for laboratory tests to have any bearing on the wild situation, hunger stress and motivation to eat novel seed must be manipulated carefully.

[11] Under its revised classification criteria, EPA designates as minor (and thus barely reportable, aside from cataloguing as "WB") any pesticide incident that kills fewer than 200 individuals of a "flocking species," 50 individuals of a songbird species, or 5 raptors.

A more interesting design involved broadcasting treated and untreated seed on small plots within an aviary, mimicking (albeit on a small scale) a field situation where one seeded field might be treated but a neighbouring one not. This was done at the highest seed treatment rate tested (2500 ppm). (In comparing this with the seed rates registered, the loading per seed is similar to the higher loading on corn seed[12]). More seed was removed from the control plots than from the treated seed plot. However, birds feeding on the treated plots '*did not react as if the seeds were distasteful or unpalatable'*. Birds feeding on the treated plots ingested seeds at a rate of 5.4 seeds per minute versus 6.9 seeds per min. on control plots. The experimenters estimated from another experiment that 84-87% of the total insecticide load was not consumed but left behind on the hulls. In later work, however, the same Bayer-sponsored research team (Avery et al. 1997) found that house finches (*Carpodacus mexicanus*), red-winged blackbirds (*Agelaius phoeniceus*) and boat-tailed grackles (*Quiscalus major*) only discarded between 15-41% of imidacloprid on treated millet, rice, sunflower or sorghum when shelling the seeds, and that there were several species-seed type combinations where seeds were eaten whole and all residues ingested.

In their 2007 regulatory review (EPA 2007), the EPA scientists quite rightly expressed misgivings about relying on any repellency to mitigate the high hazard suggested by the toxicology.

"*However, to what extent risk would be mitigated is still an uncertainty. Both studies suggested that avoidance of birds to imidacloprid treated seed is a learned response mediated by postingestional distress. The treated seed was not a sensory repellent or irritant to the birds. Although the birds did eat the treated seed and exhibited treatment related effects (ataxia and retching), effects were deemed as transitory. These effects,*
although deemed as transitory under laboratory conditions, may make the affected birds more susceptible to predation in the wild. However, to what extent this is a possibility is unknown."

Indeed, when avoidance is due to post-ingestional illness, the ability of birds to avoid the chemical is directly related to their ability to associate the contaminated feed with the illness. Whereas this may be relatively easy for them to do in a laboratory situation, it becomes much more difficult in the wild when habitual food sources have become contaminated**. There are clear parallels with the cholinesterase-inhibiting insecticides where repellency was similarly thought to reduce in-field risks. For example, the organophosphorous insecticide diazinon is extremely well avoided in the laboratory. Yet, thousands of geese and other species grazed their way to an early death on diazinon-treated turf** (Frank et al. 1991, Mineau et al. 1994).

Given that shelling is very species and seed specific, it is reasonable to assume that some species will incur the full toxicological risk by ingesting seeds without shelling them.

Mortality of partridges and pigeons with imidacloprid-treated seed has been seen in France (Berny et al. 1999), one of the few countries with an active investigation system for pesticide poisonings. Reports came in of birds appearing weak and reluctant to move. They subsequently tested positive for residues. Regardless of the exact conditions surrounding the kills (seed concentration etc.) they do suggest that any avoidance/repellency is not operating well enough under actual field conditions to prevent exposure and acute intoxication.

[12] A 2500 ppm concentration would work out to approximately 0.0875 mg/wheat seed or 0.95 mg/corn seed. On rice, this came to 0.068 mg/seed.

5.2.2. Clothianidin

Industry studies on avoidance were submitted for clothianidin. These followed the German BBA (Ministry of Agriculture) Test Guideline.

The first (Barfknecht 1998a) tested Japanese quail exposed to treated canola (rapeseed). The birds were habituated to a seed diet consisting of 50% rapeseed, 10% millet and 60% canary seed (sic - yes this adds up to more than 100%). A week before exposure, the birds were given a choice of 1:1 of their usual seed mixture and the untreated target seed type, in this case rapeseed scattered on the ground. At the beginning of exposure, the birds (4 males and 4 females) were fasted for 16 hours and then exposed to a mixture of their standard diet and clothianidin-coated rapeseed. The latter had a violet appearance. The amount of regular seed provided was calculated to represent 25% of their usual daily intake only, while treated seed made up the rest. Exposure lasted for 8 hours after which the birds were returned to a standard diet and observed for a further 14 days. The entire design was repeated four times.

According to the author, the birds showed a high rate of food intake during the first hour of exposure. He claimed that, with the exception of one bird, the standard diet only was consumed while the treated seed was left untouched. Without further information, we find this claim difficult to support. It is difficult to see how a single observer could observe 8 birds simultaneously and determine what the birds were actually pecking at – since the standard diet and treated seed were said to be spread uniformly on the floor. Following the first hour, birds were only observed at hourly intervals. No feeding activity was observed at any point between the first and 8th hour post dose. It is possible that the aviary floors were sieved and the remaining seed counted but this was not apparent from the methods. No signs of intoxication or mortality were observed but two individuals (of 32) showed enlarged spleens at necropsy. This was judged to be within normal variation.

In a second study (Barfknecht 1998b), domestic rock doves were exposed to treated corn (maize) seed at 50 g a.i./50 000 seeds. The usual diet in this case was composed of 30% maize, 21% peas, 20% barley, 18% wheat, 8% milo, 2% dari and 1% vetch. Procedures were the same as described above except that exposure was repeated on three consecutive days (8 hours/exposure period). As with the quail above, the author reported that the birds fed heavily during the first hour. However, only one bird (of 10) showed a ‘reserved interest’ on treated seeds but spat out the red-coloured treated seeds immediately after picking them up. We believe this to be a ‘one off’ observation without much actual relevance given that the mechanism of avoidance of neo-nicotinoids is understood to be post-ingestional illness. No signs of intoxication or mortality were observed. However, 2 of 10 birds showed enlarged spleen at necropsy, and 4 of the 10 showed reduced gonad size ascribed to the fact that breeding condition had not been induced in those birds[13] (the study was run in May/June). Unfortunately, there were no control birds with which to compare this population and the author did not ascribe any importance to those findings.

In a third study (Barfknecht 2000), rock doves were exposed to treated corn again but at half of the concentration as the previous experiment (25 g a.i./50 000 seeds). This time, birds were either given untreated seed only or treated seed only. Food consumption was measured from 3 days pre-exposure to three days post exposure. On the day of exposure, food intake (as a proportion of body weight) was said to be 5.14% of body weight for control birds and 2.32% for the treated seed. In

[13] Bird species tested here are typically brought into breeding readiness by photoperiod (the ratio of the daily light to dark period). Failure to do so may be an indication of some interference with the endocrine system.

terms of food weight, this meant that individual birds feeding on treated seed consumed between 0 and 20.5g of seed on exposure day (mean = 11.7g) compared to 20.4 to 34.8 (mean = 26.1 g) for the control birds. This led the author to conclude that the seeds were partially avoided.

On the whole, we support the US EPA view that avoidance of treated seed has not been sufficiently well demonstrated in a realistic field context to believe that this will mitigate any high toxicological risk.

6. Could neonicotinoid insecticides be disrupting food chains and affecting birds indirectly?

It has been suggested by the Dutch toxicologist Henk Tennekes (2010) that the neonicotinoid insecticides and other systemic products represent a 'disaster in the making' because of their potential to affect birds through reductions of their food supply.

This matter has several sub-questions related to it. The first is whether it is indeed feasible to affect bird populations though a reduction of their food supply. A small review of the subject is called for here.

6.1. Have reductions in the available food supply been shown to affect birds?

Food supply (i.e. abundance and availability) can affect habitat selection, reproductive success and survival in birds (Simons and Martin 1990, Martin 1987, Johansson and Blomqvist 1996, Brickle et al. 2000, Moller 2001, Hole et al. 2002, Nagy and Holmes 2004, Strong et al. 2004, Boatman et al. 2004, Morris et al. 2005, Nagy and Holmes 2005, Britschgi et al. 2006, Hart et al. 2006, Zanette et al. 2006, Golawski et al. 2008, Selas et al 2008, Dunn et al. 2010, Poulin et al. 2010). In cases where food availability has not been to found to affect life history it is either considered to be superabundant (Powell 1984, Greer and Anderson 1989, Miller et al. 1994, Rauter et al. 2000), or in the case of reproductive success, adults are considered to compensate for low food availability by travelling further to find food, or spending more time foraging (Adams et al. 1994, Howe et al. 2000, Martin et al. 2000, Bradbury et al. 2003, Zalick and Strong 2008). Given that reproductive success and survival are key components of population growth and stability, one might be tempted to assume that bird populations will readily show a response to the food supply. There certainly are examples of bird species whose populations track irruptive pests – e.g. wood warblers and spruce budworm in eastern North America. Showing this link in an agricultural context is harder.

Most of the detailed work on the effects of food supply on farmland birds has been carried out in the UK. Evidence that food supply can affect reproductive success of farmland birds in the UK is fairly strong, but links with population declines are weaker. The best documented example of food supply affecting farmland birds is the example of the gray partridge (*Perdix perdix*). Herbicide use reduces the abundance of invertebrates in farm fields, including the abundance of preferred invertebrate prey items critical to the growth and survival of gray partridge chicks. Simulation modelling shows that declines in gray partridge populations can be wholly explained by herbicide use in farmland (reviewed by Potts 1986).

Insecticide use leading to reduced invertebrate food abundance has been linked to reductions in reproductive success of at least four farmland passerines in the UK: corn bunting (*Miliaria*

calandra), yellowhammer (*Emberiza citrinella*), whinchat (*Saxicola rubetra*) and reed bunting (*Emberiza schoeniclus*) (Brickle et al. 2000, Brickle and Peach 2004, Morris et al. 2005, Hart et al. 2006, Dunn et al. 2010 but see Bradbury et al. 2000, Bradbury et al. 2003). However, in contrast to the gray partridge, changes in invertebrate abundance alone do not fully explain population declines for these species. In fact, reproductive success for these species increased during time periods when populations were declining (Siriwardena et al. 2000). Population declines have instead been linked to reduced over-winter survival, linked to reduced seed availability (Peach et al. 1999, Siriwardena et al. 2000, Butler et al. 2010). The gray partridge therefore remains the only clear example of pesticide-induced insect food reductions affecting a bird population directly.

An example of pesticide-induced effects possibly more relevant to a broad contamination of the aquatic environment by neonicotinoid insecticides is the work carried out in the Camargue region of France following the use of the biological insecticide Bti (*Bacillus thuringiensis israelensis*) for the control of mosquitoes (Poulin et al. 2010, Poulin 2012, Poulin et al. 2012). Despite the very selective nature of Bti (being toxic to mosquitoes and some midges only), the research team documented clear impacts on the broader avian food web, especially spiders and other predator species, as well as breeding success reductions in house martins (Delichon urbicum) nesting nearby.

However, it is important to note that the ability of a bird population to maintain itself is dependent on rate of re-nesting attempts and post-fledgling survival as well as success of single nesting attempts (Setchfield et al, 2012). Rate of re-nesting attempts has been shown to be affected by food supply (Nagy and Holmes 2005), and can have a major effect on annual reproductive success (Nagy and Holes 2005, Setchfield et al. 2012). Post-fledgling survival is also thought to be closely tied to food availability (Sullivan 1989, Simons and Martin 1990, Yackel Adams et al. 2006), although very little is known about this stage since juveniles are very difficult to follow or study. Survival rates of gray partridges include this vulnerable stage, since these birds are precocial and leave the nest very soon after hatching.

Although invertebrate food supply has been suggested as a mechanism for increased abundance and species richness of birds on organic farmland in North America (Freemark and Kirk 2001, Beecher et al. 2002), and for reductions in the reproductive success of tree swallows (*Tachycineta bicolor*) as the proportion of intensive agriculture in the landscape increases (Ghilain and Bélisle 2008), very little research has been conducted on diet, foraging habitat or food supply of farmland birds in North America. Farmland breeding birds in North America are known to use crop fields, hay fields and boundary features, such as hedgerows, for foraging (Best et al. 1990, Boutin et al. 1999; Puckett et al. 2009). Boutin et al. (1999) surveyed birds in corn, soybean, apple orchards and vineyards in southern Ontario and found that of 14 species regularly observed within field centers and in edges adjacent to crop fields, most species were observed in edges more than expected based on habitat availability. Vesper sparrows (*Pooecetes gramineus*) nesting in corn and soybean fields forage most often within 1 m of weedy or unplanted areas, and prefer fields with crop residue over bare fields, presumably due to increased food supply (Rodenhouse and Best 1994). Song sparrows (*Melospiza melodia*) nesting in hedgerows adjacent to hay, corn and soybean in eastern Ontario were found to obtain approximately 40% of invertebrate nestling food from crop fields and 60% from hedgerows and hayfields (Girard et al. 2012). However, Zalick and Strong (2008) examined food supply for savannah sparrows (*Passerculus sandwichensis*) in mown and unmown hayfields and found no effect of food reductions on reproductive success. In eastern Ontario, organic soybean fields were found to support greater biomass of soil-dwelling invertebrates important for feeding nestlings than conventional soybean fields, but reproductive success of song

sparrows nesting in hedgerows in this region was not affected by local invertebrate food availability (Girard 2012).

There has been a small amount of experimental manipulative work on the food supply of breeding birds in North America, but this work has occurred in forests or native grasslands, rather than in intensively managed pastures or cropland. In grasslands, the effects of experimental reductions in food supply due to insecticide application have had little or no effects of reproductive success of birds (Powell 1984, Adams et al. 1994, Martin et al. 1998, Martin et al. 2000). Girard (2012) found that differences in soil-dwelling invertebrate biomass between organic and conventional fields was greatest in the fields themselves, rather than in the field edges or hedgerows, suggesting that birds that most depend on the fields will be most affected by invertebrate food reductions. For forest species, as mentioned above, the rise and fall of warbler species in response to budworm outbreaks remains one of the best indications of the impact of food supply on populations.

In summary, the link between impacts on the insect food of birds and population declines of farmland bird species is difficult to establish unequivocally, save for the evidence linking the grey partridge to both insecticide and herbicide use. Studies linking reductions in house martin breeding success and mosquito control are directly relevant to the issue of broad aquatic contamination from the neonicotinoid insecticides. Nevertheless, existing literature suggests that it is difficult to predict the relative importance of food supply during the breeding season compared to other risks such as habitat loss, food supply during migration and during winter, predation or even direct losses from poisoning or disturbances such as mowing or tillage. Each species responds to a different set of stressors and it is likely that reasons behind many of farm bird declines are multi-factorial. Farmland species are already well adapted to use multiple, irregular food sources that may collapse overnight as a result of agricultural operations, whether tillage, mowing or insecticide use; these species already take a large proportion of their food outside of actively cropped (and pesticide-treated) areas. Insecticides registered for agriculture before the advent of neonicotinoids, whether organochlorines, cholinesterase inhibitors or synthetic pyrethroids, were all rather indiscriminate in the type of insects they killed and sudden drops in food availability following insecticide treatment were undoubtedly commonplace before the neonicotinoids became so dominant in insect control. On the other hand, systemic insecticides such as the neonicotinoids might be game-changers (Francisco-Bayo et al. 2013). Because of their persistence in plant tissue, there is some evidence that they may affect terrestrial insect populations to a greater extent than non-systemic products. Systemic insecticides can be returned to the soil and remobilised in succession crops. The impacts on terrestrial food chains may therefore be much longer-lived and pernicious than those we have seen with other types of insecticides. Not only can these questions not be answered with the information made available through the registration process – but the questions themselves have not even been considered (save a few comment by EPA scientists on ‘structural and functional changes’ to ecosystems – see section 1.3). Generally speaking, an over-efficient removal of insects in crop fields is seldom seen as a matter of serious concern by regulators – especially in North America. The indirect impacts of pesticides are not considered in registration reviews – whether the US or anywhere else in the world.

In his book, the Dutch toxicologist Henk Tennekes (2010) makes the case that the contamination of surface water by neonicotinoids is so widespread in the Netherlands (and possibly elsewhere in Europe), that loss of insect biomass on a continental scale is behind many of the widespread declines that are being seen, be they of marsh birds, heath or meadow birds or even coastal species. This suggests that we should be looking at possible links between neonicotinoid

insecticides and birds, not on a farm scale, but in the context of whole watersheds and regions. Impacts from the neonicotinoids may very well be further afield than the arable area on which they are used and many of those impacts may be mediated through the aquatic environment. Because aquatic impacts are considered during product registration reviews, it is reasonable to ask whether the potential impact of neonicotinoids to aquatic life has been assessed correctly.

7. How toxic are the neonecotinoids to aquatic life?

In terms of scale of use, clothianidin and the other more recently- registered neonicotinoid insecticides thiamethoxam, acetamiprid and thiacloprid have probably overtaken imidacloprid. Yet, much more is known about imidacloprid, and a lot of the toxicity information being published now features that active ingredient almost exclusively. By necessity, much of this review will emphasize imidacloprid. We suggest (see 7.4) that the other neonicotinoids can be assessed through comparison with imidacloprid.

7.1. Has the toxicity of imidacloprid to aquatic life been properly assessed?

In carrying out a risk assessment, it is customary for regulators to pick a critical toxicity value (or reference level) against which to compare exposure estimates or empirical water residue measurements. Various methods are used, some more scientifically rigorous than others.

In asking whether the USEPA properly assessed the aquatic risks posed by imidacloprid, it may be unfair to go back to the very first registration reviews because, clearly, we know much more about the product now than we did when it was first registered in the mid-1990s. A more recent evaluation of the active ingredient came in 2007 when EPA was considering requests to expand the use of this active ingredient, notably for soybeans, peanuts, kava, millet, oats, artichoke, wild raspberry, and cane berries (USEPA 2007a). At the time, imidacloprid was already registered for a variety of leafy and fruiting vegetables, pome fruits, cotton, potatoes, hops, pecans, cucurbits, citrus, and tobacco, and had been studied extensively.

In this 2007 risk assessment, the EPA stated that "*imidacloprid is categorized as very highly toxic (0.069 - 0.115 ppm) to freshwater invertebrates on an acute basis.*" This was based on two freshwater species tested by the registrant in the early 1990s; the lower value was used to compute risk ratios with predicted exposure levels. By 2007, there were already a host of studies in the open literature showing acute toxicity levels as low as 0.003 ppm (see annex 1). Despite the fact that *Daphnia* had been shown to be a very insensitive species compared to other aquatic invertebrates, this was the only chronic data examined or required by EPA; on that basis, EPA concluded that "*imidacloprid exposure to freshwater invertebrates can potentially result in growth effects at 3.6 ppm.*" The NOEC for that same study was given as 1.6 ppm – a full 23 times higher than acute toxicity levels. Fortunately, when it came time to compute final risk ratios, the EPA scientists abandoned this value in favour of a value of 0.001 ppm obtained through an acute toxicity value and an acute/chronic extrapolation factor.

As luck would have it,[14] the marine invertebrate species that happened to be tested proved to be more sensitive to the pesticide and reference levels were deemed to be lower in the marine

[14] In relying on a handful of test species, it is clear that current regulatory assessments have more to do with a game of chance than with good science.

environment. The 2007 EPA review stated: "*Imidacloprid is very highly toxic to estuarine/marine invertebrates (mysid shrimp) on an acute basis (0.037 ppm)*" and "*chronic exposure of imidacloprid to estuarine/marine invertebrates can result in growth and survival effects (0.0013 ppm)*."

In fact, all of these reference levels are at least an order of magnitude too high (see below) and totally fail to protect the aquatic environment. **This example highlights the problems of blindly adhering to strict review protocols that ignore much of the accumulated scientific evidence and scientific insights available from the open literature in favour of a few outdated studies carried out by the registrant.** By the time risk quotients are calculated by EPA scientists, it is difficult to know how much the selection of specific reference levels has a bearing on registration decisions. Despite the incomplete use of available data by EPA in 2007 and the inherent underestimation of risk, calculated risk quotients for all proposed new uses exceeded EPA's chronic 'level of concern.' Yet, it appears that all new uses were approved for registration.

In December of 2008 (EPA 2008), the EPA launched a re-evaluation of imidacloprid. It is clear from the re-evaluation notice that the emphasis is to be on honeybees. Despite an acknowledgment of imidacloprid's high aquatic toxicity, no requirements are set out for a better characterisation of aquatic risk.

The EU (EFSA 2008) based its final 2008 risk assessment of imidacloprid on the most sensitive of two species tested (*Daphnia magna* and *Chironomus riparius*) as well as on a mesocosm study. The use of mesocosm results sets the EU process apart from that used by EPA. The European regulatory body proceeded to calculate their risk ratios with the following:

- Acute risk: 24 h EC50 of 55.2 ug/l
- Chronic risk: EC5 (emergence) for 28 d exposure of 1.9 ug/L
- Community risk: NOEC of 0.6 ug/l given DT50 of 5.8-13 d in the system studied. The LOEC was 1.5 ug/l but, at this concentration, no recovery was seen at the conclusion of the experiment. The Agency suggested that a safety factor of 1-3 would be appropriate along with the NOEC value cited above, giving an approximate value of 0.2 ug/l on which to compare calculated or empirical water concentrations.

In Canada, the CCME[15] developed non-regulatory water quality guidelines for imidacloprid in 2007. For freshwater bodies, they used the same *Chironomus* emergence study but retained the EC15 (emergence) of 2.25 ug/l to which they applied an arbitrary safety factor of 10. They therefore proposed an interim freshwater protection level of 0.23 ug/l. For the marine environment, they only had acute studies. They retained a 48h LC50 of 13 ug/l for the salt marsh mosquito to which they applied a safety factor of 20 on the grounds that imidacloprid is non-persistent in water[16]. The interim proposed guideline for saltwater environments was therefore set at 0.65 ug/l.

It is more difficult to assess the adequacy of the PMRA's assessment of aquatic risk from imidacloprid. That Agency often does not make its assessments public and the two documents available for imidacloprid (PMRA 1997, 2001) not provide any details.

[15] Canadian Council of Ministers of the Environment. A federal/provincial entity which, among other things, sets proposed (i.e. non-binding and non-regulatory) 'action levels' for concentrations of various chemicals in water in order to protect both human health and the environment.

[16] However, as the main degradation pathway is photolytic, this may not be a safe assumption in all bodies of water; e.g. turbid ones.

It is appropriate to question the continued separation of freshwater and marine endpoints in assessing aquatic toxicity. Maltby et al. (2005) explored the differences between toxicity estimates from distributions generated with data for freshwater and saltwater crustaceans for 10 well characterized insecticides. No significant differences were seen between estimates from these habitats. Even though saltwater species tended to be more sensitive, this was ascribed to the make-up of taxa most represented in the two habitats rather than any fundamental (toxicologically-driven) salt vs. freshwater difference.

7.2. Towards a more scientific approach of assessing toxicity information

A critical failure of existing regulatory evaluation protocols is that they typically look at data generated from a very small number of species. For example, submitted crustacean data may be for *Daphnia* only. By relying on a single indicator species, interspecies differences in susceptibility are not adequately addressed and, as argued above, much is left to chance. This is especially true in the case of pesticides with targeted (receptor-based) modes of action, such as the neonicotinoids. For this review, we opted to consider the ever-growing body of data from the published literature in addition to the few species mandated by regulatory authorities. The disadvantage of using these data is that they may be of varying quality and protocols may not be as standardised as those data mandated by regulatory agencies. (However, most of these studies are published and have therefore gone through a peer-review process which may indeed be more rigorous than regulatory scrutiny.) The advantage is that the published studies more fully represent the range of species likely to be exposed, thus providing a measure of the differences in sensitivity of aquatic organisms at large.

Once these data are assembled, the most credible way of determining a critical toxicity endpoint is through a species sensitivity distribution (see section 2 for an introduction to this topic). Species sensitivity distributions were generated separately for aquatic insects and crustacea and we derived HC5 (hazardous concentration) values, using the ETX 2.0 software (van Vlaargingen et al. 2004).

Data were obtained from regulatory documents as well as the primary literature. It was not always possible to obtain the source information so the study details were not always available. However, even standardised tests can show wide variations in results. This argues for being inclusive when it comes to test results. In assembling data, priority was given to 96h test duration, the lowest of EC50 or LC50 if both were measured, and technical versus formulated material in that order. Geometric means were computed where several equally acceptable values were available. To derive water quality criteria, the U.S EPA (Stephan et al. 1985) recommended the use of EC50 measures based on death <u>or</u> immobilization[17] to better reflect the total severe acute adverse impact of the test material on the test species. Sanchez Bayo and Goka (2006) reported that the effective dose (EC50 – immobilisation) was 100-600X lower than the LC50 (true death) with imidacloprid specifically. They recommended that EC50 values should be used in risk assessment and suggested that the gap between EC50 and LC50 might be greater with neonicotinoids than with other classes of pesticides. Beketov and Liess (2008a) found that with neonicotinoids and other neurotoxic

[17] The distinction can be difficult to make with some organisms. If an organism is sufficiently incapacitated and fails responding when gently prodded, it is to be classified as dead whether or not it is clinically dead.

insecticides, drift of invertebrates[18] was already considerable at water concentrations 1/10 of the LC50.

For reasons outlined in the previous section, marine and freshwater species were considered together.

7.2.1. A quick note on test variability and repeatability

There is a tendency in regulatory circles to become overly preoccupied with the accuracy and precision of any given toxicity test value. This fixation is driven by various factors: risk quotients are derived from very few tests, they may need to be defended in the courts, internal agency guidelines insist on stringent test conditions and therefore expect perfect repeatability etc. In reality, whether dealing with aquatic invertebrates or warm-blooded vertebrates, experience shows us that there could be significant test-to-test variation, even when those tests are conducted under carefully standardised conditions.

As an example, Table 7.1 illustrates the various test results obtained for 48h static or static renewal acute tests with imidacloprid and *Daphnia magna,* the best known and best characterised aquatic test species.

Table 7.1. Toxicity test results for 48h EC50 and LC50 values for Daphnia magna exposed to imidacloprid. TECH refers to technical material; FORM to a formulated end product. All toxicity values are given in ug/l of active substance.

Form of the a.i.	Measure	Toxicity (ug/l)	CL	Probit slope	Source	Reference	Comments
TECH	LC50	10,440	6,970-17,710	1.86	Original publication	Song et al. 1997	Test at 27 degrees C
TECH	LC50	17,360	12,510-30,050	1.86	Original publication	Song et al. 1997	Test at 20 degrees C
FORM	LC50	30,000	28,000-44,000		Original publication	Tisler et al. 2009	
FORM	EC50 (immobility)	43,265	34,302-53,592		Original publication	Hayasaka et al. 2012	
TECH	LC50	56,600	34,400-77,200		Original publication	Tisler et al. 2009	
TECH	LC50	64,873			Original publication	Sanchez-Bayo 2009	

[18] Defined as the organisms being sufficiently impaired to detach from the substrate and be carried downstream by the current. The removal of aquatic life from stretches of a stream represents an ecologically undesirable effect.

TECH	EC50 (immobility)	85,200	75,000-113,000	11	One liner 2004, 2007; Pesticide Manual, EPA 2007 assessment	Young 1990 (Bayer)	
TECH	EC50	>32,000			Agritox	Bayer France	

Based on these data, 10X differences within species should not be surprising. This level of variation also underscores the importance of obtaining multiple tests on multiple species in order to derive a credible critical toxicity threshold for environmental protection.

7.2.2. Other factors influencing sensitivity of organisms

Life stage

Yokoyama and colleagues (2009) showed that sensitivity could vary greatly between different larval instars[19] of the same species with younger instars tending to be more sensitive, possibly because of greater surface to mass ratios. For example, sensitivity of the caddis fly *Cheumatopsyche brevilineata* to imidacloprid dropped by 5.1-5.7 fold as the larvae matured from first to fifth instar. Interestingly, these authors showed that for an organophosphorous and carbamate insecticide, individuals from cleaner urban streams were much more sensitive than those taken from agricultural areas. This did not hold true for imidacloprid however.

Technical vs. formulated insecticide

The technical product is the pure form of a pesticide synthesised by the registrant. Because it is synthesized under industrial conditions, its level of purity typically approaches 95-99%. The pesticide purchased by the consumer contains the active ingredient to which has been added: solvents, emulsifiers, chemicals to help the droplets stick to or penetrate plant surfaces, etc. This final (formulated) product typically contains 40-80% active ingredient – but this can sometimes be much lower. The formulants are often called 'inerts' but they are often nothing of the sort toxicologically. There are enough comparable test data with imidacloprid to provide a comparison of technical vs. formulated material (Table 7.2).

Table 7.2. A comparison of acute toxicity values for technical and formulated imidacloprid. All values corrected to ug/l in active ingredient.

Species	Endpoint	Value for technical material (ug/l)	Value for formulated material (ug/l)	Reference
Americamysis bahia	96h LC50	38	159	EPA One liner
Daphnia magna	24h LC50	97,900	38,000	Tisler et al. 2009
Daphnia magna	48h LC50	56,600	30,000	Tisler et al. 2009
Hyalella azteca	96h LC50	65.4	9.7/17.4	Stoughton et al. 2008
Chironomus tentans	96h LC50	5.4	5.75	Stoughton et al. 2008

[19] An insect's period of postembryonic growth between molts.

On that basis, we feel justified in pooling data from both technical and formulated material in the same distributions (see below).

Temperature
Song and colleagues obtained almost identical values for 48h LC50 values with *Aedes aegypti* at either 20 or 27 degrees C. This does not argue for strong temperature-dependence as is the case for pyrethroid insecticides for example. On the other hand, Mohr and colleagues (2012) obtained more pronounced effects on an assemblage of benthic species from imidacloprid pulses in their summer applications which they attributed to higher water temperatures.

Light
Because photolysis is the main mode of degradation for imidacloprid, the amount of illumination provided during testing is expected to be critical to the results. Because water clarity is variable in nature, the extrapolation from lab to field will be very difficult as a result. Light levels are seldom reported in the test data. For this reason, the Dutch government (RIVM 2008) in its assessment of imidacloprid toxicity rejected all tests conducted in the light unless concentrations were empirically verified. Sanchez-Bayo and Goka (2006) found that values obtained in the dark could be two-fold lower than values obtained in the light. This is not that great a difference given some of the information shown above on repeat testing. Therefore, we chose not to restrict data in the same way. Because we were not as strict with the test data, some of the studies may have underestimated the toxicity of imidacloprid compared to what it could be in turbid or strongly coloured water.

Season
Season was found to be one of the most important factors affecting the toxicity of imidacloprid to the amphipod *Gammarus roselli* (Bottger et al. 2012). Depending on test conditions, the 96h EC50 varied from 1.9 to 129 ug/l. Small hungry individuals in the spring were found to be the most sensitive and tests manipulated to mimic those conditions gave results that best approximated what was observed in the field.

7.3. Deriving HC5 values for imidacloprid

The ETx software was used to determine the HC5 or hazardous concentration based on available acute and chronic toxicity data.

7.3.1. Acute data

All aquatic toxicity data are given in appendix 1. The following tables summarise the data entered into the calculation of HD5 values.

Table 7.2. Imidacloprid. Summary of acute toxicity values in ug/l for crustacean species.

Taxonomic_SD	Study Time (h)	FORM	Measure	Toxicity SD_ppb
Ceriodaphnia dubia*	48	FORM	LC50	2.07
Cypridopsis vidua	48	TECH	EC50	3

Ilyocypris dentifera	48	TECH	EC50	3
Cypretta seurati	48	TECH	EC50	16
Gammarus roselli	96	UNKNOWN	EC50	29
Americamysis bahia	96	FORM	LC50	36
Hyalella azteca	96	TECH	LC50	65
Gammarus pulex	96	TECH	LC50	350
Palaemonetes pugio	96	TECH	LC50	417
Ceriodaphnia dubia*	48	FORM	EC50	572
Gammarus fossarum	48	FORM	LC50	800
Chydorus sphaericus	48	TECH	EC50	832
Ceriodaphnia reticulata	48	FORM	EC50	5553
Asellus aquaticus	48	FORM	LC50	8500
Daphnia magna	48	TECH	EC50/LC50	35539
Daphnia pulex	48	FORM	EC50	36872
Moina macrocopa	48	FORM	EC50	45271
Artemia sp.	48	TECH	LC50	361230

*Both values for Ceriodaphnia were kept because of their wide divergence and the apparent validity of both independent tests performed on different continents.

The wide inter-species range in recorded toxicity with imidacloprid is notable. Mayer and Ellerseck (1986) looked at in-house acute toxicity tests for 82 pesticides and chemicals. In all test species confounded, the average ratio between the lowest and highest LC/EC50 was 256X (868X for insecticides only). The highest recorded spread was 166,000X for an insecticide. The ratio for imidacloprid values is greater than 174,000X. Also remarkable is the low sensitivity of the cladoceran *Daphnia magna*. This is the most common test species on which much of the aquatic risk assessment is usually based. There is evidence that cladocera as a group are insensitive to neonicotinoid insecticides (Hayasaka et al. 2012) although there is an alternate study which found the cladoceran *Ceriodphnia dubia* to be among the most sensitive species tested (Chen et al. 2009).

Table 7.3. Imidacloprid. Summary of acute toxicity values in ug/l for aquatic insect species.

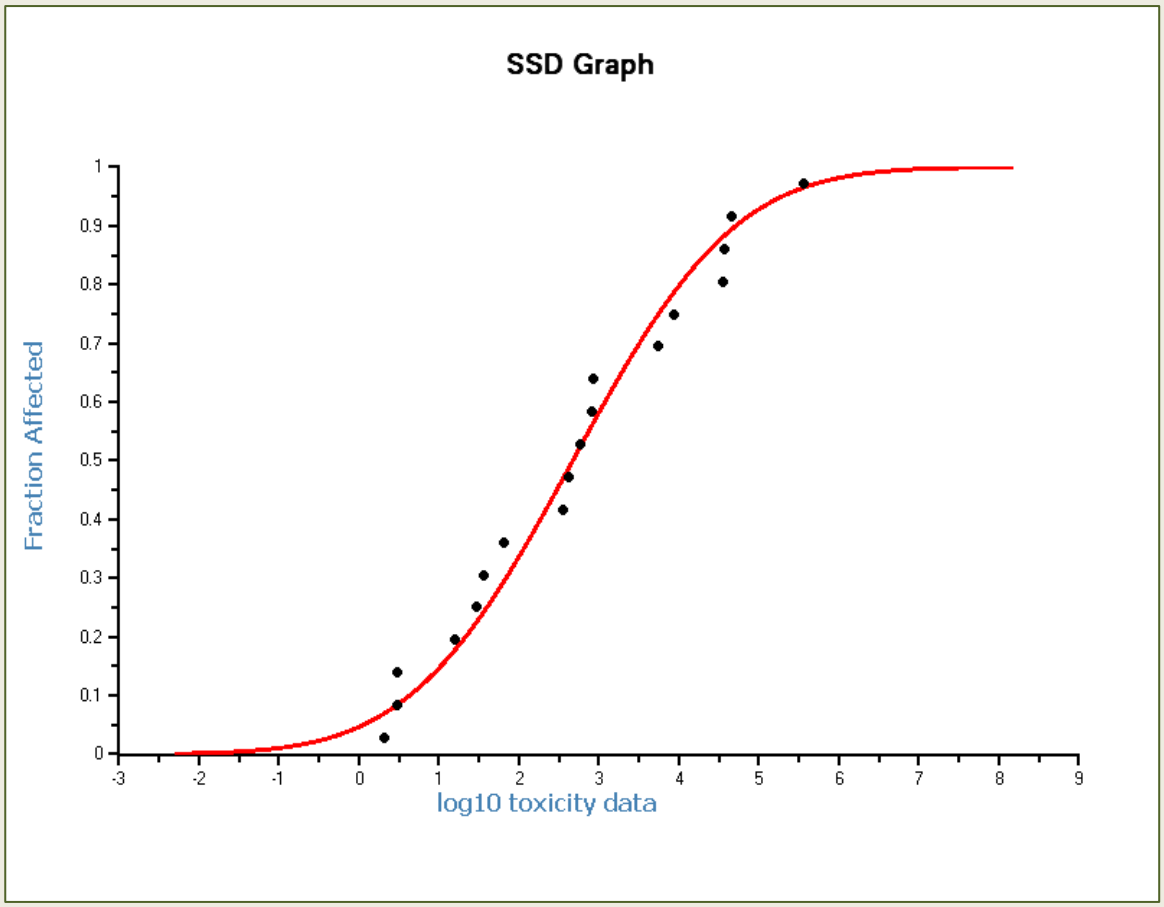
FORM_SD	Taxonomic_SD	Study Time (Value)	Study time (Unit)	Measure	Toxicity SD_ppb
FORM	Epeorus longimanus	96	h	LC50	0.65
FORM	Chironomus dilutus	96	h	EC50	2.65
FORM	“Heptageniid mayfly”	96	h	LC50	3.7

TECH	Simulium latigonium	96	h	LC50	3.73
TECH	Cheumatopsyche brevilineata	48	h	LC50	6.59
TECH	Chironomus tentans	96	h	LC50	7.8
TECH	Simulium vittatum	48	h	LC50	8.09
TECH	Baetis rhodani	48	h	LC50	8.49
FORM	Chironomus riparius	96	h	EC50	12.9
TECH	Aedes taeniorhynchus	48	h	LC50	13
TECH	Aedes aegypti	48	h	LC50	44

A species sensitivity distribution based on the normally distributed acute data returns an HC5 of 1.01 ug/l for crustacea (0.06-6.8) and an almost identical 1.02 ug/l for aquatic insects (0.31-2.06). Despite the overlap, the insects appear to have a much lower sensitivity variance – i.e. more similarity in response. A pulse of imidacloprid in the ug/l range would therefore be expected to affect a larger proportion of the insect community.

Dragonfly on wheat by Jim Occi, BugPics, Bugwood.org

The following figure illustrates the species sensitivity distribution for imidacloprid and crustacean species.



For the combined dataset of aquatic insects and crustacea, the calculated HC5 is 0.22 (0.03-1.0). However, the data do not fulfill the condition of normality; forcing a normal distribution[20] may not be the best way to proceed. Using slightly different methods which involve collapsing data within genera before applying a species sensitivity distribution, Nagai and colleagues (2012) arrived at a similar value with an HC5 of 0.43 ug/l.

7.3.2. Chronic data

There are enough chronic toxicity data for imidacloprid to run a species sensitivity distribution (Table 7.4). Although they address slightly different endpoints, most deal with survival and reproduction over a 21-28 day period. The HC5 for NOEC values is calculated to be 0.029 (0.00038-0.28).

Table 7.4. Imidacloprid. Available chronic data for aquatic invertebrate species.

Form of the pesticide	Taxon	Species	Study Time (d)	Expo-sure type*	Measure	Value ug/l	Source	Reference
	Crustacea	Mysidopsis bahia	28		EC50 (body length)	0.3	Stoughton et al. 2008	Cox 2001 and Felsot & Ruppert 2002

[20] A normal distribution is a continuous probability density function symmetrical around a mean of 0 and with a standard deviation of 1. It is the 'standard bell curve' often used to characterise a variable subject to random influences.

TECH	Crustacea	Americamysis bahia			NOEC (growth and survival)	0.6	EPA 2007 assessment	Ward 1990 (Bayer)
TECH	microcosm	Chironomidae and Batidae		S, S	NOEC	0.6	EFSA 2008 Scientific report	
TECH	Insecta	Chironomus tentans	28		EC50 (emergence)	0.91	Original publication	Stoughton et al. 2008
TECH	Insecta	Chironomus riparius	28	S	EC50 (emergence)	3.11	EFSA 2008 Scientific report	
FORM	Crustacea	Hyalella azteca	28	SR	LC50	7.08	Original publication	Stoughton et al. 2008
TECH	Crustacea	Gammarus pulex	28	S	NOEC (swimming behavior)	64	Draft assessment report from Germany (Rapporteur State) 2005	
TECH	Crustacea	Daphnia magna	21	SR	NOEC (repro)	1800	EPA 2007 assessment EFSA 2008 Scientific Report	Young 1990 (Bayer)

* S = Static; SR = Static with renewal; S,S = 2 applications at 21 d interval.

Another way to approach the problem is to consider the acute-chronic ratio for the compound and apply this to the appropriate acute toxicity endpoint. This is scientifically much more credible than accepting a chronic toxicity endpoint that is much higher than most acute toxicity endpoints merely because it was determined for a species that happened to be insensitive.

There are four species for which we can derive an acute-chronic ratio. This ratio is lower in the crustacea - 2.5 in *Hyalella* and 5.5 in *Gammarus* but much higher in the two *Chironomus* species studied to date – 17.7 and 75.8. The latter values, applied to the most sensitive insect species tested to date (*Epeorus*) would return a chronic toxicity value of 0.0086 ug/l (using a factor of 75.8) to 0.037 ug/l (using a factor of 17.7).

It is clear that a more credible consideration of all the species toxicity information collected to date suggests that the toxicity of imidacloprid to aquatic invertebrates has been greatly underestimated by EPA (summary in tables 7.5 and 7.6). Effects on aquatic invertebrates are likely to be substantial indeed at sub ppb levels of water contamination. Sanchez-Bayo and Goka (2006) reported that, in rice mesocosms, all zooplankton species were eliminated as long as water concentrations remained above 1 ug/l.

Table 7.5. A summary of reference concentrations (in chronological order) for acute (peak) exposure of imidacloprid in freshwater environments.

Source	Reference level against which exposure concentrations are to be	Justification

	compared for freshwater environments (ug/l)	
EPA (2007) (US)	35	Lowest of three tests examined – to which a factor of 2 has been applied in keeping with the 0.5 LOC (Level of Concern) for a risk quotient
EFSA (2008) (Europe)	0.55	Lower of two species tested to which factor of 100 has been applied in keeping with Annex VI triggers for the Toxicity/Exposure Ratio.
RIVM (2008) (Netherlands – non regulatory)	0.2	Maximum acceptable concentration from short term exposure or exposure peaks – based on mesocosm study and 3X safety factor
Nagai et al. 2012	0.43	HC5 but with SSD methodology which combines species within the same genus – also with 50% confidence
EPA (2012)* (US – non regulatory)	35	Aquatic life benchmark – presumably same methodology as regulatory review
This report	1.01	HC5 (with 50% confidence) for acute exposure in crustacea
This report	1.02	HC5 (with 50% confidence) for acute exposure in insects
This report	0.22	HC5 (with 50% confidence) for acute exposure in all aquatic invertebrates (ignoring lack of normality)

* http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.
Htm. Accessed December 2012.

Table 7.6. A summary of critical toxicity levels for aquatic invertebrates exposed to chronic (3-4 week) exposures to imidacloprid.

Source	Reference level against which averaged exposure concentrations are to be compared for freshwater environments (ug/l)	Justification
EPA (2007) (US)	0.5	Obtained with an acute/chronic ratio and applying a factor of 2 for the usual LOC. (Using the usual chronic NOAEC for Daphnia would have meant accepting a value of 800 – much higher than the acute value)
CCME (2007) (Canada – non regulatory)	0.23	EC15 for the most sensitive of two freshwater species tested chronically to which a factor of 10 has been applied
EFSA (2008) (Europe)	0.2 – 0.6	NOEC from microcosm study (same study used for deriving an acute criterion in the Netherlands) to which a 1-3 safety factor has been applied based on expert deliberations
RIVM (2008) (Netherlands – non regulatory)	0.067	Maximum permissible concentration for long term exposure derived from lowest NOAEC value and factor of 10. This replaces an older value of 0.013 ug/l.
EPA (2012)* (US – non regulatory)	1.05	Aquatic life benchmark – methodology uncertain
This report	0.029	Distribution analysis of NOECs for chronic studies on 7 single species and one species assemblage.
This report	0.0086	The higher of two empirically-determined acute-chronic ratios for insects applied to the most sensitive insect species of 8 tested to date

* http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.
Htm. Accessed December 2012.

Based on our assessment as well as that of various jurisdictions around the world, it is clear that the US EPA has underestimated the toxicity of imidacloprid to aquatic invertebrates by over an order of magnitude. Severe impacts to aquatic environments are expected from short term (pulse) exposures as low as 0.2 ug/l (ppb) and chronic exposures to concentrations at least 10 times lower.

7.4. Deriving critical water concentrations with other neonicotinoid insecticides

Only two other neonicotinoid insecticides have a sufficient amount of data to fit to a distribution (annex 1) – but then only by pooling all invertebrates (crustacea and insecta).

Tables 7.7 and 7.8 provide the data entered into ETx to derive an HC5 value. Thiamethoxam is at the limit of credibility given the small sample size and the fact that one of the values is a limit value. Nevertheless, we believe that this is a more reasonable approach than simply basing a critical concentration on one or two standard species as is currently the case (especially when the main test species is known to be insensitive).

Table 7.7. Thiamethoxam acute toxicity data for aquatic invertebrates.
TECH = technical material. See annex 1 for details.

Taxon	Species	Study Time (h)	Form	Measure	Toxicity (ug/l)
Crustacea	Daphnia magna	48	TECH	EC50	>106000*
Crustacea	Chaoborus sp.	48	TECH	EC50	180
Crustacea	Americamysis bahia	96	TECH	EC50	5400
Insecta	Cloeon sp.	48	TECH	EC50	14
Insecta	Chironomus riparius	96(?)	TECH	EC50	35

* Value entered as such regardless of >

Table 7.8. Thiacloprid acute toxicity data for aquatic invertebrates.
FORM = formulated material; ANALYTICAL = analytical grade material. See annex 1 for details.

Taxon	Species	Study Time (h)	Form	Measure	Toxicity (ug/l)
Crustacea	Daphnia magna	24	FORM	LC50	4100
Crustacea	Asellus aquaticus	24	FORM	LC50	153
Crustacea	Gammarus	24	FORM	LC50	190

	pulex				
Insecta	Sympetrum striolatum	24	FORM	LC50	31.19
Insecta	Notidobia ciliaris	24	FORM	LC50	6.78
Insecta	Simulium latigonium	24	FORM	LC50	5.47
Insecta	Culex pipiens	24	FORM	LC50	5.76
Crustacea	Gammarus pulex	96	ANALYTICAL	LC50	350
Insecta	Baetis rhodani	96	ANALYTICAL	LC50	4.6

HC5 (with 50% confidence) values are estimated as 0.74 and 0.80 ug/l for thiamethoxam and thiacloprid respectively. With their own data (7 species tested), Beketov and Liess (2008b) had determined an HC5 value of 0.72 ug/l. Given the small number of species tested, this certainly places these compounds in the same general range as imidacloprid.

Rather than attempt to derive unique values for the other compounds that are based on very little data, we propose a comparative approach; i.e. how do the various neonicotinoids compare to imidacloprid where comparable data exist. Table 7.9 provides data where species, formulation and test duration were a reasonable match.

Table 7.9. Comparison of neonicotinoid acute toxicity to aquatic invertebrates. Crustacea in normal type, insect species are in bold. All data derived with technical material unless otherwise stated. All times as indicated in table unless otherwise specified. IMI = Imidacloprid, ACE = Acetamiprid, THC = Thiacloprid, CLO = Clothianidin, THM = Thiamethoxam, DIN = Dinotefuran.

Species	Study Time (h)	End-point	IMI (ug/l)	ACE (ug/l)	THC (ug/l)	CLO (ug/l)	THM (ug/l)	DIN (ug/l)
Daphnia magna	48	EC50	35,539	49,800	43,777	109,523	>106,000	1,000,000
Americamysis bahia	96	LC50	36	66	31	51	6900	790
Gammarus pulex	96	LC50	350	50	350			
Asellus aquaticus	48	LC50	8500*		153* **			
Hyalella azteca	96	LC50	65		37			
Chironomus riparius	48	EC50	20*			22	35	
Simulium latigonium	96	LC50	3.7	3.7	5.5* **			

Baetis rhodani	48	LC50	8.5		4.6***			

* formulated
** 24h
*** 96h

The table highlights more similarities than differences between the active ingredients. Whereas clothianidin and thiamethoxam appear at first blush to be less toxic to crustacea, the *Chironomus* data suggest that they are equally toxic to nontarget aquatic insects. Acetamiprid and thiacloprid appear to be very similar to imidacloprid. There isn't enough information for dinotefuran to say one way or the other. From the point of view of protecting aquatic communities, we suggest that the critical values derived in section 7.3 for the protection of aquatic ecosystems against imidacloprid could easily apply to all other neonicotinoids. Indeed, because of the similarity in mode of action, we suggest that the critical concentration values derived for imidacloprid should be applied to the sum of all neonicotinoid residues.

We believe that the above is a more credible approach than relying on a few aberrant data points as the EPA has done repeatedly. For example, in their 2003 assessment of clothianidin for corn and canola seed treatments (US EPA 2003) the agency used an NOAEC of 42 ug/l based on the chronic life cycle test in daphnia for the protection of fresh water environments. This is despite the fact that by the time of the 2003 review, Daphnia had been shown to be comparatively insensitive to both imidacloprid and clothianidin; we now know this insensitivity extends to all neonicotinoids. The 42 ug/l value for chronic exposure retained by EPA in 2003 is actually higher than the 22 ug/l value that was retained for acute effects. If nothing else, a chronic reference level that is twice as high as the acute reference level in the same receiving waters should have rung a few alarm bells. **Once again, this leads one to conclude that EPA's approach to the assessment of aquatic risk is scientifically unsound and places aquatic environments at risk.**

In the Netherlands, Van Dijk (2010) reports that chronic reference values were set independently by the government for each of the neonicotinoids: 0.025 ug/l for thiacloprid, 1 ug/l for thiamethoxam, 14 ug/l for clothianidin and, the older value of 0.013 ug/l for imidacloprid set in 2007 but reviewed upwards in 2008 (table 7.6). We question whether the data are sufficient to ascribe a different aquatic toxicity to each of these active ingredients.

7.4.1. Degradates or metabolites

Most of the neonicotinoid insecticides have complex degradation pathways in soil or in receiving waters. There are a few cases where this degradation needs to be taken into account when assessing the full toxicity of the active ingredient in the environment. The most obvious example is the production of clothianidin as a major degradation product of thiamethoxam. The higher aquatic toxicity of the TZNG metabolite of clothianidin also needs to be considered[21] as well as that of the NOA407475 metabolite of thiamethoxam (Annex 1). Even when of equal or lesser toxicity than the parent material, metabolites must be considered because they may prolong the toxicity profile of the insecticide.

[21] Acute toxicity to Daphnia magna is given as 640 ug/l relative to approx. 110,000 ug/l for the parent material.

7.5. Concerns that 'standard approaches' to risk assessment are not adequate for neonicotinoid insecticides.

As seen above, the US EPA and, to a lesser extent, other regulatory bodies have grossly underestimated the toxicity of neonicotinoids to aquatic environments. Critical concentrations at which effects are expected are much lower than currently assumed by regulators. However, there is even more reason to be concerned about these compounds in the aquatic environment.

Sanchez-Bayo and Goka (2007) and Sanchez-Bayo (2009) argued, as have some before them[22] that the conventional method of assessing toxicity at fixed exposure times makes it difficult to extrapolate to exposures of different duration – whether shorter pulse exposures or prolonged exposures resulting from compound persistence. Using imidacloprid specifically, Sanchez-Bayo showed that a 48h LC50 of 390 ug/l for the ostracod *Cypridopsis* dropped 100 fold to 4 ug/l after a 5 day exposure period. This time dependence is not unique to neonicotinoids. However, the more toxicity is shown to be a function of time (keeping concentration equal), the more it can be argued that the compound is having an irreversible mode of action. This argument was made by Tennekes (2010) who likened the toxicity of neonicotinoid insecticides to a 'one hit' model of chemical carcinogenesis. Tennekes went on to describe how neonicotinoids closely follow Haber's rule which states that the product of exposure concentration and duration is a constant. In theory, this means that an infinitesimally small dose can result in a toxic effect provided duration of exposure is sufficient. He argued that the toxicity of neonicotinoids to both bees and aquatic organisms showed this characteristic. These insecticides bind almost irreversibly to invertebrate cholinergic receptors – which incidentally makes them very powerful insecticides and helps explain why their use has increased so dramatically over time. Despite Bayer Corporation's protestations that the mode of action of imidacloprid is not irreversible (Maus and Nauen 2011), Tennekes (2011) counter-argued successfully that evidence to date shows otherwise (despite minor deviations, the insecticide is dangerously close to showing irreversible activity) and even used some of Bayer Corporations' earlier reports on imidacloprid's mode of action to make his point.

Of course, what is of interest is the internal (i.e. at the receptor level) exposure rather than the external (i.e. test medium) exposure. **Possibly the most troublesome piece of evidence on neonicotinoid insecticides to date is that of Beketov and Liess (2008b) studying the toxicity of thiacloprid to several aquatic invertebrate species. What they reported is that the apparent LC50 to various test species dropped dramatically merely by extending the post-exposure observation period. The most extreme example of delayed mortality was for Gammarus pulex where the calculated LC50 was 50X lower after observing the exposed individuals for 17d even though exposure in all cases was for 24h only.** Similarly, Stoughton et al. (2008) compared a 96h pulse of imidacloprid with a prolonged observation period in clean water with a continuous 28d exposure. The 96h pulse was intended to mimic a realistic runoff scenario. The calculated NOAEC was identical under both exposure scenarios in one of the two species tested (the amphipod *Hyaella azteca*); continuous exposure proved more damaging for the midge *Chriromus tentans*.

These types of observation do lend credence to Tennekes's comment on irreversibility of action and increase our concern with exposure to the neonicotinoids even if those are pulse exposures. There

[22] These authors provide a good review of time-dependent approaches to toxicity estimation and references going back to the 1930s.

has been at least another effort to look at the effect of a pulsed neonicotinoid exposure. Mohr and colleagues (2012) exposed stream mesocosms to weekly imidacloprid pulses of 12 ug/l. The most sensitive species in the system was affected following the first pulse whereas effects on other taxa were more gradual and increasingly evident after 2 or 3 pulses. Pond mesocosms have effect levels that are much lower than this but the authors argued that effect concentrations are not that dissimilar once a time weighted concentration approach is used in the case of the pulse exposure.

Tennekes and Sanchez-Bayo finally collaborated in 2011 to reiterate the points made above and argued that neonicotinoids are far more dangerous than other insecticides of higher toxicity. This is a key point because the neonicotinoids have replaced insecticides (such as synthetic pyrethroids) of very high aquatic toxicity (see section 7.5.1.).

7.5.1. A quick comparison of the toxicity of neonicotinoids and older insecticides to aquatic ecosystems

Whiteside and colleagues (2008) compared the toxicity of all insecticides registered at the time in Canada to aquatic environments. They ran all products through a simplified runoff model assuming maximum label rates and a standard application scenario, and assessed the acute risk of registered products to fish, crustacea, insects and plants through a 'weighted community score.' They weighted fish more heavily than invertebrates and invertebrates more heavily than algae – reflecting the ease with which these ecosystem components could be replaced if lost. Because the toxicity of neonicotinoids to fish is quite low compared to either pyrethroid or organophosphorous insecticides, the neonicotinoids fared quite well when compared to a number of older insecticides they have replaced (Table 7.10).

Table 7.10. Comparison of aquatic toxicity and relative aquatic community risk (after Whiteside et al. 2008) of neonicotinoid insecticides and several of the insecticides they have replaced.

Active ingredient	Fish HC5	Crustacea HC5	Aquatic insect HC5	Weighted community risk score
tefluthrin	0.0101	0.000961		8700
dimethoate	12.6	0.010	14.7	2900
methamidophos	16100	0.0196		660
diazinon	56.9	0.191	2.98	380
chlorpyrifos	0.966	0.05	0.350	200
carbofuran	72.3	18.0	1.01	98
terbufos	1.41	0.180	1.40	40
deltamethrin	0.254	0.00147	0.0122	8.3
imidacloprid	16000	0.704 (1.01*)	1.40 (1.02*)	4.4
malathion	48.2	0.417	3.30	4.4
methomyl	610	14.3	6.23	4.4
acetamiprid	10600	28.7		0.08
clothianidin	10500	38.9		0.03
carbathiin	232	1090		0.01
thiamethoxam	10900	427		0.00
thiacloprid				NA

* Updated value based on this report

Again, this comparison may be misleading if the chronic risk of neonicotinoids is different, whether for reasons invoked by Tennekes and Sanchez-Bayo above – or because of exposure characteristics. Exposure will be reviewed briefly below. Care must therefore be exercised before concluding (as have some authors – e.g. Barbee and Stout 2009) that the neonicotinoids are an improvement over older classes of insecticides. **Whereas neonicotinoids are clearly less acutely toxic to fish than many other insecticides, we might expect fish to be affected indirectly though efficient and prolonged removal of aquatic invertebrates.**

7.5.2. Sublethal and delayed effects of neonicotinoids

All pesticides have the potential to cause effects at doses that are not immediately lethal. However, there is some evidence that neonicotinoids are more of an issue here than other registered pesticides. The issue of disorientation of honeybees at extremely low exposure levels suggests that their mode of action (i.e. the quasi-irreversible binding of neonicotinic synapses) causes behavioural effects. Alexander et al. (2007) showed that short (12h) exposure pulses of 1 ug/l and higher caused feeding inhibition in mayflies. Even pulse exposures as low as 0.1 ug/l affected the size of the adults at emergence (Alexander et al. 2008). Englert and colleagues found that predator-prey interactions and leaf litter breakdown were affected at concentrations of thiacloprid between 0.5 and 1 ug/l in a simple laboratory ecosystem. Pestano et al. (2009) found effects on respiration in chironomids an order of magnitude below lethal levels.

7.5. Will exposure levels be high enough to cause problems in aquatic environments?

7.5.1. The regulatory view

In its earliest review of imidacloprid (USEPA 1994b), the Agency reviewers already had concluded that the chemical's mobility, solubility and persistence were a concern for groundwater contamination and aquatic systems. This concern was echoed in most if not all reviews carried out since that time; e.g. "*EFED has concluded that the available data on imidacloprid shows that the compound is mobile and persistent, has potential to leach to ground water, and also presents concerns for transport to surface water via runoff. In addition to the persistence issue, EFED also has a concern for imidacloprid residual carry-over to other crops after the previous year's application*." (USEPA 2007a)

In Canada, imidacloprid was first registered in 1995 although a number of data gaps existed at the time. The PMRA updated its review in 2001 (PMRA 2001). Their review determined that "*imidacloprid is classified as persistent under agricultural field crop conditions according to the classification scheme of Goring et al. (1975), with a DT50 in soil in the order of 1-2 years.*" They went on to compare imidacloprid to atrazine, a problematic well known aquatic contaminant, the latter having a much shorter 'official' DT50 of 120 days. The PMRA also acknowledged the high probability of both surface and groundwater contamination with imidacloprid.

Given some of the data presented below, it appears that regulatory agencies in Canada, the US, and EU were absolutely correct in their early assessment. Yet they proceeded to allow a multitude of labeled registrations under varied agronomic conditions. In 2001, the PMRA stated that they were willing to entertain label extensions provided these new uses were "*in low environmental risk situations or critical need uses in the context of sustainable pest management programs and where*

mitigative measures can be incorporated into product labelling." Unfortunately, imidacloprid is still registered for a wide range of field, horticultural and orchard crops.
In their latest re-evaluation of imidacloprid (EFSA 2008), European Regulators appeared to be trying to 'bend over backwards' to make the compound pass their aquatic triggers. Even after the application of draconian mitigation steps in the water modeling work (e.g. reducing drift by 95%) they were unable to make common uses of the insecticide (e.g. tomatoes) not trigger their criteria for concern. As discussed above, the situation would be worse still if they adopted more realistic toxicity reference levels.

The situation is largely repeated with clothianidin, and to a lesser extent thiamethoxam (see section 1). Regulators fully expected these compounds to have an impact on the aquatic environment.

7.5.2. Empirical data to date

A review of data on groundwater contamination is beyond our scope; however, as of 1997, Bayer was already reporting concentrations of imidacloprid as high as 1 ug/l in California groundwater (Bacey 2003). USEPA (2008a) reported detections ranging from 0.2 to 7 ug/l in New York State. In Quebec, samples from wells in potato-growing areas were reporting levels as high as 6.4 ug/l and detections in 35% of 28 wells sampled (Giroux 2003). Detection of three imidacloprid metabolites was also reported. Data are sparser and just emerging with the other neonicotinoids. Huseth and Groves (2013) reported contamination with thiamethoxam in Wisconsin wells in 2008 and 2009. The levels ranged as high as 9 ug/l with several wells having values above 1 ug/l. **These are levels at which we would expect <u>acute</u> effects on aquatic invertebrates -- this may be totally unprecedented in the history of pesticide registration to have groundwater samples show such a high biological activity to aquatic systems.**

There isn't much empirical data for surface water monitoring for the neonicotinoids. The most comprehensive effort is the recently published data by Starner and Goh (2012) who reported on imidacloprid alone in three irrigated agricultural regions of California (Imperial Valley, Salinas and Santa Maria). They sampled 23 rivers, small creeks or drains. The data are reproduced below (Table 7.11) by sampling site (the original publication listed residues by date). Based on crops grown in the areas, the authors believe that most of the contamination is from the production of lettuce and, to a lesser extent, cole crops and wine grapes.

Table 7.11. Imidacloprid water monitoring results from agricultural watersheds in California (Starner and Goh 2012).

Date	Site	Time	Imidacloprid conc. (ug/L)	Max. for site (ug/l)
May-17-10	27-7	11:45:00	1.02	
June-07-10	27-7	11:45:00	0.544	
April-25-11	27-7	11:45:00	0.581	
June-13-11	27-7	12:00:00	2.09	
July-19-11	27-7	10:15:00	0.157	2.09
May-17-10	27-8	12:30:00	0.443	

June-07-10	27-8	12:30:00	0.626	
April-25-11	27-8	12:15:00	0.372	
May-16-11	27-8	12:10:00	0.787	
June-13-11	27-8	12:45:00	0.44	
July-19-11	27-8	11:00:00	0.635	
August-30-11	27-8	12:00:00	0.35	0.787
April-26-11	27-9	13:30:00	ND	ND
October-05-10	13-10	13:00:00	0.353	
October-11-10	13-10	15:00:00	0.301	0.353
May-17-10	27-10	13:50:00	1.03	
June-07-10	27-10	13:45:00	1.24	
April-25-11	27-10	13:45:00	3.05	
May-16-11	27-10	13:30:00	2.06	
June-13-11	27-10	14:00:00	0.57	
August-30-11	27-10	13:15:00	1.3	3.05
April-26-11	27-11	12:15:00	0.272	
June-14-11	27-11	7:30:00	0.2	
July-19-11	27-11	8:20:00	0.114	
August-30-11	27-11	14:15:00	0.13	0.272
October-05-10	13-22	11:30:00	0.133	
October-11-11	13-22	12:45:00	0.262	0.262
October-11-11	13-23	13:30:00	3.29	3.29
October-11-11	13-24	17:00:00	0.241	0.241
October-05-10	13-25	10:45:00	0.08	
October-11-11	13-25	11:15:00	0.114	0.114
October-05-10	13-56	12:20:00	0.276	0.276
October-11-11	13-56	14:15:00	0.269	
October-05-10	13-69	9:45:00	0.602	
October-11-11	13-69	10:25:00	0.789	0.789
October-11-11	13-71	9:40:00	0.559	0.559
October-11-11	13-73	12:00:00	ND	ND
April-26-11	27-13	14:00:00	ND	ND
May-17-10	27-14	15:50:00	ND	
April-25-11	27-14	15:30:00	ND	
May-16-11	27-14	15:30:00	0.05	
June-13-11	27-14	15:45:00	ND	
July-19-11	27-14	14:20:00	ND	
August-30-11	27-14	15:45:00	ND	0.05
June-14-11	27-50	10:40:00	0.167	0.167
May-17-10	27-66	14:45:00	0.223	
June-07-10	27-66	14:30:00	0.647	
April-25-11	27-66	14:30:00	0.418	
May-16-11	27-66	15:00:00	0.488	

June-13-11	27-66	15:00:00	0.334	
June-14-11	27-66	13:15:00	0.203	
July-18-11	27-66	12:20:00	0.178	
August-30-11	27-66	15:15:00	0.162	0.647
May-17-10	27-70	13:00:00	0.489	
June-07-10	27-70	13:00:00	0.924	
April-25-11	27-70	13:00:00	2.09	
May-16-11	27-70	12:45:00	1.79	
June-13-11	27-70	13:20:00	0.48	
July-19-11	27-70	11:55:00	1.03	
August-30-11	27-70	12:30:00	0.45	2.09
June-08-10	40-13	15:15:00	0.544	
August-31-11	40-13	13:45:00	0.578	0.578
June-08-10	42-48	13:00:00	0.723	
August-31-11	42-48	12:00:00	1.24	1.24
June-08-10	42-49	13:40:00	0.168	0.168
June-08-10	42-50	12:15:00	0.938	
June-08-10	42-50	14:15:00	0.876	
May-17-11	42-50	10:45:00	1.11	
May-17-11	42-50	11:45:00	1.18	
May-17-11	42-50	12:45:00	1.38	
May-17-11	42-50	13:45:00	1.26	
May-17-11	42-50	14:15:00	1.21	
August-31-11	42-50	11:10:00	0.984	
August-31-11	42-50	12:30:00	0.842	
August-31-11	42-50	14:20:00	0.878	1.38

Most of the samples are above any reasonably set reference level for acute effects and at least an order of magnitude higher than a chronic effect level (see tables 7.5 and 7.6). **Yet, most remarkable is the fact that on sites where multiple samples were taken, concentrations remain consistently high and often above acute impact levels throughout the entire season.** Having rearranged the data by site makes this easy to see (Table 7.11). This is exactly what we would expect from a compound either used repeatedly throughout the growing period or a compound with very high persistence being gradually released to the aquatic environment after any rain or irrigation period. It is notable that grab samples such as these never reveal true maxima (by chance alone, how could a grab sample find the maximum?) so the situation (already looking very bleak) is worse than depicted. As the authors point out, a true picture would require that other neonicotinoids as well as the many imidacloprid degradates be measured as well.

Hladik and Calhoun (2012), in a methods-oriented report for the USGS, provide data on two Georgia streams: Scope creek and the Chattahoochee River sampled between October 2011 and April 2012. A full interpretation of the results will be given in a later publication for this ongoing sampling effort, but Scope creek was described as being primarily urban. On this site, imidacloprid was detected in 86% of the samples at concentrations ranging from 4.5 (essentially the detection limit) to

35.3 ng/l. The Chattahoochee River drains a much larger area of mixed forest, urban and agricultural areas. Imidacloprid was detected in 60% of the samples at concentrations ranging from 3.4 to 10.1 ng/l. The other neonicotinoid insecticides acetamiprid, clothianidin, thiacloprid and thiamethoxam were not detected at detection limits ranging from 3.6 to 6 ng/l.

An earlier effort (2007-2008) to sample imidacloprid in drinking water supplies (Smith 2011) had revealed that imidacloprid was one of the more frequently detected pesticides from the Hobbs and Stony Brook basins in Massachusetts. Most samples were composites taken during storm flow. One of the 5 sampling stations on the reservoir tributaries recorded imidacloprid in all of the base flow samples and in 83% of the storm flow samples (detection limit of 0.06 ug/l) with a maximum detected level estimated to be 1.21 ug/l.

These last two sampling efforts highlight the fact that, because of the use of imidacloprid on turf and ornamentals, we cannot discount urban areas as sources of aquatic contamination.

In its review of imidacloprid in Canada, the CCME (2007) reported the results of early monitoring efforts by Environment Canada to assess runoff from potato fields in Eastern Canada (Table 7.12). These results were inconsistent, with early detections reaching as high as 11.9 ug/l but later samples showing either lower or no residues. Detection limits were often high, however, meaning that the frequency of detection was consistently underestimated.

Table 7.12. Summary of early sampling for imidacloprid by Environment Canada in runoff and surface waters in proximity to potato fields. Based on unpublished reports reviewed by CCME 2007.

Location	Year	Type of sample	No. samples	Detection limit (ug/l)	No. positive	Highest level detected (ug/l)	Source cited in CCME (2007)
Prince Edward Island	2001-2002	Runoff		0.5		11.9	Denning 2004
Prince Edward Island	2003-2004	Runoff	45	1.0	0		Murphy and Mutch 2005
New Brunswick	2003-2004	Runoff	42	2.0	0		Murphy and Mutch 2005
Nova scotia	2003-2004	Runoff	18	2.0	0		Murphy and Mutch 2005
Prince Edward Island	2003-2005	Surface waters (stream)	82	0.2	0		Murphy et al. 2006
Nova scotia	2003-2005	Surface waters (stream)	48	0.2	0		Murphy et al. 2006
New Brunswick	2003-2005	Surface waters (stream)	57	0.2	2	0.3	Murphy et al. 2006
New Brunswick	2003-2005	Runoff and surface water – single site				0.3	Hewitt 2006

The first effort to look for a wider suite of neonicotinoids in Canada was in the Fall (October 4-15) of 2011. For seed treatment uses, this would be 5-6 months after application. Single samples were

taken from streams in southern Ontario draining either urban or rural areas, the latter being either orchard-dominated or field-crop dominated. The data are summarised in Table 7.13.[23]

Honeybees by Jessica Lawrence, Eurofins Agroscience Services, Bugwood.org

[23] We are indebted to the following individuals for use of their unpublished data: John Struger and John Kraft, Environment Canada Water Quality Monitoring and Surveillance (WQM&S) – Ontario; and Josey Grabuski, Steve Cagampan and Ed Sverko, Environment Canada National Laboratory for Environmental Testing (NLET) – Burlington.

Table 7.13. Environment Canada surveillance data for neonicotinoid insecticides; Fall 2011.

		URBAN OR TURF SITES							
	Minimum Detection Limit (ng/L)	**taylor 4-11**	**indian 5-11**	**highland 5-11**	**credit 5-11**	**mimico 5-11**	**spencer 4-11**	**kossuth 5-11**	**indian 13-11**
Analytes		**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**
clothianidin	**1.76**	nd	nd	nd	nd	nd	<MDL	<MDL	nd
thiacloprid	**0.49**	nd	nd	nd	nd	nd	nd	nd	nd
thiamethoxam	**1.39**	nd	nd	nd	nd	nd	<MDL	<MDL	nd
imidacloprid	**1.28**	2.13	4.72	<MDL	1.66	nd	nd	1.31	3.49
dinotefuran	**3.28**	nd	nd	nd	nd	nd	nd	nd	nd
TOTAL		**2.13**	**4.72**	**0.00**	**1.66**	**0.00**	**0.00**	**1.31**	**3.49**

		ROW CROPS					POTATOES PRIMARILY			VINES AND ORCHARDS
	Minimum Detection Limit (ng/L)	**welland 4-11**	**20bailey 4-11**	**20westbrk 4-11**	**LThames 5-11**	**LGrand 5-11**	**innisfil 13-11**	**nott-baxter 13-11**	**nott-SR10 13-11**	**vineland 4-11**
Analytes		**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**	**ng/L**
clothianidin	**1.76**	2.22	32.6	nd	19.9	7.52	nd	<MDL	nd	34.8
thiacloprid	**0.49**	nd	nd	<MDL	nd	nd	nd	nd	nd	3.49
thiamethoxam	**1.39**	6.46	174	nd	7.87	2.11	6.13	1.75	1.78	<MDL
imidacloprid	**1.28**	nd	26.9	nd	6.14	6.56	6.03	4.63	2.95	9.02
dinotefuran	**3.28**	nd	nd	nd	nd	nd	nd	nd	nd	nd
TOTAL	**8.20**	**8.68**	**233.00**	**0.00**	**33.90**	**16.20**	**12.20**	**6.38**	**4.73**	**47.30**

Although the highest reported value (0.23 ug/l total neonicotinoids) from a row crop site is much lower than imidacloprid concentrations reported by Starner and Goh (2012) for California, we need to remember that these values represent water concentrations 5-6 months after use, at least in the case of the thiamethoxam seed treatment likely responsible for the highest level recorded downstream from the Ontario corn/soy field. This clearly puts us into the concentration range where chronic effects are likely. Water collections were extended to the full summer in 2012 (J. Struger, pers. comm.) but these data are not yet available. It is interesting to see that detected residues follow predicted use patterns: urban and turf sites showing primarily imidacloprid; agricultural sites showing a mixture of the three main products.

Huseth and Groves (2013) analysed leachate samples (collected at a depth of 75 cm) following the use of thiamethoxam. The insecticides had been applied to potato seed pieces before planting or applied as a foliar spray afterwards. For one of the two years of the study, leachate concentrations averaged between 10-15 ug/l regardless of application method; in the following year they averaged approximately 5 ug/l.

The most worrisome analysis is that of Van Dijk (2010) for the Netherlands. Based on national monitoring data for water analyses from 1998 to 2007, she reports that imidacloprid was detected as high as 325 ug/l[24] with the bulk of detections falling between 0.013 and 1.6 ug/l[25]. She was able to match these monitoring data to aquatic invertebrate species abundance data (another national monitoring scheme in the Netherlands). She was able to see a clear inverse relationship between imidacloprid residues and the abundance of diptera. Non-significant differences were also seen in coleopteran, amphipoda and odonata. To be fair, a few positive relationships were also seen (especially hydracarina), suggesting that some species might be more affected than others and that imidacloprid may be affecting the relative competing ability of different taxa. This is well known from mesocosm work where insensitive taxa can exhibit large increases as a result of release from competition or predation.

Unfortunately, Van Dijk (2010) could not assess whether neonicotinoids had resulted in temporal changes in invertebrate abundance over the decades of use because, even in the Netherlands, the historical data proved inadequate to the task.

The special case of prairie potholes

[24] This is based on the author reporting that the highest concentration detected was 25,000 times the older Dutch reference value of 13 ng/l.
[25] In the Netherlands, major uses of imidacloprid include flower bulbs – a large industry in that country – as well as potatoes and chicory.

The case has long been made that pesticide application to, or runoff into, small prairie wetlands (sloughs) could have disastrous consequences on waterfowl as well as other aquatic bird species that depend on the rich supply of invertebrates for egg production as well as chick growth and development (Mineau et al. 1987; Sheehan et al. 1987, 1995). The agricultural areas of the prairies are critical to North American waterfowl populations. Euliss and Mushet (1999) sampled wetlands in cropland and grassland in North Dakota and confirmed that wetlands in cropland areas were much more likely to be devoid of cladocera and have reduced numbers of key invertebrate species recognised as waterfowl food.

Morrissey and Main (2010) concluded that the highest intensity of neonicotinoid use in the Canadian prairies overlaps directly with areas of high wetland density. Sediment and macroinvertebrate collections as well as a tree swallow nest box study are underway. In June of 2012, they also sampled a number of wetlands. In all, 63% of their samples were positive, with the following maxima being reported: imidacloprid 0.19 ug/L, thiamethoxam 1.1 ug/L, clothianidin 2.3 ug/L, acetamiprid 0.044 ug/L[26]. Seed treatments in canola were the principal sources.

8. Putting it together: Next steps

8.1. Priority research directions

A rigorous analysis of avian trends in North America, and attempts to link these to neonicotinoid uses, is beyond what we can accomplish in this review. As discussed in section 3, avian declines are likely to be multi-faceted and respond to many factors, both here and on their wintering grounds. The analysis will not be simple.

As discussed more fully in the section below, we believe that it is essential to design biochemical assays that will allow diagnosis of poisonings in wildlife. It is also critical to assess the potential of neonicotinoids to affect avian reproduction given the laboratory evidence to date. The hypothesis that neonicotinoid exposure might result in increased vulnerability of wildlife to pathogens deserves further investigation, as well.

It is clear that we are witnessing contamination of the aquatic environment at levels that will affect aquatic food chains. This has a clear potential to affect consumers of those aquatic resources, be they birds, fish or amphibians. Based on this review, a few priority avenues of research are indicated:

- For population trend analyses, we recommend expanding the assessment from farmland/grassland species (the usual place where researchers start looking for pesticide impacts) to those species known to be more reliant

[26] We are indebted to Dr. Christy Morrissey and her research team at the University of Saskatchewan for these early (preliminary) results.

on the aquatic or emergent insect food supply even if further from cropland.

- The case of prairie potholes was mentioned above. Because these are static water bodies often surrounded by field crops, they are the ideal testing ground for looking at aquatic impacts resulting from the use of neonicotinoids in cereal and oilseed crops.

- Aerial insectivores, as a group, are currently experiencing widespread population declines. Their dependence on emergent insects is well known; seeing whether population declines can be linked to the increased contamination of aquatic systems with neonicotinoids may be a worthwhile direction, especially in light of the existing work linking poor reproductive success in some species with prey reduction following mosquito control operations.

Any analysis will need to refer to pesticide use statistics. This will be difficult to carry out in Canada because these data are not collected. However, some simplifying assumptions can be made as to the increasing popularity of the neonicotinoid seed treatments since their introduction in the early 2000s. For US-based analyses, information on pesticide use does allow for enquiries into the role of pesticides in bird declines (Mineau end Whiteside, 2013). However, we do not believe current USDA pesticide surveys cover seed treatment chemicals if applied to the seed by commercial seed treatment operations. This is a serious knowledge gap.

8.2. Needed changes to the regulatory system

This review has shown how current regulatory procedures are inconsistent, scientifically outmoded, and prone to the vagaries of chance. There is a significant disconnect between the red flags raised by scientists who evaluate the neonicotinoid ingredients and the risk managers who approve the neonicotinoid product registrations. This problem has been raised previously in the context of the lethal impact of insecticides to birds (Mineau, 2004).

Simply put, EPA has not been heeding the warnings of its own toxicologists. Internal Agency reviews voice major concerns about neonicotinoid risks, particularly with respect to developmental and reproductive toxicity. Their official cautions would be even more dire if EPA scientists went beyond their antiquated protocols and correctly assessed the full extent of the impacts. For example, risk

assessment methods for birds fail to fully account for the interspecies variation in toxicity, underestimating acute risk by up to 10 fold for the universe of species beyond mallards and bobwhites. As for aquatic invertebrates, EPA has underestimated the toxicity of imidacloprid by over an order of magnitude, because of the Agency's failure to consider data from the peer-reviewed literature. The Agency has grossly underestimated the toxicity of the other neonicotinoids as well, in part due to the Agency's reliance on a test species, *Daphnia magna*, that is uniquely insensitive to neonicotinoids.

Recent studies in the U.S. and Europe have shown that small amounts of neonicotinoids from treated seeds can cause disorientation, suppressed immunity, and early death in honeybees. This report makes clear that birds – critical agents in the control of agricultural pests -- are adversely affected as well. A single seed treated with imidacloprid is enough to kill a blue-jay-sized bird, and less than one corn seed per day treated with any of the neonicotinoid insecticides is sufficient to cause reproductive abnormalities. This is extremely worrisome given the extensive use of neonicotinoids as seed treatments for corn, soy, canola, and increasingly for cereals. As this report shows, unlimited quantities of these treated seeds are readily available to birds while regulators mistakenly assume that exposure can be minimized by label statements or adherence to good agricultural practices.

Neonicotinoid-treated seeds present a lethal risk for the birds that eat them. Yet when a state or county officer receives a report of dead birds or other wildlife, the inspector has no way of determining whether neonicotinoids contributed to the death. There is no readily available biomarker for neonicotinoids as there is for cholinesterase inhibitors such as the organophosphorous pesticides. It is astonishing that EPA would allow a pesticide to be used in hundreds of products without ever requiring the registrant to develop the tools needed to diagnose poisoned wildlife. It would be relatively simple to create a binding assay for the neural receptor which is affected by this class of insecticides.

It is perplexing, as well, that EPA does not require registrants to report any bird kills involving fewer than 200 of a "flocking species," 50 individuals of a songbird species, or 5 raptors. The agency's 1997

revisions to its incident reporting requirements under FIFRA section 6(a)2 essentially place the Agency in a state of enforced ignorance in this regard. These feeble reporting requirements and the failure to require the development of basic biomarkers help keep the government in the dark on a range of pesticide effects on wildlife.

The neonicotinoids are systemic, persistent in soils (and thus prone to accumulation from year to year), and susceptible to runoff and groundwater infiltration. These physical properties and their near-ubiquity in pest control products have led to strikingly high groundwater contamination levels, already beyond the threshold found to kill many aquatic invertebrates. The resulting effects on birds and other organisms are cause for concern. It is clear that these chemicals have the potential to affect entire food chains.

Neonicotinoids have been suspended for some uses in several European countries. The European Commission and the British government are currently taking steps to assess the risks. Meanwhile the U.S. continues to sanction new uses. There is evidence that U.S. regulators historically have waited far too long to impose needed restrictions on toxic insecticides responsible for millions of bird deaths per year (Mineau 2004) and that these chemicals likely contributed to the significant decline of grassland birds in North America (Mineau and Whiteside, 2013). Given the red flags raised by this new class of pesticides, a serious independent review of the neonicotinoids is warranted, one that goes well beyond the effects on honeybees.

The results of this study and others have led American Bird Conservancy and partners in the National Pesticide Reform Coalition to urge the EPA to take the following actions:

- Suspend all applications of neonicotinoids pending independent review of these products' effects on birds, terrestrial and aquatic invertebrates, and other wildlife.
- Expand its re-registration review of neonicotinoids beyond bees to include birds, aquatic invertebrates, and other wildlife.
- Ban the use of neonicotinoids as seed treatments.
- Require that registrants of acutely toxic pesticides develop the tools necessary to diagnose poisoned birds and other wildlife.

Northern Bobwhite by Bill Hubick

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California Quail chick by Precocial, wikimedia.org

Caddisfly by Bruce Marlin, Wikimedia.org

ANNEX 1 – Toxicity of imidacloprid to aquatic invertebrates

Under Form of pesticide: FORM = formulation, TECH = Technical a.i., DEG = degradate. Under study type: A = Acute, C = Chronic. Under exposure type: S = Static, F = Flow through, SR = Static renewal.

Chemical	ai	Form	Taxon1	Taxon2	Common Name	Taxonomic	Age	AGE Class	Study Time (Value)	Study time (Unit)	Study type	Exposure type	Measure	Qualifier for Toxicity	Toxicity (ug/l)	CL (in original units)	Probit slope	Source	Reference	Study Date	Notes
Acetamiprid	Analytical grade	TECH	Crustacea	Amphipoda	Scud	Gammarus pulex			96	h	A	S	LC50		50	30.0-90.0		Original publication	Beketov and Liess 2008b		
Acetamiprid	99	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	S	EC50		49800	46-62	1.48	One liner 2007, EU 2004 review		1998	
Acetamiprid	EXP 60707A (20%)	FORM	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	S	EC50	>	15900			EU 2004 review			
Acetamiprid		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			24	d	C		NOEC (reproduction)		5000			EU 2004 review		1998	
Acetamiprid	99.9	TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 hr	LARVAE	96	h	A	F	LC50		66	0.056-0.082	4.5	One liner 2007		1998	
Acetamiprid		TECH	Insecta		Midge	Chironomus riparius			28	d	C		NOEC (emergence & development)		5			EU 2004 review			
Acetamiprid	Analytical grade	TECH	Insecta		Black fly	Simulium latigonium		LARVAE	96	h	A	S	LC50		3.73	1.54-9.05		Original publication	Beketov and Liess 2008b		
Acetamiprid (IC-0 Metabolite)	99.7	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	SR	EC50	>	95100	N.A.	N.A.	One liner 2007, EU 2004 review		1997	
Acetamiprid (IM-1-2 Metabolite)	99.6	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	SR	EC50	>	99800	N.A.	N.A.	One liner 2007, EU 2004 review		1997	
Acetamiprid (IM-1-4	98.7	DEG	Crustacea	Branchiopo	Water flea	Daphnia magna	<24	LARVAE	48	h	A	SR	EC50		43900	34.8-55.9	3.56	One liner 2007		1997	

Metabolite)				da			hr														
Acetamiprid (IM-1-4 Metabolite)	98.7	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr		48	h	A		EC50		43900	34.8-55.9	3.56	One liner 2007, EU 2004 review		1997	
Acetamiprid (IM-1-4 Metabolite)	99.6	DEG	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 hr	LARVAE	96	h	A	F	LC50		19000	15-24	4.5	One liner 2007		1997	
Acetamiprid (IM-1-4 Metabolite)		DEG	Insecta		Midge	Chironomus riparius			48	h	A		LC50		76000			EU 2004 review			
Clothianidin	97.6	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	S	EC50	>	119000	N.A.		One Liner 2007, EPA 2003 assessment for corn and canola	Palmer 2000	2000	
Clothianidin	97.6	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A		EC50		109523	N.A.		EPA 2003 Fact Sheet		2000	
Clothianidin		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C		NOEC (number of young)		120	N.A.	N.A.	EU 2005 Summary; PMRA 2011			
Clothianidin		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C		NOEC (number of young)		42	N.A.	N.A.	EPA 2003 assessment for corn and canola	Noack et al. 1998	1998	
Clothianidin		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C		LC50		17300	N.A.	N.A.	PMRA 2011			
Clothianidin	97.6	TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 hr	LARVAE	96	h	A	F	LC50		51	0.044-0.057		One Liner 2007, EPA 2003 Fact sheet; PMRA 2011		2000	
Clothianidin		TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia			39	d	C		NOEC (number of young)		5.1		N.R.	EPA 2003 assessment for corn and canola; PMRA 2011	Drottar et al. 2000		Note factor of 10 from acute data. Very different from Daphnia ratio of >2800
Clothianidin		TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia			39	d	C		EC50 (reproduction)		7.6		N.R.	PMRA 2011	Drottar et al. 2000		
Clothianidin		TECH	Insecta		Midge	Chironomus riparius			28	d	C		EC15 (larval emergence)		0.72			EU 2005 Summary; Footprint			Footprint DB gives this as the NOEC

																		DB; PMRA 2011			
Clothianidin		TECH	Insecta		Midge	Chironomus riparius			28	d	C		EC50 (emergence)		1			PMRA 2011			Footprint DB gives this as the NOEC
Clothianidin		TECH	Insecta		Midge	Chironomus riparius			48	h	A		EC50		22			EPA 2003 Fact Sheet, corn and canola assessment	Mattlock 2001		
Clothianidin		TECH	Insecta		Midge	Chironomus riparius			48	h	A		EC50		29			EU 2005 Summary			
Clothianidin		TECH	microcosm			micocosm					C		EAC		3.1			EU 2005 Summary			
Clothianidin MNG metabolite	99	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	S	EC50	>	100800	N.A.	N.A.	EPA 2003 corn and canola assessment.	Hendel 2000	2000	Note that One Liner 2007 refers to this value as TZNG
Clothianidin TNG metabolite	95.1	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	S	EC50	>	115200	N.A.	N.A.	EPA 2003 corn and canola assessment.	Hendel 2000	2000	
Clothianidin TZNG metabolite	99	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	S	EC50		640	N.A.	N.A.	EPA 2003 corn and canola assessment	Hendel 2000	2000	
Clothiniadin MU metabolite		DEG	Insecta		Midge	Chironomus riparius	2-3 D	LARVAE	48	h	A		LC50	>	83600	NA	NA	EPA 2003 corn and canola assessment.			Note possible inconsistency between test species in naming of metabolites
Clothiniadin TMG metabolite	98.2	DEG	Insecta		Midge	Chironomus riparius	2-3 D	LARVAE	672	h	C	S	LC50	<	18	NA	NA	One liner 2005	BAY	1998	
Clothiniadin TZMU metabolite		DEG	Insecta		Midge	Chironomus riparius	2-3 D	LARVAE	48	h	A		LC50	>	102000	NA	NA	EPA 2003 corn and canola assessment.			Note possible inconsistency between test species in naming of metabolites
Clothiniadin TZNG metabolite		DEG	Insecta		Midge	Chironomus riparius	2-3 D	LARVAE	48	h	A		LC50		386	NA	NA	EPA 2003 corn and canola assessmen			Note possible inconsistency between

																		t.			test species in naming of metabolites
Dinotefuran	99.2	TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 Hr		96	h	A	F	LC50		790	0.49-1.0	NA	One Liner 2007		2001	
Dinotefuran			Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 Hr		48	h	A		EC50	>	110600	NA	NA	One Liner 2007		2001	
Dinotefuran	97.26	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 Hr	LARVAE	48	h	A	S	EC50	>	968300	NA	NA	One Liner 2007		2000	
dinotefuran		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna		UNKNOWN	48	h	A		EC50		1000000			FOOTPRINT DB			
Dinotefuran		TECH	Crustacea	Malacostraca	Crayfish	Orconectes nais	NR	UNKNOWN	48	h	A		LC50		4840			Pesticide Manual (14th ed)			
dinotefuran	NR	TECH	Crustacea	Malacostraca	Crayfish	Orconectes nais	NR	UNKNOWN	48	h	A		LC50		7071.067812			PM 2000			
Imidacloprid	96.2	TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	Juv	JUVENILE	96	h	A	F	LC50		38	0.0260-.046	N.R.	One liner 2004, 2007	TES	1990	
Imidacloprid	22.7	FORM	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 hr	LARVAE	96	h	A	S	LC50		159	0.138-0.046	N.R.	One liner 2004, 2007	TES	1992	
Imidacloprid			Crustacea	Malacostraca	Saltwater shrimp	Americamysis bahia			96	h	A		LC50		37			Stoughton et al. 2008	Cox 2001 and Felsot & Ruppert 2002		
Imidacloprid	96.2	TECH	Crustacea	Malacostraca	Saltwater shrimp	Americamysis bahia			96	h	A		LC50		34.1			CCME 2007	Ward 1990b (Bayer)		
Imidacloprid	240 g/L	FORM	Crustacea	Malacostraca	Saltwater shrimp	Americamysis bahia			96	h	A		LC50		36			CCME 2007	Lintott 1992 (Bayer)		
Imidacloprid	96.2	TECH	Crustacea	Malacostraca	Saltwater shrimp	Americamysis bahia			96	h	A		LC50		37.7			CCME 2007	Ward 1990b (Bayer)		
Imidacloprid	>95%	TECH	Crustacea		Brine shrimp	Artemia sp.			48	h	A		LC50		361230	307830-498090	3.47	Original publication	Song et al. 1997		Test at 27 degrees C
Imidacloprid		FORM	Crustacea		Isopod	Asellus aquaticus			48	h	A	S	LC50		8500			Original publication	Lukancic et al. 2010		
Imidacloprid	40.00%	FORM	Crustacea	Cladocera	Cladoceran	Ceriodaphnia dubia			48	h	A	S	LC50		2.07	1.14-3.30	0.78	Original publication	Chen et al. 2009		
Imidacloprid	99.50%	TECH	Crustacea			Chydorus sphaericus			24	h	A	S	EC50 (immobility)		1469	250-8619		Original publication	Sanchez-Bayo and Goka 2006		
Imidacloprid		FORM	Crustacea	Amphipoda	Scud	Hyalella azteca	2-9 d		28	d	C	SR	LC50		7.08	2.95-16.98		Original publication	Stoughton et al. 2008		Observation time extended to 28 d

Imidacloprid	99.50%	TECH	Crustacea			Chydorus sphaericus			48	h	A	S	EC50 (immobility)		832	274-2522		Original publication	Sanchez-Bayo and Goka 2006		
Imidacloprid	99.50%	TECH	Crustacea			Cypretta seurati			24	h	A	S	LC50		732	456-1176		Original publication	Sanchez-Bayo and Goka 2006		Note wide spread between immobility EC50 and LC50
Imidacloprid			Crustacea		Saltwater shrimp	Americamysis bahia			28	d	C		EC50 (body length)		0.3			Stoughton et al. 2008	Cox 2001 and Felsot & Ruppert 2002		
Imidacloprid	99.50%	TECH	Crustacea			Cypretta seurati			24	h	A	S	EC50 (immobility)		46	13-161		Original publication	Sanchez-Bayo and Goka 2006		Note wide spread between immobility EC50 and LC50
Imidacloprid	99.50%	TECH	Crustacea			Cypretta seurati			48	h	A	S	LC50		301	187-485		Original publication	Sanchez-Bayo and Goka 2006		Note wide spread between immobility EC50 and LC50
Imidacloprid	99.50%	TECH	Crustacea			Cypretta seurati			48	h	A	S	EC50 (immobility)		16	7-39		Original publication	Sanchez-Bayo and Goka 2006		Note wide spread between immobility EC50 and LC50
Imidacloprid	99.50%	TECH	Crustacea	Ostracoda		Cypridopsis vidua			24	h	A	S	LC50	>	4000			Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50
Imidacloprid	99.50%	TECH	Crustacea	Ostracoda		Cypridopsis vidua			24	h	A	S	EC50 (immobility)		8	1.3-47		Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50
Imidacloprid	99.50%	TECH	Crustacea	Ostracoda		Cypridopsis vidua			48	h	A	S	LC50		715	365-1400		Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50
Imidacloprid	99.50%	TECH	Crustacea	Ostracoda		Cypridopsis vidua			48	h	A	S	EC50 (immobility)		3	0.5-15		Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50
Imidacloprid		TECH	Crustacea	Branchiopo	Water flea	Daphnia magna			24	h	A		LC50		97900	81.4-127.7		Original publication	Tisler et al. 2009		

				da																	
Imidacloprid		FORM	Crustacea	Branchiopoda	Water flea	Daphnia magna			24	h	A		LC50		38000	32-48		Original publication	Tisler et al. 2009		
Imidacloprid	95.4	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24 hr	LARVAE	48	h	A	S	EC50 (immobility)		85200	75-113	11	One liner 2004, 2007; Pesticide Manual, EPA 2007 assessment	Young 1990 (Bayer)	1990	
Imidacloprid	>95%	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		LC50		17360	12510-30050	1.86	Original publication	Song et al. 1997		Test at 20 degrees C
Imidacloprid		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		LC50		56600	34.4-77.2		Original publication	Tisler et al. 2009		
Imidacloprid		FORM	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		LC50		30000	28-44		Original publication	Tisler et al. 2009		
imidacloprid	NR	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	NR	UNKNOWN	48	h	A		EC50	>	32000			Agritox	Bayer France		
Imidacloprid		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		LC50		64873			Original publication	Sanchez-Bayo 2009		
Imidacloprid	>95%	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		LC50		10440	6970-17710	1.86	Original publication	Song et al. 1997		Test at 27 degrees C
Imidacloprid		FORM	Crustacea		Scud	Gammarus fossarum			48	h	A	S	LC50		800			Original publication	Lukancic et al. 2010		
Imidacloprid	Analytical grade	TECH	Crustacea	Amphipoda	Scud	Gammarus pulex			96	h	A	S	LC50		350	210-570		Original publication	Beketov and Liess 2008b		Drift seen at approx. 1/10 of LC50
Imidacloprid			Crustacea	Amphipoda	Scud	Gammarus roselli		ADULTS	96	h	A		EC50		29			Mohr et al. 2012	R. Boettger, pers. Comm.		
Imidacloprid		TECH	Crustacea	Amphipoda	Scud	Hyalella azteca			48	h	A		EC50		115			EPA 2007 assessment	England & Bucksath 1991 (Bayer)	1991	Stoughton et al. using the same industry source give EC50 of 55
Imidacloprid		TECH	Crustacea	Amphipoda	Scud	Hyalella azteca	2-9 d		96	h	A	S	LC50		65.43			Original publication	Stoughton et al. 2008		
Imidacloprid		FORM	Crustacea	Amphipoda	Scud	Hyalella azteca	2-9 d		96	h	A	S	LC50		17.44			Original publication	Stoughton et al. 2008		
Imidacloprid		FORM	Crustacea	Amphipoda	Scud	Hyalella azteca	2-9 d		96	h	A	S	LC50		9.74	5.56-17.05		Original publication	Stoughton et al. 2008		Observation time extended to 28 d

Imidacloprid		TECH	Crustacea	Amphipoda	Scud	Hyalella azteca			96	h	A		LC50		526			Stoughton et al. 2008	England & Bucksath 1991 (Bayer)	1991	Reporting error? Much higher than 48h value.
Imidacloprid		TECH	Crustacea	Amphipoda	Scud	Gammarus pulex			28	d	C	S	NOEC (swimming behaviour)		64			Draft assessment report from Germany (rapporteur State) 2005			
Imidacloprid	95.9	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C	SR	NOEC (Reproduction)		1800			EPA 2007 assessment, EFSA 2008 Scientific Report	Young 1990 (Bayer)	1990	
Imidacloprid		FORM	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C	SR	NOEC (Reproduction)		5000			Original publication	Jemec et al. 2007		NOEC for protein content of 2500
Imidacloprid		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C	SR	NOEC (Reproduction)		2500			Original publication	Jemec et al. 2007		NOEC for protein content of 1250
Imidacloprid	99.50%	TECH	Crustacea			Ilyocypris dentifera			24	h	A	S	LC50		759	337-1709		Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50
Imidacloprid		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			10	d	C		LC50		9500			Original publication	Sanchez-Bayo 2009		
Imidacloprid	99.50%	TECH	Crustacea			Ilyocypris dentifera			24	h	A	S	EC50 (immobility)		5	1-25		Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50
Imidacloprid	96.2	TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia					C		NOEC (growth and survival)		0.6			EPA 2007 assessment	Ward 1990 (Bayer)	1990	
Imidacloprid	99.50%	TECH	Crustacea			Ilyocypris dentifera			48	h	A	S	LC50		214	98-463		Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50
Imidacloprid	99.50%	TECH	Crustacea			Ilyocypris dentifera			48	h	A	S	EC50 (immobility)		3	0.2-48		Original publication	Sanchez-Bayo and Goka 2006		Note large difference between EC50 and LC50

Imidacloprid	99.50%	TECH	Crustacea		Grass shrimp	Palaemonetes pugio		LARVAE	96	h	A	SR	LC50		308.8	273.6-348.6		Original publication	Key et al. 2007		
Imidacloprid	99.50%	TECH	Crustacea		Grass shrimp	Palaemonetes pugio		ADULTS	96	h	A	SR	LC50		563.5	478.1-664.2		Original publication	Key et al. 2007		
Imidacloprid		TECH	Insecta		Midge	Chironomus tentans		LARVAE	48	h	A		EC50		69			EPA 2007 assessment	Gagliano 1991 (Bayer)	1991	
Imidacloprid		TECH	Insecta		Midge	Chironomus riparius			24	h	A		LC50		55.2			EFSA 2008 Scientific report			
Imidacloprid		TECH	Insecta		Midge	Chironomus riparius			28	d	C	S	EC50 (emergence)		3.11			EFSA 2008 Scientific report			
Imidacloprid	Confidor SL 200	FORM	Insecta		Midge	Chironomus riparius			28	d	C	S	EC50 (emergence)		3.6			EFSA 2008 Scientific report			
Imidacloprid		FORM	Insecta		Mayfly	Epeorus longimanus		LARVAE	24	h	A	S	LC50		2.1			Original publication	Alexander et al. 2007		
Imidacloprid		FORM	Insecta		Mayfly	Epeorus longimanus		LARVAE	96	h	A	S	LC50		0.65			Original publication	Alexander et al. 2007		
Imidacloprid		FORM	Insecta		Midge	Chironomus riparius		LARVAE	96	h	A	S	EC50		12.9			Original publication	Pestana et al. 2009		Anti-predator behavious compromised
Imidacloprid		TECH	Insecta		Midge	Chironomus tentans		LARVAE	96	h	A	S	LC50		5.75			Original publication	Stoughton et al. 2008		
Imidacloprid		FORM	Insecta		Midge	Chironomus tentans		LARVAE	96	h	A	S	LC50		5.4			Original publication	Stoughton et al. 2008		
Imidacloprid		TECH	Insecta		Midge	Chironomus tentans		LARVAE	28	d	C		EC50 (emergence)		0.91	0.73-1.12		Original publication	Stoughton et al. 2008		Observation period extended to 28 d
Imidacloprid		TECH	Insecta		Midge	Chironomus tentans		LARVAE	96	h	A		LC50		10.5			Stoughton et al. 2008	Gagliano 1991 (Bayer)	1991	
Imidacloprid		FORM	Insecta		Mayfly	Heptageniid mayfly'		LARVAE	96	h	A		LC50		3.7			Leblanc et al. 2012	Leblanc et al. 2010 (unpublished thesis)		
Imidacloprid		FORM	Insecta		Midge	Chironomus dilutus		LARVAE	96	h	A		LC50		2.65			Original publication	Leblanc et al. 2012		
Imidacloprid		FORM	Insecta		Midge	Chironomus tentans		LARVAE	10	d	C		LC50		3.17			Stoughton et al. 2008	Gagliano 1991 (Bayer)	1991	
Imidacloprid	>95%	TECH	Insecta		Freshwater mosquito	Aedes aegypti			48	h	A		LC50		44	41-47	4.02	Original publication	Song et al. 1997		Test at 27 degrees C
Imidacloprid	>95%	TEC	Insecta		Freshwat	Aedes aegypti			48	h	A		LC50		45	42-48	4.33	Original	Song et al.		Test at 20

		H			er mosquito													publication	1997		degrees C
Imidacloprid	>95%	TECH	Insecta		Saltwater mosquito	Aedes taeniorhynchus			48	h	A		LC50		13	10.0-16.0	3.63	Original publication	Song et al. 1997		Test at 27 degrees C
Imidacloprid	>98%	TECH	Insecta		Black fly	Simulium vittatum			48	h	A	S	LC50		8.09			Original publication	Overmyer et al. 2005		Geomean from 3 separate tests based on measured concentrations
Imidacloprid	200 g/L	FORM	Insecta			Pteronarcys dorsata		LARVAE	14	d	C		LC50		70.1			van Dijk 2010	Kreutzweizer et al. 2008		Not found with ref provided
Imidacloprid	Analytical grade	TECH	Insecta		Black fly	Simulium latigonium		LARVAE	96	h	A	S	LC50		3.73	1.54-9.05		Original publication	Beketov and Liess 2008b		
Imidacloprid	Analytical grade	TECH	Insecta		Mayfly	Baetis rhodani		LARVAE	48	h	A	S	LC50		8.49	4.45-16.20		Original publication	Beketov and Liess 2008b		Drift seen at approx. 1/10 of LC50
Imidacloprid		TECH	microcosm			microcosm					C	S (2X at 21 d interval)	NOEC		0.6			EFSA 2008 Scientific report			Based on toxicity to chironomids and Batidae
Imidacloprid	20.00%	FORM	Crustacea	Cladocera	Cladoceran	Ceriodaphnia dubia			48	h	A	SR	EC50 (immobility)		572	290-841		Original publication	Hayasaka et al. 2012		
Imidacloprid	20.00%	FORM	Crustacea	Cladocera	Cladoceran	Ceriodaphnia reticulata			48	h	A	SR	EC50 (immobility)		5553	4213-7388		Original publication	Hayasaka et al. 2012		
Imidacloprid	20.00%	FORM	Crustacea	Cladocera	Cladoceran	Daphnia magna			48	h	A	SR	EC50 (immobility)		43265	34302-53592		Original publication	Hayasaka et al. 2012		
Imidacloprid	20.00%	FORM	Crustacea			Daphnia pulex			48	h	A	SR	EC50 (immobility)		36872	28399-48106		Original publication	Hayasaka et al. 2012		
Imidacloprid	20.00%	FORM	Crustacea	Cladocera		Moina macrocopa			48	h	A	SR	EC50 (immobility)		45271	34378-62218		Original publication	Hayasaka et al. 2012		
Imidacloprid	Analytical grade	TECH	Insecta		Caddisfly	Cheumatopsyche brevilineata		LARVAE	48	h	A	S	LC50		6.59			Original publication	Yokoyama et al. 2009		First instar results (most sensitive). Geomean of two populations
Imidacloprid		FORM	Insecta		Midge	Chironomus riparius		LARVAE	48	h	A	S	EC50		19.9			Leblanc et al. 2012	Azevedo-Pereira et al. 2011		
Imidacloprid-5-hydroxy		DEG	Insecta		Midge	Chironomus riparius			24	h	A		LC50		668			EFSA 2008 Scientific report			
Imidacloprid-AMCP		DEG	Insecta		Midge	Chironomus riparius			28	d	C	S	EC50 (emergence)	>	105000			EFSA 2008 Scientific report			

Imidacloprid-desnitro		DEG	Insecta		Midge	Chironomus riparius			28	d	C	S	EC50 (emergence)		46000			EFSA 2008 Scientific report			
Imidacloprid-desnitro-olefine		DEG	Insecta		Midge	Chironomus riparius			28	d	C	S	EC50 (emergence)		21300			EFSA 2008 Scientific report			
Imidacloprid-nitroso		DEG	Insecta		Midge	Chironomus riparius			24	h	A		LC50		283			EFSA 2008 Scientific report			
Imidacloprid-urea		DEG	Insecta		Midge	Chironomus riparius			28	d	C	S	EC50 (emergence)		73600			EFSA 2008 Scientific report			
Thiacloprid	97.5	TECH	Insecta		Midge	Chironomus riparius	1st in	LARVAE	672	h	C	S	EC50		1.8	0.0016 -0.002	NR	One liner 2005	BCA	1996	
Thiacloprid	44SC	FORM	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 hr	LARVAE	96	h	A	F	LC50		50	0.039-0.064	NR	One liner 2007	WLI	1997	
Thiacloprid	99.3	TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 hr		96	h	A	F	LC50		31	0.027-0.037	5.06	One Liner 2007		1996	
Thiacloprid	97.2	TECH	Crustacea	Amphipoda	Scud	Hyalella azteca	14-21		96	h	A	S	LC50		37	0.03-0.05	2.62	One Liner 2007		1996	
Thiacloprid	97.2	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	1st in	LARVAE	48	h	A	S	EC50		22520	19.24-26.	3.94	One Liner 2007		1995	
Thiacloprid		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		EC50		85100			Footprint DB			
Thiacloprid		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C		NOEC		580			Footprint DB			
Thiacloprid		FORM	Crustacea	Branchiopoda	Water flea	Daphnia magna			24	h	A	S	LC50		4100			Original publication	Beketov and Liess 2008a		Extended post treatment observation period - evidence of delayed effects
Thiacloprid		FORM	Crustacea		Isopod	Asellus aquaticus			24	h	A	S	LC50		153			Original publication	Beketov and Liess 2008a		Extended post treatment observation period - evidence of delayed effects
Thiacloprid		FORM	Crustacea	Amphipoda	Scud	Gammarus pulex			24	h	A	S	LC50		190			Original publication	Beketov and Liess 2008a		Extended post treatment observation

																					period - evidence of delayed effects
Thiacloprid		FORM	Insecta		Dragon fly	Sympetrum striolatum		LARVAE	24	h	A	S	LC50		31.19			Original publication	Beketov and Liess 2008a		Extended post treatment observation period - evidence of delayed effects
Thiacloprid		FORM	Insecta		Caddisfly	Notidobia ciliaris		LARVAE	24	h	A	S	LC50		6.78			Original publication	Beketov and Liess 2008a		Extended post treatment observation period - evidence of delayed effects
Thiacloprid		FORM	Insecta		Black fly	Simulium latigonium		LARVAE	24	h	A	S	LC50		5.47			Original publication	Beketov and Liess 2008a		Extended post treatment observation period - evidence of delayed effects
Thiacloprid		FORM	Insecta		Mosquito	Culex pipiens		LARVAE	24	h	A	S	LC50		5.76			Original publication	Beketov and Liess 2008a		Extended post treatment observation period - evidence of delayed effects
Thiacloprid		FORM	Insecta		Midge	Chironomus tepperi		LARVAE	24	h	A		LC50		1.58			Beketov and Liess 2014	Stevens et al. 2005		
Thiacloprid	Analytical grade	TECH	Crustacea	Amphipoda	Scud	Gammarus pulex			96	h	A	S	LC50		350	210-570		Original publication	Beketov and Liess 2008b		
Thiacloprid	Analytical grade	TECH	Insecta		Mayfly	Baetis rhodani		LARVAE	96	h	A	S	LC50		4.6	3.74-5.66		Original publication	Beketov and Liess 2008b		
Thiacloprid (Metabolite)	97.4	DEG	Crustacea	Amphipoda	Scud	Hyalella azteca	14-21	UNKNOWN	96	h	A	S	LC50		31180	20.37-77.	1.147	One liner 2005	BCA	1997	
Thiacloprid (Sulfonic Acid metabolite)	89.9	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna	1st in	LARVAE	48	h	A	S	LC50	>	96100	NA	NA	One Liner 2007		1995	
Thiamethoxam	98.6	TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna	<24	LARVAE	48	h	A	S	EC50	>	106000	N.A.	N.A.	One liner 2007	NCP	1996	

							hr														
Thiamethoxam	99.2	TECH	Crustacea	Malacostraca	Opossum shrimp	Americamysis bahia	<24 hr	LARVAE	96	h	A	F	LC50		6900	5.8-8.4	3.8	One liner 2007	WLI	1997	
Thiamethoxam		TECH	Crustacea	Ostracoda	seed shrimp	Chaoborus sp.			48	h	A		EC50		180			EU 2006 review			
Thiamethoxam		TECH	Insecta			Cloeon sp.			48	h	A		EC50		14			EU 2006 review			
Thiamethoxam	WG25	FORM	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		EC50	>	25000	N.A.	N.A.	EU 2006 review			
Thiamethoxam	98.6	FORM	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		EC50		27300	N.A.	N.A.	EU 2006 review			
Thiamethoxam		TECH	Crustacea	Branchiopoda	Water flea	Daphnia magna			21	d	C		NOEC		100000			EU 2006 review			
Thiamethoxam		TECH	Insecta		Midge	Chironomus riparius			30	d	C		NOEC (emergence)		10			EU 2006 review			PMRA 2007 gives the chronic EC50 as 11
Thiamethoxam		TECH	Insecta		Midge	Chironomus riparius				h	A		EC50		35			PMRA 2007			Given as acute endpoint for exposure period not stated
Thiamethoxam		TECH	Crustacea			Americamysis bahia			96	h	A		EC50		5400			PMRA 2007			Given as acute endpoint for exposure period not stated. Assumed to be 96 h based on US info from same test
Thiamethoxam CGA322704		DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		EC50	>	100000			EU 2006 review			
Thiamethoxam CGA322704		DEG	Insecta		Midge	Chironomus riparius			28	d	C		NOEC (emergence)		0.67			EU 2006 review			
Thiamethoxam CGA355190		DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		EC50	>	100000			EU 2006 review			
Thiamethoxam NOA407475		DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		EC50		82900			EU 2006 review			
Thiamethoxam NOA407475		DEG	Insecta		Midge	Chironomus riparius			28	d	C		NOEC (emergence & development)	>	1000						

Thiamethoxam NOA459602	99	DEG	Crustacea	Branchiopoda	Water flea	Daphnia magna			48	h	A		EC50	>	120000			EU 2006 review			
Thiamethoxam NOA459602		DEG	Insecta		Midge	Chironomus riparius			28	d	C		NOEC (development)		50000						

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