CANADIAN WILDLIFE FEDERATION

NEONICOTINOID INSECTICIDES AND BATS

An assessment of the direct and indirect risks

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Photo credit: Sherri and Brock Fenton

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List of Acronyms

ai	Active ingredient. When used with an application rate, refers to the quantity of 'pure' (technical grade purity) pesticide applied, calculated from the concentration of 'pure' material in the numerous pesticide formulations.
Bt	Bacillus thuringiensis
BW	Body weight
CESCC	Canadian Endangered Species Conservation Council
CaDPR	California Department of Pesticide Regulation
Cal EPA	California Environmental Protection Agency
DNA	Deoxyribonucleic acid
EC	European Commission
ECOFRAM	Ecological Committee on FIFRA Risk Assessment Methods
EFSA	European Food Safety Authority
EU	European Union
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (US)
FMR	Field Metabolic Rate
IUCN	International Union for the Conservation of Nature
LD50	Lethal Dose 50; the median lethal dose; the dose required to kill half the members of a tested population after a specified test duration.
LOAEL	Lowest Observed Adverse Effect Level; lowest dose at which there was an observed toxic or adverse effect.
mRNA	Messenger Ribonucleic Acid
NOAEL	No Observed Adverse Effect Level; highest dose at which there was not an observed toxic or adverse effect
OMAFRA	Ontario Ministry of Agriculture Food and Rural Affairs
OP	Organophosphates
PMRA	Canadian Pest Management Regulatory Agency
ppm	Parts per million
RQ	Risk Quotient. The ratio of a point estimate of exposure and a point estimate of effect. Exposure is calculated from estimated environmental concentrations on foodstuffs as well as feeding rates. Toxicity refers to an effect level obtained from toxicity testing (e.g. LD50 or NOAEL).
RUD	Residue per Unit Dose. Residue levels (usually on plant or insect prey) standardised to a common pesticide application rate, usually 1 lb a.i./acre (US) or 1 kg a.i./hectare (Canada, Europe)
SARA	Species at Risk Act

TIM	Terrestrial Investigation Model (USEPA)
TRex	Terrestrial Residue EXposure (T-REX) model; USEPA program to calculate risk (RQs) to wild birds and mammals from residues on avian and mammalian food items given single or multiple applications of a pesticide.
UK	United Kingdom
US	United States of America
USEPA	US Environmental Protection Agency
WIA	Worldwide Integrated Assessment on the Impact of Systemic Pesticides on Biodiversity and Ecosystems; convened under the auspices of the Species Survival Commission and the Commission on Ecosystem Management of the IUCN.

Tri-coloured bat, one of three bat species listed as Endangered in Canada. Photo credit: Sherri and Brock Fenton.

Introduction

A major shift has taken place in agriculture, specifically in the way we carry out pest control. The last two decades have seen an unprecedented rise in the use of systemic insecticides – products that permeate every part of plants (either crop or non-crop species in field borders), including leaves, stems, roots, pollen, and nectar. The use of these products differs from other pesticides, in that they are being used on a prophylactic basis, whether the pest population has reached a threshold of damage or not. This is particularly true for crops grown from treated seed.

The largest class of systemic insecticides are the neonicotinoid insecticides (neonics for short). They are the most widely used insecticides in the world. The case has been made that the ubiquitous use of neonicotinoids, their high invertebrate toxicity and resulting wide-scale contamination of surface waters (because of their physico-chemical characteristics) could lead to a depletion of invertebrate food sources generally. This depletion of invertebrates could potentially extend far beyond the farm fields to entire watersheds (Mineau and Palmer 2013; Morrissey *et al.* 2015). A depletion of insect prey due to widespread and systemic use of pesticides such as neonics could impact insectivores such as bats.

Although the acute toxicity of neonicotinoid insecticides to vertebrate species is far less than that of some of its predecessors (e.g. Organophosphates (OP) and carbamate insecticides) the systemic nature of the products, their long persistence in soil, and their overuse mean that exposure could be much more prolonged. This raises questions about the possible effects of sub-chronic and chronic pesticide exposure – questions that were seldom raised with older, shorter-lived products.

As comprehensively reviewed by Jones *et al.* (2009), bats appear to be declining worldwide. This is worrisome because for centuries bats were diverse and abundant globally and they provide key ecosystem services, such as insect control in agricultural regions. A number of possible factors for these declines were reviewed by Jones *et al.* (2009), including climate change and weather extremes, habitat and landscape changes such as deforestation and agricultural intensification (e.g. loss of hedgerows and increased use of pesticides), contaminants, hunting, and disease. These authors argue that bats are ideal bio-indicators of environmental quality.

In scoping what would and would not be feasible for this review, it became clear that attempting to match geographically explicit population trends to agricultural stressors such as pesticide use was not within the realm of possibilities. Bats are difficult to census and very few bat population assessments have been carried out and only for the most common species. Fortunately, censusing methods such as bio-acoustical recording have greatly expanded the scope of possible research and, as governments and conservation groups become more organised (e.g. Loeb *et al.* 2015) this limitation may eventually disappear, but probably not for many years.

Although there are many threats to bat populations globally, a fungal disease known as Pseudogymnoascus destructans (formerly Geomyces destructans) or white-nose syndrome has dominated the conservation agenda as well as the public consciousness. In the past decade, approximately 12 million bats in the United States and Canada have died from white-nose syndrome. It was discovered in North America in 2006 and was most likely spread by human activity between Europe and North America. White-nose syndrome has devastated populations of the Little Brown Myotis (Myotis lucifugus), once the most common bat in North America, now listed as Endangered in Canada. Two other bat species have been listed as Endangered in Canada under the Species at Risk Act due to white-nose syndrome: Northern Myotis (Myotis septentrionalis) and Tri-coloured Bat (Perimyotis subflavus).

Eskew and Todd (2013) drew a parallel between the impacts of white-nose syndrome on bats and another dermatophytic fungus currently advancing through North America – the chytrid fungus Batratrachochytrium dentrobatidis in frogs. Both fungal pathogens appear to be recent introductions that are host-generalists and which appear to have abiotic reservoirs that ensure their environmental persistence outside of a host. These authors point to life-cycle characteristics in both bats and amphibians that encourage infectivity, namely seasonally-high densities and rates of contact as well as depressed immune function brought about by hibernation.

Rather than seeing the two fungal diseases as a mere temporal coincidence, Mason *et al.* (2013) hypothesized that neonicotinoid insecticides might be at the root of these disease problems through their effects on immune function. In their provocative paper, they pointed also to other recent infective agents, namely ranaviruses in frogs as well as Mycoplasma gallisepticum, Trichomonas gallinae, Suttonella ornithocola and other pathogens newly found in North American and European songbird species. They based their case on the temporal coincidence of these parasitic diseases with the concomitant explosion in the use of neonicotinoid insecticides, the geographical correspondence between the first documented chytrid and ranavirus infections in California with the presence of agricultural lands upwind of infected regions, and the finding of an association between fish ectoparasites and imidacloprid use in rice paddies (Sanchez-Bayo and Goka 2006). Bayer Corp. reported that imidacloprid, the first neonicotinoid insecticide, made termites more susceptible to pathogenic soil fungi – although the mechanism there was purported to be through a disruption in grooming behaviour rather than through an impact on their immune system (Bayer undated). In addition, several researchers have now shown experimentally that neonicotinoids at sub lethal dose can disrupt immune function in bees (e.g. Alaux *et al.* 2010; Videau *et al.* 2011; Pettis 2012, Di Prisco *et al.* 2013, Aufauvre *et al.* 2014). Sanchez-Bayo *et al.* (2016a) and Pamminger *et al.* (2018) provide comprehensive reviews. Finally, there is some indication that, mechanistically at least, neonicotinoids can alter immune function in mammals. This will be reviewed in more detail below.

Another contemporaneous review by Quarles (2013) independently made the suggestion that current in-use insecticides and other environmental contaminants, either directly or indirectly (through food shortages and its consequences on immune function) might have predisposed bat populations to the current epidemic of white-nose syndrome.

The purpose of this review is to examine the various lines of evidence that the new systemic insecticides may be putting Canadian bats at risk. Specifically, this review will investigate the risk and magnitude of neonicotinoid exposure for bats foraging in agricultural landscapes, and assess the resulting direct risk to bats by constructing a formal risk assessment following an exhaustive review of what is known currently about the mammalian toxicology of neonics. The indirect effects of neonicotinoid insecticides in bats will also be explored by reviewing the case for insect declines and the probable involvement of neonicotinoid insecticides in these declines. Section 2 presents details of bat biology that are relevant to the risk assessment; Section 3 presents evidence for indirect effects of neonicotinoids on bats, and; Section 4 provides an assessment of the direct impacts of neonicotinoids on bats. Section 4 is further partitioned into a summary of known impacts of pesticides on bats and the presentation of a framework for a bat-centric assessment for the five neonicotinoid pesticides registered in Canada.

2. Aspects of Bat Biology Relevant to this Review

All Canadian bats are strictly insectivorous. General physiological features of various bat species occurring in Canada will be sufficient to explore potential impacts of neonicotinoid insecticides on bats. Based on Loeb *et al.* (2015), there are 19 species in Canada, including six species listed as a species At Risk under the federal Species at Rick Act (Table 2.1).

Table 2.1. North American bat species and their conservation status (Modified after Loeb *et al.* 2015)

Scientific name	Common name	Presence in Canada	Status in Canada	Presence in the U.S.	Status in the U.S.	Presence in Mexico	Status in Mexico
Antrozous pallidus	Pallid Bat	+	SARA Threatened	+	—	+	—
Artibeus jamaicensis	Jamaican Fruit-eating Bat	-	—	+	—	+	—
Choeronycteris mexicana	Mexican Long-tongued Bat	-	—	+	—	+	Threatened
Corynorhinus rafinesquii	Rafinesque's Big-eared Bat	-	—	+	—	-	—
Corynorhinus townsendii	Townsend's Big-eared Bat	+	CESCC Sensitive	+	C. t. ingens and C. t. virginianus Endangered	+	—
Eptesicus fuscus	Big Brown Bat	+	CESCC Secure	+	—	+	—
Euderma maculatum	Spotted Bat	+	SARA Special Concern	+	—	+	Special Protection
Eumops floridanus	Florida Bonneted Bat	-	—	+	Endangered	-	—
Eumops perotis	Greater Bonneted Bat	-	—	+	—	+	—
Eumops underwoodii	Underwood's Bonneted Bat	-	—	+	—	+	—
Idionycteris phyllotis	Allen's Big-eared Bat	-	—	+	—	+	—
Lasionycteris noctivagans	Silver-haired Bat	+	CESCC Secure	+	—	+	Special Concern
Lasiurus blossevillii	Western Red Bat	-b	—	+	—	+	—
Lasiurus borealis	Eastern Red Bat	+	CESCC Secure	+	—	+	—
Lasiurus cinereus	Hoary Bat	+	CESCC Secure	+	L. c. semotus Endangered	+	—
Lasiurus ega	Southern Yellow Bat	-	—	+	—	+	—
Lasiurus intermedius	Northern Yellow Bat	-	—	+	—	+	—

Lasiurus seminolus	Seminole Bat	-	—	+	—	-	—
Lasiurus xanthinus	Western Yellow Bat	-	—	+	—	+	—
Leptonycteris nivalis	Mexican Long-nosed Bat	-	—	+	Endangered	+	Threatened
Leptonycteris yerbabuenae	Lesser Long-nosed Bat	-	—	+	Endangered	+	Threatened
Macrotus californicus	California Leaf-nosed Bat	-	—	+	—	+	—
Molossus molossus	Pallas' Mastiff Bat	-	—	+	—	+	—
Mormoops megalophylla	Peter's Ghost-faced Bat	-	—	+	—	+	—
Myotis auriculus	Southwestern Myotis	-	—	+	—	+	—
Myotis austroriparius	Southeastern Myotis	-	—	+	—	-	—
Myotis californicus	California Myotis	+	CESCC Secure	+	—	+	—
Myotis ciliolabrum	Western Small-footed Myotis	+	CESCC Secure	+	—	+	—
Myotis evotis	Long-eared Myotis	+	CESCC Secure	+	—	+	—
Myotis grisescens	Gray Myotis	-	—	+	Endangered	-	—
Myotis keenii	Keen's Myotis	+	CESCC May be at Risk	+	—	-	—
Myotis leibii	Eastern Small-footed Myotis	+	CESCC May be at Risk	+	—	-	—
Myotis lucifugus	Little Brown Myotis	+	SARA Endangered	+	—	-	—
Myotis melanorhinus	Dark-nosed Small-footed Myotis	+	Not assessed	+	—	+	—
Myotis occultus	Arizona Myotis	-	—	+	—	+	—
Myotis septentrionalis	Northern Myotis	+	SARA Endangered	+	Threatened	-	—
Myotis sodalis	Indiana Myotis	-	—	+	Endangered	-	—
Myotis thysanodes	Fringed Myotis	+	CESCC May be at Risk; SARA Special Concern	+	—	+	—
Myotis velifer	Cave Myotis	-	—	+	—	+	—
Myotis volans	Long-legged Myotis	+	CESCC Secure	+	—	+	—
Myotis yumanensis	Yuma Myotis	+	CESCC Secure	+	—	+	—
Nycticeius humeralis	Evening Bat	-	—	+	—	+	—

Nyctinomops femorosaccus	Pocketed Free-tailed Bat	-	—	+	—	+	—
Nyctinomops macrotis	Big Free-tailed Bat	-	—	+	—	+	—
Parastrellus hesperus	Canyon Bat	-	—	+	—	+	—
Perimyotis subflavus	Tri-colored Bat	+	SARA Endangered	+	—	+	—
Tadarida brasiliensis	Brazilian Free-tailed Bat	+c	—	+	—	+	—

+ = Species present, - = Species not present, — = No special status.

Note: Scientific and common names follow Wilson and Reeder (2005, Mammal species of the world. 3d ed. Baltimore: Johns Hopkins University Press. 2 vol.) except for those species whose taxonomy has been revised since publication of that document.

[a] SARA = Species at Risk Act, Schedule 1; CESCC = Canadian Endangered Species Conservation Council assessment of the state of biodiversity nationally, considering provincial status ranks (Canadian Endangered Species Conservation Council (CESCC). 2011. Wild species 2010: the general status of species in Canada. National General Status Working Group. 302 p. http://publications.gc.ca/ collections/collection_2011/ec/CW70-7-2010-eng.pdf. [Date accessed: May 2015].). This latter ranking system affords no protection to bats federally, but is indicative of expert assessment of the status of each species. In addition, some species are protected under provincial legislation.

[b] Recent genetic evidence confirms this species has not been found in Canada (Nagorsen and Paterson 201; an update of the status of red bats, Lasiurus blossevillii and Lasiurus borealis, in British Columbia. Northwestern Naturalist. 93: 235–237.).

[c] Recently discovered in British Columbia, especially Salt Spring Island, (Ommundsen *et al.* 2017. Northwestern Naturalist 98:132-136.)

2.1 Bats as Generalist Predators

There is a growing consensus that most insectivorous bat species in North America are generalist predators feeding on a wide range of insect taxa and switching prey preferences in response to availability. The advent of molecular bar-coding tools for analysis of fecal samples has contributed to this understanding. For example, a comprehensive dietary analysis of 56 Eastern Red Bats captured in Pinery Provincial Park in southern Ontario identified at least 127 different prey species in five orders and 16 families (Clare *et al.* 2009). Insect prey species were seldom identified more than once and a species accumulation curve showed no sign of plateauing – an indication that the authors were merely scratching the surface with their sample size.

Clare repeated the exercise with the Little Brown Myotis with the collaboration of several colleagues across Canada (Clare et al 2014). They identified nearly 600 distinct insect species, 30% of which could be identified. Not surprisingly, prey species differed seasonally and geographically. Repeat samples in the same location and time of year for different years also showed large differences.

Whereas some might argue that the broad food preferences of many bat species reduces their risk of being affected by food shortages brought about by insect depletions (section 3.1 below), it is likely that rich and complex food webs are essential, even for generalist predators. For example, it has been shown that the temporal interplay of multiple terrestrial and aquatic insect resources is critical to insectivorous bird communities; i.e. birds are able to switch from terrestrial to aquatic sources and vice versa in response to availability (Nakano and Murakami 2001). Foraging in bats (as in many insectivorous bird species) is very energetically-demanding and requires very high insect densities to be profitable. There is some evidence that bats are often food-limited, which results in a complete cessation of feeding activity and night-time roosting while waiting for feeding conditions to improve (Anthony *et al.* 1981). Diverse and abundant food resources from land and insect hatches from freshwater systems are likely important for maintaining North American bat populations.

The two main foraging strategies of insectivorous bats are aerial hawking and gleaning from surfaces. Several species appear to show plasticity in that regard and can use either strategy even if one dominates (Ratcliffe and Dawson 2003). In the context of pesticide exposure, we propose that gleaning from plant surfaces would result in greater exposure potential (see section 4.4).

Bats are generalist predators. Little Brown Myotis foraging. Photo Credit: Sherri and Brock Fenton.

2.2. Food Habitat of Bats in Agricultural Landscapes

Bats are commonly present in landscapes dominated by agriculture and the value of bats in providing ecosystem services has been an increasing area of study. In temperate regions, bats eat up to 600 insects a night and so provide a vital pest-control service that is valued at approximately US$3.7 billion a year for North American farmers (Boyles *et al.* 2011).

Traditional analysis of insectivorous bats' food habits (through the microscopic identification of insect parts in bat feces) have shown that bats consume many prominent agricultural pest species (reviewed in Kunz *et al.* 2011). It is therefore reasonable to assume that consumption of these insect pests may represent a source of pesticide exposure – especially where there is a delay between treatment and insect mortality, where the insects were sub-lethally exposed or when insects are externally contaminated on their wings, hairs or scales by dust or droplets. The broad contamination of terrestrial and aquatic systems by neonicotinoid insecticides ensures that a broad brush of insect species beyond the pest species will be contaminated (see section 4.4. below). Kunz *et al.* (2011) cited some key pest species and crops where bat predation might occur: June Beetles (grasses, cereals, sugar beet, soybeans and potatoes), Wireworms (most crops), Leafhoppers and Plant Hoppers (rice, potatoes, grapes, almond, citrus and row crops), Corn Rootworms/Spotted Cucumber Beetles (corn, spinach, cucurbits), Stinkbugs (fruit trees, corn, cereals and vegetables), Cutworms (most crops), Tortrix moths (fruit and nut trees), and Snout Moths (nut and fruit trees, cranberries).

A cucumber beetle, one of the many agricultural pests that bats feed on. Photo credit: Canadian Wildlife Federation Photo Club.

As mentioned earlier, the use of genetic tools such as DNA barcoding has greatly aided the study of bat food habits by allowing the identification of soft-bodied insects such as moths to the species level. Kunz *et al.* (2011) have reviewed case studies including the impact of Brazilian Free-tailed Bats on herbivorous insects in coffee plantations. Recent studies in that system had shown that much of the insect removal previously attributed to birds was from bats instead. Kunz *et al.* (2011) also referenced a number of other studies showing that bats use agroecosystems for foraging.

McCracken *et al.* (2012) argue that, as generalist predators, bats perform very large ecosystem services in agriculture. For vast acreages of annual crops – our dominant form of agriculture today – generalist predators are able to survive periods between irruptions of the main pest species by switching to alternative pest species. They looked at free-tailed bats in Texas and their relationship to corn earworms/cotton bollworm – Helicoverpa sp. – and documented a correlation between the number of pests regionally and the proportion of bat feces with earworm DNA, showing that the bats tracked moth abundance in space and time. The authors also documented the importance of late fall insect flights on the ability of the bats to accumulate fat reserves for their migratory flights south. The same argument could presumably be presented on the importance of abundant irruptive insect species to hibernation readiness and survival in more Northern species of bats.

Sirami *et al.* (2013) reviewed several studies where agricultural crops offered foraging opportunities for bats, notably vineyards, olive groves, cotton fields, cacao and banana plantations. However, they stressed the importance of natural features (woodlots, hedgerows or tree lines) as well as natural or impounded bodies of water as focal points for foraging activity even though foraging in their own study was as intensive in the crop areas surrounding artificial wetlands than over the wetlands themselves. Similarly, Stahlschmidt *et al.* (2017) documented the use of orchards, vineyards and field crops by foraging bats in Germany; although foraging intensity was highest along forest edges there was no difference between agricultural and non-agricultural sites. Their conclusion was not so much about the benefit of bats for pest control in cropland but a concern for potential exposure of bats to the suite of pesticides applied on those crops. We will be looking at this in detail in section 4.

Natural habitat on farmland provide important foraging opportunities for bats. Photo Credit: Getty Images.

Dagenais (2016) found that bats in the Okanagan valley of British Columbia used vineyards as much as they did natural habitat. Vineyards were in close proximity to cliff roosting areas and she postulated that bats (especially lactating females) might have foraged as close to their roosts as possible for energetic reasons. However, she was not able to distinguish actual foraging from travel to and from foraging areas.

Not all studies show a heavy use of crops by bats. Insectivorous bats in rural Sicily foraged over water and riparian vegetation but appeared to avoid intensively farmed vineyards (Di Salvo *et al.* 2009). Studies on the endangered Indiana Bat have shown that, in agriculturally-dominated areas, the bats tend to concentrate their foraging in riverine areas and in small woodlots (Kniowski *et al.* 2014) within the agricultural matrix. It was not clear from these studies whether insect abundance was lower in the agricultural areas than the natural habitats.

It is logical to think that site and time-specific prey availability will dictate how much bats will use agricultural crops; pesticides clearly can affect local prey availability. Indeed, of all the components of agricultural intensification, pesticides (specifically insecticides and fungicides) emerge as the most significant factor in reducing biodiversity in agro-ecosystems (Geiger *et al.* 2010). In a more bat-directed study, Wickramasinghe *et al.* (2003) compared bat activity over organic fields with that of paired conventionally-grown fields in the United Kingdom. They found that bat activity overall was 61% higher on organic farms although bat diversity did not differ. The differential use of bodies of water on organic and conventional farms was most pronounced; suggesting to us that the reduced potential of surface water on conventional farms to produce insects – most likely as a result of pesticide contamination – was responsible for the reduced bat foraging opportunities. As part of the same study, Wickramasinghe *et al.* (2004) found that agricultural intensification in the form of agrochemical use had a clear negative impact on nocturnal insect communities; they argued that bats are resource-limited and suffer population impacts as a result of the invertebrate loss that accompanies agricultural intensification. Put *et al.* (2018) arrived at similar results in a study near Ottawa, Ontario: bat activity is consistently higher over organic soybean fields than over conventional soybean fields paired for size, hedgerow length and nearby cover type. Here again, insect prey abundance appears to be the causal link.

Fuentes-Montemayor *et al.* (2011) looked at promoted agri-environmental schemes in the UK (having to do with hedgerow and streambank management as well as the creation of 'beetle banks' and species-rich grasslands) but found that these schemes did not improve foraging opportunities for bats and indeed did not improve use by the locally-dominant bat species of the Pipistrellus genus. Froidevaux *et al.* (2017), unlike results presented above, did not find higher bat activity in organic vineyards. However, they stated that early-summer insecticide sprays were mandatory for all vineyards, whether conventional or organic. Their statistical models showed the proximity to water courses to be the strongest explanatory factor for bat activity. The re-introduction of structural elements to increase habitat heterogeneity in agricultural landscapes has been proposed to help bat species more generally (Frey-Ehrenhold *et al.* 2013).

2.3 Ecosystem Services

One key aspect in assessing the value of generalist predators is whether they can reduce the need for conventional treatment such as pesticide application. Unfortunately, the main feature of recent insect pest control has been the prophylactic treatment of vast acreages with systemic pesticides – or, in some cases, the use of transgenic crops that also contain an insecticide whether or not justified by pest pressure. The idea that prophylactic treatments could result in a costly reduction of pest control services when generalist predators decrease their activity in treated areas has been raised by several authors. For example, Federico *et al.* (2008) looked at the advent of Bt cotton crops (then 95% of the total cotton crop area) and asked through a modeling exercise whether this method of pest control nullified the agronomic value of bat predation. They found that the agronomics of both Bt and conventional cotton production was more profitable when large numbers of insectivorous bats were present.

They also argued that bat predation on surviving insect pests in Bt cotton would delay the onset of the inevitable resistance that accompanies any development of a new insecticide class, a long term benefit not quantified in the model. Extending data computed for cotton-dominated agro-ecosystems to other cropland, Boyles *et al.* (2011) estimated that bats are worth between 3.7 and 53 USD$ billion per year.

Closer to a Canadian crop scenario, Maine and Boyles (2015) used night-time exclosures to assess the impact of bats on corn pests in Illinois. They also proposed to test the critical assumption that predation on adult moths by bats would result in a reduced number of eggs laid and thence the number of larvae infesting the crop and the level of crop damage. They found that bats appeared to follow insect abundance and areas under exclosures had, on average 59% more corn earworm larvae per ear of corn and 56% more damaged kernels/ear. Because of the high variance, the 20% increase in overall yield in the exclosures was not significant; on the other hand, the increased level of fungal infection and mycotoxin as a result of insect damage was significant.

Now that we have demonstrated the importance of agricultural land to bat foraging, the next section focuses on the indirect effects of pesticides including neonicotinoids on bats.

Townsend's Big-eared Bat. Photo credit: Sherri and Brock Fenton.

3. Assessing the Indirect Effects of Pesticides on Bats

3.1 The Evidence for Insect Declines

Recent scientific papers have documented the global decline of insect species. Dirzo *et al.* (2014) developed a global index for invertebrate abundance that indicated a 45 percent decline over the last forty years. The German Krefeld Society analyzed long term datasets in 63 locations within protected areas of Germany (Hallmann *et al.* 2017). They used Malaise traps to assess the flying insect biomass between 1989 and 2016 and, through an elegant statistical analysis, reported a dramatic decline in abundance of insects over this period – by 77% as a weighted yearly average or as much as 82% in the summer season. The authors looked at various factors such as climate, land use and local habitat to explain those declines. Whereas the number of frost days and winter precipitation explained some within and between-year variation, it did not explain the decline over time. Indeed, the period under study saw a gradual increase in temperature and reduction in wind, which should have been beneficial to insect numbers. In addition, whereas habitat type explained variations seen in insect numbers, the overall loss rate was similar in all habitats.

Factoring in habitat changes within 200m of trap sites (from aerial photographs) revealed a few weak interactive effects: notably that insect declines were more marked at sites in proximity to grassland habitat than sites with a high proportion of forests or arable land. The presence of arable land had a negative effect on insect biomass, and although coverage of arable land decreased over time (while forest area increased), the authors did not see an expected increase in insect biomass. Rather than an expected 8% increase in insect biomass over time due to decreasing arable land, a 77% decline in insect biomass was observed. The authors of the study surmised that agricultural intensification (pesticide use, tillage, fertilizer use, loss of field margins and frequency of treatments) was the most plausible reason for the decline since most of the sampling locations were fragments of protected habitats within larger agricultural landscapes.

Wickramasinghe *et al.* (2004) compared the nocturnal insect community of paired conventional and organic fields in the UK. They found higher abundance in pastures, water and woodland habitats (the latter only when dry weight was compared) of organic farms compared to the same habitats on conventional farms. The abundance of Lepidoptera and Diptera correlated with bat activity on those same farms. Pesticide use was the most important difference between the two farm types; unfortunately, the authors were not able to completely separate a possible habitat structure effect, organic farms having slightly higher hedges on average.

Agricultural intensification is associated with increased use of pesticides and decreased insect abundance. Photo Credit: Getty Images.

One interesting feature of the German Krefeld Society data on which the Hallmann *et al.* (2017) analysis was based is that hoverflies, as a group, showed a particularly steep decline (Vogel 2017). They are pollinators highly dependent on nectar and pollen and are therefore vulnerable to impacts of systemic products such as the neonicotinoids. Indeed, in her reporting and analysis of the Krefeld and other data, Vogel (2017) singles out neonicotinoids as likely culprits.

Subjectively, there is reason to believe that much broader insect declines are occurring here as they are in Europe. On both continents, the 'car screen effect', the fact that one need not clean squashed bugs on car windscreens the way we had to in earlier times has been a subject of conversation – in biological circles at least if not automotive circles. Despite improvements in car aerodynamics, there is a strong feeling that a generalised decline in flying insects on both continents is real (Vogel 2017).

3.2 Importance of Aquatic Insects to Bats

Bats are known to feed on many insects of aquatic origin. For example, Clare *et al.* (2009) identified Ephemeroptera, Neuroptera, and possibly Trichoptera in fecal samples from Northern Red Bats in southern Ontario (Clare *et al.* 2009). Little Brown Myotis are known to forage extensively over

water on aquatic insect hatches (reviewed in Clare *et al.* 2014). The latter study indicated that the most frequent food items were species of chironomids and Ephemeroptera, which are amongst the most sensitive taxa to neonicotinoids (Morissey *et al.* 2015, Sanchez-Bayo 2016b).

Studies by Wickramasinghe *et al.* (2003, 2004) and Kniowski *et al.* (2014) emphasize the importance of water bodies for foraging. The findings of reduced insect availability and foraging opportunities associated with surface water on conventional farms compared to organic ones is a clear indication that disruption of aquatic food webs as a result of pesticide contamination is likely affecting bats.

A Mayfly, of the order Ephemeroptera. One of the many aquatic insect species in the bat diet and sensitive to neonicotinoid pesticides. Photo credit: Canadian Wildlife Federation Photo Club.

3.3 Indirect Effects of Neonicotinoids on Bats

This section reviews the evidence for indirect effects of neonicotinoid pesticides on bats, primarily through the mechanism of decline in insect (i.e. bat food) abundance.

3.3.1 The Risk Case for the Involvement of Neonicotinoids in Insect Declines

Mineau and Palmer (2013) argued that neonicotinoids likely caused serious impacts on invertebrate populations. Notwithstanding the fact that the predecessors of neonicotinoids (e.g. organophosphorous, carbamate and pyrethroid insecticides) had clear documented local impacts,

Mineau and Palmer (2013) argued that the risk from neonicotinoids was both wider in scope and longer in duration as a result of very high toxicity to insects and other invertebrates, very long persistence in soils, very high systemic activity and high water solubility and runoff potential. The broad scale and prophylactic use of neonicotinoids (e.g. Douglas and Tooker 2015) is clearly cause for concern given the issues associated with dust production at seeding (see section 4.4.2 below). Possibly as a result of this dust contamination, wind-erodable surface soil and/or surface runoff, studies have shown extensive contamination of pollen supplies and vegetation in field edges at levels that can exceed those in the crop proper (e.g. Botias *et al.* 2015; Long and Krupke 2016; Tsvetkov *et al.* 2017).

There is now strong evidence that the neonicotinoids and, possibly, other recent systemically active insecticides can alter aquatic food webs. Neonicotinoids are highly water-soluble, low soil binding and non-volatile and therefore only a small portion of neonicotinoid active ingredient applied to seeds is taken up by plants. These properties cause leaching into surface waters, ground water, streams and ponds via spray drift and run-off (Goulson 2013; Anderson *et al.* 2015). Neonicotinoids have become ubiquitous in aquatic environments across agricultural landscapes (Mineau and Palmer 2013; Main *et al.* 2014; Anderson *et al.* 2015; Morrissey *et al.* 2015; Miles *et al.* 2017, Struger et al 2017; Bradford et al, 2018; Hladik *et al.* 2018).

Aquatic insect larvae are sensitive to acute and chronic exposures to neonicotinoids (Morrissey *et al.* 2015). Reported effects on aquatic invertebrate larvae include lethality, feeding inhibition, reduced growth, mobility impairment and delayed emergence (Goulson 2013; Morrissey *et al.* 2015). In a review of impacts of neonicotinoids on aquatic systems, Sánchez-Bayo *et al.* (2016b) summarized strong evidence that the concentration of neonicotinoids in aquatic systems globally is causing the decline of many populations of invertebrates and is affecting the structure and function of aquatic ecosystems. Consequently, vertebrates such as bats, which depend on aquatic invertebrate hatches as an important source of food are likely being affected by neonicotinoids.

Mineau and Palmer (2013) commented unfavourably on the way in which the aquatic toxicity potential of neonicotinoids had been assessed by various regulatory authorities, especially the US Environmental Protection Agency (USEPA). This was a result of their outdated assessment methodology and their ignoring of literature values in favour of a few industry values obtained for a few species known to be insensitive to neonicotinoid insecticides. The analysis of Mineau and Palmer (2013) was expanded and published as Morrissey *et al.* (2015); a similar review of the evidence was published in 2016 (Sanchez-Bayo *et al.* 2016b). One key argument is that the aquatic toxicity of clothianidin and thiamethoxam, the two main seed treatment neonicotinoids in Canada, can be assumed to be similar to that of imidacloprid, the neonicotinoid insecticide with the most comprehensive data set. Based on Morrissey *et al.* (2015), deleterious effects on the aquatic environment (loss of aquatic and emergent insect resources) are expected from pulse exposures as low as 0.2 µg/l of combined neonicotinoid residues and chronic exposures an order of magnitude or more lower. The USEPA (2017) reassessed its position on imidacloprid and reduced their acute

reference value from 35 µg/l to 0.77 µg/l and their chronic reference value from 1.05 µg/l to 0.01 µg/l. This certainly brings the EPA closer to international standards when assessing the potential impacts from the extensive aquatic contamination that results from neonicotinoid uses.

Morrissey *et al.* (2015) demonstrated that scientifically-derived reference levels were often exceeded in various water monitoring exercises – and not just for imidacloprid. For clothianidin for example, Main (2014) reports values as high as 3.1 µg/L from sloughs in canola-growing areas following the use of seed treatments; Samson-Robert *et al.* (2014) found levels as high as 55.7 µg/L in puddles on seeded fields; Schaafsma *et al.* (2015) measured levels as high as 16.2 µg/L in ditches outside a seeded field and 3.25 µg/L in puddles potentially as far as 100 m from the fields. Whiting *et al.* (2014; 2015) documented clothianidin residues in runoff a full 156 days after planting – and this, at one-fifth of the allowable treatment rate. For thiamethoxam, Main *et al.* (2014) found values up to 1.49 µg/L from sloughs around canola fields; Samson-Robert *et al.* (2014) found levels as high as 63.4 µg/L in puddles on seeded fields; and Schaafsma *et al.* (2015) measured levels as high as 7.5 µg/L in ditches outside a seeded field and 16.5 µg/L in puddles outside their Ontario field. The latter two measurements were all the more remarkable because they were measured pre-plant and therefore indicated contamination from the previous growing season. Higher levels were recorded in puddles within the field area.

Recent samples taken from a variety of waterbodies in crop and non-crop sites within an agricultural landscape in Indiana (Miles *et al.* 2017; with 2018 correction) found concentrations of clothianidin averaging 0.101 µg/L (all sites combined; with samples taken weekly for eight weeks). Interestingly, the highest concentrations of clothianidin that were detected (0.45-0.67 µg/L) were from small lentic woodland bodies of water well away from the seeded corn and soybean fields. One of these sites (PMA W) apparently received drainage from nearby fields; how the other got contaminated is unknown. Regardless, levels in these wetlands were higher than those reported in any of the ditch samples taken nearer the seeded fields. These data reinforce the emerging concern that aquatic life in watersheds in proximity to agricultural lands are at serious risk from inputs (whether dust or runoff) from agricultural fields in the watershed at large. These impacts are likely to cause cascade effects in consumer species including bats.

These results are all the more striking when one considers that 'grab' samples, such as the above, seldom reflect peak residue levels. Indeed, it has been shown that, even when taken weekly, water samples will likely underestimate peak concentrations by one to three orders of magnitude (Xing *et al.* 2013). In addition, it is reasonable to assume that the effects of different neonicotinoid insecticides act in a cumulative fashion on exposed invertebrates. Finally, extending the exposure period (as found in monitored watersheds) dramatically increases the toxicity of (and the effect posed by) neonicotinoids (Tennekes 2010a; Sanchez-Bayo 2009). This last point has not been factored into current assessments despite its overwhelming importance and there are strong arguments that they should (Sanchez-Bayo and Tennekes 2017; Hladik *et al.* 2018).

Clothianidin, thiamethoxam, and imidacloprid, and their associated products, were re-evaluated recently by the Health Canada Pest Management Regulatory Agency (PMRA), which recommended a complete phase out of all outdoor uses of these neonicotinoids on food and feed crops including seed treatments and outdoor ornamentals due to the evidence of serious harm to aquatic species and ecosystems (PMRA 2016b; PMRA 2018).

The US EPA (2017) has similarly concluded that imidacloprid levels are frequently above levels at which aquatic taxa will be negatively affected. The EPA's re-evaluation of clothianidin and thiamethoxam was not concluded at the time of publishing this report.

3.3.2 The Developing Evidence for Bona Fide Impacts from Neonicotinoid Insecticides

Not surprisingly, actually demonstrating a direct cause and effect between the use of neonicotinoids and the loss of invertebrate biomass at landscape scales is not an easy task. The work of Van Dijk *et al.* (2013) is often cited because of their conclusion that neonicotinoid insecticides affect aquatic invertebrate levels in Dutch landscapes. However, this conclusion was criticized by Vijver and van Den Brink (2014) who argued that van Djik *et al.* (2013) failed to consider residues of other potentially toxic pesticides by concentrating on neonicotinoids alone. It is clearly difficult to separate the effect of neonicotinoid residues from those of other pesticides also found contaminating surface waters in watersheds with heavy agricultural and/or industrial activity. However, the most convincing analysis was that of Hallman *et al.* (2014) who looked at insectivorous bird population trends, also in the Netherlands. In order to get over any criticism that their study was merely correlative, Hallman *et al.* (2014) divided the time period of their analysis into pre- and post-neonicotinoid periods. They found that neonicotinoid concentrations explained bird declines and that these site-specific declines were not seen before the introduction of the neonicotinoids, despite the use of other toxic insecticides such as organophosphorous or pyrethroid products. The other interesting point in the Hallman *et al.* study is that the neonicotinoid concentration at which regional bird declines are being seen (0.194 µg/L) is exactly where we would have predicted to see an effect based on the analysis of Morrissey *et al.* (2015) presented above (deleterious effects having been predicted to occur at 0.20 µg/L of summed neonicotinoid concentration or higher).

There is now reasonably strong evidence (Gilburn *et al.* 2015) that neonicotinoid insecticides, and seed treatments in particular, are driving declines of butterfly species in the UK where extensive datasets on butterfly numbers are available. In an analysis similar to the Hallman *et al.* (2014) study, Gilburn *et al.* (2015) showed that even increasing population trends in several species were reversed following the introduction of neonicotinoids; areas with low neonicotinoid use did not show the same extent of insect declines.

Similar results were obtained in a study conducted in California (Forister *et al.* 2016). Neonicotinoid use was the best predictor to explain dramatic declines in butterflies beginning in 1997 (neonicotinoid use began in 1995 in the region). Other insecticide classes were also examined but did not show the temporal association with butterfly declines. The overall effect of neonicotinoids

was equal to (but clearly additive to) that of land conversion (habitat loss) and species showing the strongest negative association with neonicotinoid use experienced the most severe declines. These tended to be the smaller-bodied species and those with the fewest number of generations per year. Lepidoptera, especially small-bodied species are clearly important food items for bats.

Similarly, Woodcock *et al.* (2016) were able to show that the use of neonicotinoids in oilseed rape (canola) in the United Kingdom explained extinction rates of wild bee species. Several studies have now appeared showing a correlative link between neonicotinoid use (specifically clothianidin, thiamethoxam and thiacloprid) and the presence and viability of both managed and wild pollinator species as well as beneficial insect predatory and parasitoid species (see detailed review in Pisa *et al.* 2017). As shown by Tappert *et al.* (2017) working on parasitoid wasps, behavioural effects (seen at 1% of the contact LD50 in their study) can have negative consequences on reproduction and the ability of various species to maintain their populations even if contamination levels stay below lethal levels – which unfortunately, they do not.

There is evidence to support the claim that, although insects have suffered from agricultural intensification and habitat loss generally, neonicotinoids in particular have had an important role in accelerating insect declines. The evidence is especially compelling for pollinators because this is where most of the research has been concentrated. However, it is clear that many of the same findings of lethal or deleterious sub lethal effects in pollinating species apply to other insect species as well. This is occurring at a time when the loss of feeding opportunities for bats are known to be reaching crisis levels; viz. the catastrophic declines in moth species in agricultural regions of Britain (Conrad *et al.* 2006).

Evidence that neonicotinoids are a strong contributor to insect declines should not come as a surprise. Their use has exploded in the last two decades as Figure 1 shows.

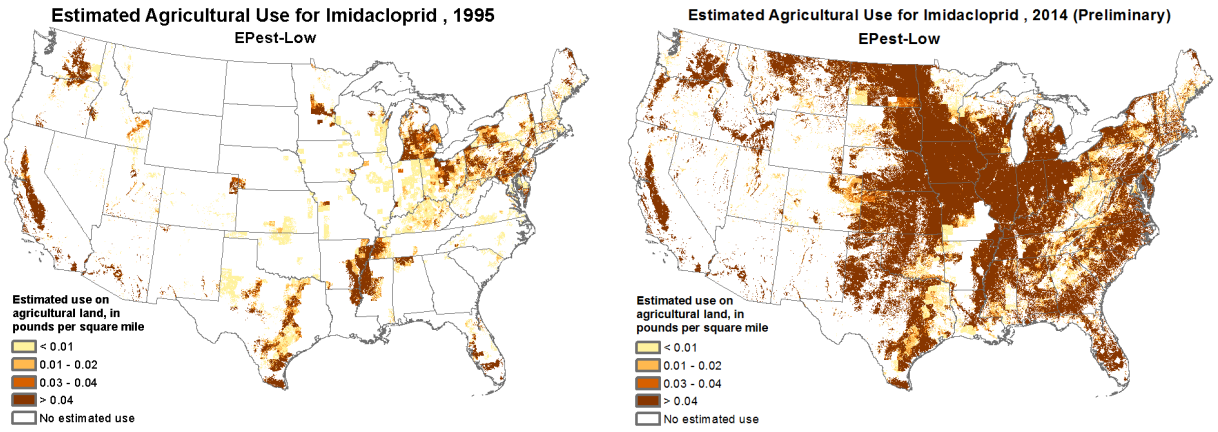

Fig.1: Comparison of estimated minimum use of imidacloprid in 1995 and 2014 according to the water program of the US Geological Survey. Source: (https://water.usgs.gov/nawqa/pnsp/usage/maps).

As early as 2008, the USEPA (USEPA 2008) in one of its reviews of thiamethoxam went as far as to predict "structural and functional changes of both the aquatic and terrestrial ecosystems" following registration of the insecticide. The senior author of the current report, despite having read pesticide risk assessments for almost forty years now, had never encountered such a broad statement of concern in a formal regulatory assessment. It is unfortunate indeed that this EPA scientist's views fell on deaf ears. Similarly, Tennekes's (2010b) self-published assessment of a 'disaster in the making' was roundly criticized for being alarmist and not distinguishing correlation from causation – but it now appears to have been correct, if not in all of the details, at least in the fundamentals.

Since then, a number of other institutions have come to similar conclusions. The 'Task Force on Systemic Pesticides' which was comprised of a large group of independent scientists (including this senior author) under the auspices of the IUCN carried out its 'Worldwide Integrated Assessment on the Impact of Systemic Pesticides on Biodiversity and Ecosystems (WIA)' and arrived at the conclusion that deleterious effects are expected on a wide range of aquatic species from current levels of neonicotinoid water contamination (van der Sluijs *et al.* 2015, Pisa *et al.* 2015). The European Academies Science Advisory Council (2015) made up of 29 independent scientists nominated by their respective countries concluded that the current widespread use of neonicotinoids has "severe negative effects on non-target organisms" and that there is "clear scientific evidence for sublethal effects of very low levels of neonicotinoids over extended periods". Most recently, an updated assessment of the exploding literature by the WIA (Pisa *et al.* 2017) reiterated the strengthened evidence for the potential of this class of insecticide to "greatly decrease populations of arthropods in both terrestrial and aquatic environments." As for Sanchez-Bayo (2016b), referenced earlier, his conclusion summarizes the level of concern aptly:

"Negative impacts of neonicotinoids in aquatic environments are a reality…. The decline of many populations of invertebrates, due mostly to the widespread presence of waterborne residues and the extreme chronic toxicity of neonicotinoids, is affecting the structure and function of aquatic ecosystems. Consequently, vertebrates that depend on insects and other aquatic invertebrates as their sole or main food resource are being affected."

4. Assessing the Direct Effects of Neonicotinoid Insecticides on Bats

The current section presents a framework for assessing effects of acute, subacute and chronic (season-long) exposure of bats to neonicotinoids. To do this, several components are compiled:

- Evidence for pesticide exposure in bats
- Application rates of the five neonicotinoids registered for use in Canada;
- Evidence for estimating neonicotinoid residue on insects following foliar application, seeding application, and residues not associated with a recent application;
- Proposing insect residue values for a risk assessment;
- Estimating daily consumption rates of insects by bats;
- Identifying effect levels for each of the five neonicotinoids registered for use in Canada from toxicological studies;
- Proposing toxicity reference values for a bat risk assessment

4.1 Framework for a Proposed Bat-centric Risk Assessment

When assessing the risk of pesticides to wild mammals, the USEPA (2013) uses both the LD50 (the lowest available for tested mammalian species) as well as the two generation rat NOAEL (The 'No Adverse Effect Level'). These endpoints are then compared to expected dietary intakes. For acute scenarios, the highest safety factor that is applied to the ratio of predicted exposure to endpoint (the risk quotient – or RQ) is a factor of 10, but only for endangered species. For all other species, no risk is 'officially expected' unless the daily calculated residue intake is greater than half the LD50. For chronic scenarios, some risk is considered possible if daily intake (at peak residue concentration) exceeds the chronic NOAEL. The EU has a somewhat similar approach but with a few minor differences (EFSA 2009a).

In our opinion, the approaches described above are inadequate for our needs. In the wild, exposed individuals showing any signs of toxicosis run a high risk of predation or demise. It has long been thought that sub-lethal debilitation was an important factor in explaining wildlife mortality, at least with neurotoxic insecticides (see several of the chapters in Mineau 1991; a review of the impact of cholinesterase-inhibiting insecticides). An acute risk assessment based on exposed mammals reaching a laboratory-derived LD50 seriously underestimates risk to those species. For a flying species especially, deficits in locomotor ability and alertness (which typically occurs at relatively low levels of exposure – Eidels *et al.* 2016) will quickly lead to injury and/or death. Conversely, comparing chronic effect levels to residues in insects immediately post-spray as is currently done undoubtedly introduces a fair degree of over-protection. Because concerns of chronic toxicity in

mammalian wildlife are repeatedly triggered following many evaluations, they become meaningless; these routine 'risk exceedances' are then likely to be dismissed (or ignored) by registration authorities.

In order to more accurately examine the risk to bats from the proliferation of neonicotinoid insecticides, we propose to develop acute, subacute and chronic (season-long) ingestion endpoints based on the existing corpus of mammalian toxicology research. For the acute scenarios, we propose to consider newly established, evidence-based no effect levels rather than LD50 values. These could come from acute lethality studies – or observations made following any other study where a single acute dose was delivered – such as the neurotoxicity screens. Sub-acute endpoints will be NOAELs obtained in studies of approximately 3 months in duration or less and chronic endpoints will be taken from the two generation (rat) studies. In human risk assessments, endpoints from rat or rabbit developmental studies are often used to calculate acute reference doses even though dosing takes place in a repeated fashion during conception, pregnancy and lactation. Unless otherwise indicated, we have opted to consider those endpoints in association with a sub-chronic assessment. For the purpose of this bat assessment, we have placed emphasis on endpoints expected to give rise to serious deficits in a wild population; that is: deficits expected to reduce the life expectancy or breeding potential of exposed individuals.

With organophosphorous insecticides, it was typical for wildlife risk assessments to put more emphasis on acute exposure risk scenarios. Whereas this might be appropriate for non-persistent pesticides with short environmental half-lives, we do not think that it is sufficient to account for the demonstrated exposure profile of systemic pesticides such as the neonicotinoids; they have been shown to have delayed effects and a long environmental persistence and therefore expose wildlife species in a sub-chronic or even chronic fashion. In their study of a non-farming human population living in an agricultural area of central Japan (Gunma prefecture), Marfo *et al.* (2015) found a prevalence of abnormal electrocardiographs, finger tremors, memory loss, headache, fatigue and muscle pain/weakness/spasm associated with neonicotinoid metabolites in the urine of patients. Exposure was thought to originate from consumption of locally-treated fruits, vegetable and especially tea; toxicity signs eventually disappeared when patients were told to avoid locally-produced foods. It is not difficult to make the case that neurological symptoms such as these would likely interfere with foraging and normal activities in bat species exposed through consumption of contaminated insects.

4.2 Pesticides and Bats

To date, most of the information on bats and pesticides concerns legacy organochlorine compounds or surface biocides used for treating wood, the latter affecting bats in attic colonies.

These issues have been reviewed in detail (e.g. Clark and Shore 2001) and there is no reason to discuss these any further. One key aspect of bat's susceptibility to these lipophilic compounds were related to yearly fat cycles and release of fat reserves during hibernation.

In a study more pertinent to modern insecticides, Eidels *et al.* (2016) dosed Big Brown Bats with chlorpyrifos and reviewed the (scant) available information on the sensitivity of bats to modern pesticides (OPs only) as well as the few known incidents where bat mortality has been linked to organophosphorous use. The latter are useful to indicate that exposure to agricultural pesticides is real and has been documented, at least occasionally.

Secord *et al.* (2015a) report what we believe to be the only detection of a neonicotinoid in bat tissue: thiamethoxam at 33 and 51 ng/g in two collected specimens. More information was included in subsequent correspondence with the senior author:

"I just checked and these two bats were M. sodalis collected on February 10, 2009 from a mine in northern NY - described as adults with apparent WNS (white-nose syndrome)." (A. Secord, pers. comm. 13 Sept. 2017)

The presence of residues at that time of the year suggests a much longer persistence than expected, possibly as a result of the bats going into torpor. This raises a question about how well the murine or dog toxicology database (reviewed below) can represent a heterothermic bat species (alternating seasonally between torpor and euthermy) given that the metabolism of ingested residues may differ substantially between those two periods.

Bats are exposed to pesticides by consuming pesticide residues on their prey. Photo credit: Sherri and Brock Fenton.

4.3 Canadian Application Rates of the Neonicotinoid Insecticides of Concern

For the purpose of this assessment, we opted to look at the five main neonicotinoid insecticides registered to date: clothianidin, acetamiprid, thiacloprid, imidacloprid, and thiamethoxam. Documents and labels compiled by the Pest Management Regulatory Agency (PMRA) or provincial authorities (e.g. Ontario or Saskatchewan) were consulted to generate maximum application rates. Three use categories were considered because of their potential to expose foraging bat species: airblast for fruit tree treatments, foliar sprays and seed treatments in all other crops. Maximum application rates in some cases were for non-food uses as specified in Table 4.1.

Table 4.1. Maximum Canadian labeled rates for the five neonicotinoid insecticides being assessed.

Active ingredient	Application type	Maximum labeled rate (g a.i./ha)	Source(s)	Notes
Clothianidin	Airblast	210	PMRA 2011	
	Foliar	350	PMRA 2011	
	Seed treatment	99	Poncho 600FS (PCP#274531) OMAFRA 2017	Corn: Poncho label of 1.25 mg/kernel seeded at 79000 kernels/ha
Acetamiprid	Airblast	168	PMRA 2010	
	Foliar	84	PMRA 2010	
	Seed treatment	45	PMRA 2010 Vault 50FS (PCP#28119)	Canola: Max seeding rate of 9 kg seed/ha after PMRA 2010
thiacloprid	Airblast	210	PMRA 2007b Calypso 480SC (PCP#28429)	
imidacloprid	Airblast	91	PMRA 2001b	
	Foliar	330	PMRA 2001b	Highest application rates to non-crop areas such as turf, lawns
	Seed treatment	196	Gaucho 600FL (PCP#26124) OMAFRA 2017	Corn: Gaucho label of 800g a.i./100kg seed, average seed weight of 3230 seeds/kg and seeding rate of 79000 seeds/ha.
thiamethoxam	Airblast	96	Actara 25WG (PCP#28408)	
	Foliar	700	Flagship (PCP#30723)	Highest application rate is for ornamentals
	Seed treatment	41.7	Cruiser Maxx (PCP#29127) Saskatchewan Agriculture 2016	Durum wheat: 30 g thiamethoxam/100 kg seed with seeding rate of 139 kg/ha

1 PCP#xxxxx refers to the legally-binding label registered in Canada. They are available at: http://pr-rp.hc-sc.gc.ca/ls-re/index-eng.php

4.4 Pesticide Residues in Insects

4.4.1 Residues after a Foliar Application

Given the relative paucity of empirical data on expected residue levels in insects after a pesticide application, it was initially proposed by the USEPA (following Hoerger and Kenaga (1972) and Kenaga (1973)) to use data from plant materials having a similar surface to mass ratio as insects; e.g. a beetle might be equivalent to a grain of wheat. On that basis, it was assumed in countless risk assessments performed in the U.S. and elsewhere that residues in small insects (standardized as fresh weight residues for a 1 kg /ha application of active ingredient – or 1.121 lb/acre) would range from a mean of 29 ppm to a maximum of 52 ppm. For large insects, these values were 2.7 and 8.9 ppm respectively. It should be noted that objections have been raised over the years about the assumption that residue levels are linearly related to application rate – i.e. that residues can be standardized to a common application unit (typically kg of pesticide active ingredient/ha – or lbs/acre in the U.S.). Many factors other than simple surface area should theoretically affect residue concentrations on surfaces; e.g. the shape and texture of the receiving surface, the characteristics of the spray, droplet spectrum etc. Some results have suggested that increased droplet 'capture' could be expected at lower rates of application (A. Hart and H. Thompson; pers. comm.). In 1994, the USEPA revised its predictive values for sprayed plant materials (Fletcher *et al.* 1994; Pfleeger *et al.* 1996) finding that the amount of residues on many plant surfaces had been underestimated. It should be noted that, for plant material, the definitive analysis was the subsequent Canadian analysis of Baril *et al.* (2005) who allowed the data to define meaningful plant categories based on gross morphology of the crop, branching pattern as well as height, leaf shape and size. They argued that, although predictively weak and variable, the linear 'Residue per Unit Dose' (RUD) concept was still the most reasonable choice for risk assessment purposes. Their recommendations form the basis of the latest EU procedures. We will use the RUD concept here also. Predicted insect residue levels can then be calculated as: RUD value (ppm/kg a.i./ha) * Application rate (kg a.i./ha).

The new 1994-96 analysis of plant residues created concern among pesticide registrants because of the risk that insect residue predictions would therefore be assessed upwards, leading risk assessments to predict a significant risk more often – especially in small insectivorous bird or mammal species that have a high food consumption relative to their body mass. Pesticide registrants consequently funded a number of studies to look at residues in insects following pesticide applications; this effort proved timely and was incorporated into USEPA's ECOFRAM (1999) project, an attempt to improve ecological risk assessments by incorporating principles of variability through the use of probability distributions rather than discrete (often worst case) estimates. These industry data suggested average, geometric mean and maximum initial residue levels of 6.4 ppm, 2.4 ppm and 60.5 ppm for all insects confounded, when exposed to a 1 kg a.i./ha foliar application of a pesticide. Although many of the samples were taken from pitfall traps – and therefore from insects still mobile and able to be trapped – both US and European regulators believed these results

not to be biased because they matched other industry-funded data. However, these latter data were obtained in part by 'pinning down' insects, thereby preventing them from obtaining residues, either through direct contact or ingestion.

Subsequent research on residues in sprayed locust nymphs (Story *et al.* 2013) has since confirmed that, some insects at least, can accumulate residues by secondary uptake (whether moving through sprayed substrates or through dietary uptake) before they become debilitated; therefore, maximum residues are generally reached 3 h to 24 h post-spray and it is unlikely that artificially immobilized insects reflect realistic maximum residue levels any more than pitfall traps can. Our data suggest that sampling of (debilitated) insects for risk assessment purposes should mimic predation (we 'pecked' at prey items with forceps) and should take place over a longer and different time course than the aforementioned studies.

European risk assessors, in particular, had turned to those mid-1990s industry studies to revise downwards predicted residue levels in insects (European Commission 2002). This change affected all ecological risk assessments performed from the mid-1990s onward. Incidentally, this is the period of time during which all of the neonicotinoid insecticides that concern us here were assessed and registered. It is less clear whether the USEPA changed its initial screening procedures (Tier 1) to accommodate the new industry studies although they had been using the new industry values in their higher tier probabilistic assessments (e.g. their Terrestrial Investigation Model (TIM)). As late as December 2008, USEPA's Tier 1 web assessment tool TRex still used some of the older insect residue estimates based on the Kenaga 1972 and Fletcher *et al.* 1994 vegetation values (User's guide T-Rex Version 1.4.1; December 11 2008).

Currently, there is now a clear separation between USEPA and European thinking on insect residue levels expected following a pesticide application. After much back and forth, the EU adopted the following from EFSA recommendations (EFSA 2009a) (Table 4.2).

Based on the work by Story et al (2013) on locust nymphs referenced earlier – the latter being clear examples of 'ground-dwelling invertebrates without foliage interception' – the values in Table 4.2 (which form the basis of current EU vertebrate wildlife risk assessments) appear to be clear underestimates. For example, Story *et al.* (2013) calculated rate-normalised geometric mean concentrations of residues (retaining a single peak value per sampling site) to be 9.6 ppm, 12.6 ppm, and 14.8 ppm in live, debilitated, and dead locust nymphs, respectively. The residue-per-unit dose values reached maxima of 32 ppm in live and debilitated nymphs and 40 ppm in dead nymphs (fresh wt.). Literature values obtained in other orthopteran species are higher still (Story *et al.* 2013). A formal EU risk assessment would have predicted average and 90th percentile residues of 7.5 ppm and 13.8 ppm respectively, seriously underestimating the risk of intoxication in the many consumer species that prey on locust nymphs.

The USEPA, after reconsideration, now rejects many of the industry studies generated in the mid-1990s on the grounds of insufficient documentary evidence and because of the heavy reliance on pitfall traps (USEPA, TRex 1.5 accessed September 2017). Instead, they turned to available literature values and to submissions by various registrants pre-2007. They now propose two values for use in registration procedures – the 90th percentile of average values from the collection of studies on hand (73 ppm for a one kg a.i./ha application – or 65 ppm for a 1 lb a.i./acre application) and the 90th percentile of 90th percentile values obtained for each study (105 ppm – or 94 ppm for a 1 lb a.i./acre application).

Table 4.2. Current European recommendations for estimating insect residue levels post-spray.

Insect category	Application detail	Mean (ppm)	Standard deviation	90th percentile (ppm)	N
Ground dwelling invertebrates without interception	Ground directed applications	7.5	12.0	13.8	21
Ground dwelling invertebrates with interception	Applications directed to crop canopies, whether crops or orchards and vines	3.5	3.8	9.7	28
Insects (foliar dwelling Invertebrates** with foliar interception)	Whole season	21.0	21.6	54.1	35

** Data on aphids excluded. These were very high values but were excluded because these were not considered to be of high preference to avian predators.

All this back and forth debate on putative insect residue values may seem sterile and pointless to some – but it is actually quite important. Beyond the issue of regulatory effectiveness, having a trustworthy value to work with is the only way in which we can relate the results of toxicological research to actual risk of harm in the wild. For example, a well-publicised recent article by Hsiao *et al.* (2016) showed that Formosan Leaf Bats (Hipposideras terasensis) orally dosed with imidacloprid at a level of 20 mg/kg bw for 5 days experienced spatial memory disorders. The authors concluded that: "… agricultural pesticides may pose severe threats to the survival of echolocation bats." However, such a conclusion was premature without the benefit of an assessment that takes into account likely exposure levels in the real world. To do this requires a few assumptions – the main one being likely residue levels in insect prey. We will have more to say about this study in a later section of the paper.

To our knowledge, only one study was carried out with the stated goal of assessing the risk to foraging bats. This was the Ph.D. work of Peter Stahlschmidt in Germany referred to earlier and

published in Stahlschmidt and Brühl (2012). He measured pesticide residues in both flying insects (by means of a light trap) and foliar insects (knocked down from the orchard trees) following applications of (primarily) fenoxycarb and chlorpyrifos-methyl (Stahlschmidt and Brühl, 2012). The residue concentrations obtained, standardised to a 1 kg a.i./ha application are tabulated in Table 4.3 below.

Table 4.3. Insect residue data from treated orchards in the bat study by Stahlschmidt and Brühl (2012).

Pesticide, application replicate and sample type	Day 0 concentration (ppm)	Day 1 concentration (ppm)	Day 2 concentration (ppm)
Fenoxycarb			
Small flying insect, 1st spray	2.9	1.04	0.28
Small flying insect, 2nd spray	2.2	1.75	0.21
Small moths, 2nd spray	4.92	7.28	-
Large moths, 1st spray	2.21	0.89	0.13
Large moths, 2nd spray	1.34	0.88	0.21
Foliage-dwelling arthropods, 1st spray	57.52	10.8	11.55
Foliage-dwelling arthropods, 2nd spray	133.15	27.37	8.51
Chlorpyrifos-methyl			
Foliage-dwelling arthropods, 1st spray	4.34	-	-

The lower residue levels in flying insects were expected – because it is reasonable to expect that the more contaminated the insects, the more likely they are to be disabled and less likely to fly. The measured RUD (residue per unit dose) concentration of 133 ppm obtained with fenoxycarb in foliage-dwelling insects was, however, higher than expected based on the values documented above. Using the same methodology, the authors obtained a day 0 value of 4.3 ppm only following an application of chlorpyrifos-methyl. Regardless, this single experiment makes the USEPA chosen values of 73 ppm (to represent a worst-case average concentration) and 105 ppm (to represent a worst-case maximum concentration) look quite reasonable even if high by European (EFSA) standards. One issue of note, however: Using 90th percentiles for expected means and maxima minimises the huge variation expected from application to application. As the work of Baril *et al.* (2005) has shown, residue levels are extremely variable in time and place, even when standardised to the same substrate. A factor of 10 or even 100 is expected between different trials. In that context, the large variation seen in the Stahlschmidt and Brühl work tabulated above is not unexpected.

4.4.2 Neonicotinoid Residues in Bees

Some data exist on neonicotinoid residues on bees specifically. However, these data were collected in the context of passive hive monitoring projects. Because of the uncertainty as to how fresh the samples were and the likely loss of residues through 'weathering' (rapid loss for imidacloprid was demonstrated by Schott *et al.* (2017)), these results are qualitatively interesting but do not replace the body of work described above, much of which was carried out with the express purpose of determining reasonable residue values for risk assessment purposes. Note that the reported values below are clearly not corrected for application rates.

Calatayud-Vernich *et al.* (2016) collected dead honeybees outside of hives situated in a mixed agricultural region of Spain dominated by citrus and stone fruit. Samples were collected at flowering time when we would expect spraying to be at a minimum. Samples were only collected every week (or at best twice a week if mortality was high), so pesticide degradation was very likely to have happened. Residues of chlorpyrifos and dimethoate were the most often encountered, followed by imidacloprid – despite the fact that the product was under severe restriction at the time. Apiary samples had four different insecticides on average. Maximum concentrations of the most common insecticides were 0.751 ppm (chlorpyrifos), 0.403 ppm (dimethoate) and 0.223 ppm (imidacloprid).

Kiljanek *et al.* (2016) reported concentrations of a large number of pesticides detected in bee samples submitted after poisoning incidents in Poland. Samples from a total of 73 incidents were analysed. The most frequently detected insecticides were chlorpyrifos, dimethoate and clothianidin. When detected, average levels of most pesticides ranged from 0.002 ppm to 0.399 ppm although one fungicide (chlorothalonil) averaged 14.3 ppm. Maximum levels ranged from from 0.002 ppm to 3.29 ppm for all but chlorothalonil with a maximum of 55.8 ppm (typical application rates in fruit trees are 2-3 kg a.i./ha).

Bacandritsos *et al.* (2010) collected bees from apiary-reported June/July mortality events, reports of aberrant behaviour (trembling), and hive depopulation. Three of five samples were positive for imidacloprid, ranging from 0.014 to 0.039 ppm. The hives belonged to migratory beekeepers and were thought to have been sited on olive, citrus and other fruit orchards previously. Unfortunately, these authors did not look for the presence of imidacloprid metabolites. In their study, Codling *et al.* (2016) found that residues of the various neonicotinoid metabolites typically exceeded the quantity of parent material.

4.4.3 Neonicotinoid Residues in Insects after a Seeding Operation

Much of the use of neonicotinoid insecticides is in the form of seed treatments. It is much more difficult to predict what the possible insect residue levels will be if herbivorous insects are exposed through consumption of different plant parts rather than through direct contact with a spray application but we can surmise that their residue loads will be lower. However, the main route

of contamination of flying insects following a seed treatment use of neonicotinoids is through dust drifting from the planting equipment. The risk is thought to be higher under high humidity conditions, (Girolami *et al.* 2013; Halm *et al.* 2012). These authors theorized that the high humidity may help in the absorption of the systemic insecticides through the insect cuticles. If so, the effect may be dependent on the water solubility of different products. It is also likely that high humidity at the time of application will allow pesticide dust to more effectively stick to the bodies of the insects. In North America, it is customary for farmers to use talc or graphite as lubricants in their seeding machinery (e.g. Krupke *et al.* 2012). Although this increases the visibility of the dust cloud, it is unknown whether it changes the fundamentals of exposure. Tapparo *et al.* (2012) conducted experiments where individual bees were captured after merely flying over a corn field in the process of being sown in order to reach a food source (the entire test running for 1h). They measured amounts of 0.078-1.240 ug/bee (N=5, mean=0.570 ug/bee) for clothianidin at 1.25 mg a.i./seed and 0.128-0.302 ug/bee (N=4, mean=0.189 ug/bee) for thiamethoxam at 1 mg a.i. / seed. The same authors reported maximum concentrations of 3.65 µg/bee (approx. 36.5 ppm) obtained in previous work with imidacloprid-treated seed. These concentrations were thought to be primarily from bees picking up dust as they flew although several other routes of exposure – such as guttation fluids and ingestion of contaminated nectar and pollen – are recognised. After a few hours and normal activities in the hive, residues had dropped by an order of magnitude. (Note that although of no relevance to the current assessment, the bulk of the insecticide-laden dust on bee surfaces was thought to have been dislodged though normal hive activities. With potentially thousands of foragers returning to a hive, grams of insecticide can thus be transferred efficiently to the hive environment.) Assuming a bee weight of approximately 100mg, the maximum amount recorded by Tapparo *et al.* (2012) represents a concentration of 12.4 ppm. The authors do not provide a concentration per seed for imidacloprid but, based on U.S. labels of the same product (Gaucho), corn treatment was said to be 1.34 mg/seed. Tapparo *et al.* (2012) give seeding rates of 66,660 seeds per ha; this represents an application rate of 89.3 g imidacloprid/ha. This means that the RUD value standardised to 1 kg a.i./ha was approximately 409 ppm. Similarly, the more recent clothianidin results give a maximum RUD value of 149 ppm and mean of 68.5 ppm after the one hour seeding exposure; for thiamethoxam, the RUD had a max of 45.3 ppm and an average of 28.4 ppm. From a 'residue per unit dose' point of view, it appears that seeding results in higher contamination of insects than an equivalent spray application but, due to the lower per ha rates of application for seed treatments, we can expect a comparable level of contamination in non-target arthropods. Given that bees could be exposed to contaminated dust for a period longer than the 1 hour on test (or duration of seeding), both in the air and once the dust has settled on plant surfaces, it becomes difficult to separate exposure from dust from dietary routes – e.g. consumption of nectar, pollen, guttation fluids and other plant juices, and drinking of dew and from other contaminated water sources such as puddles (Samson-Robert *et al.* 2014).

Krupke *et al.* (2012) analysed samples of honeybees from kills associated with the planting of treated maize seed. Clothianidin levels in dead and dying bees were much lower than in the

Tapparo *et al.* (2012) study, ranging from .0038 to .013 ppm. However, Tapparo *et al.* (2012) also mentioned that dead bees sampled in the hive after sowing had residue levels below detection limits. Clearly, the more relevant scenario from a risk assessment point of view are levels of insecticide dust retained by flying insects as they alight on nearby vegetation at dusk and become prey to foraging bats.

4.4.4 Neonicotinoid Residues in Insects not Associated with Recent Applications.

Hladik *et al.* (2016) collected native bees either in grasslands in proximity to wheat fields or inside the wheat fields proper in northeastern Colorado. Most samples were collected from late July to early September a long time after any seeding operation. Thiamethoxam was the most frequently detected neonicotinoid. The highest concentrations were detected in bees from grasslands. They averaged 0.1 ppm and had a sample maximum of 0.31 ppm. Imidacloprid and clothianidin were also detected but at lower concentrations.

Codling *et al.* (2016) collected live bee samples from apparently healthy apiaries with a mixture of canola, forage crops such as alfalfa and wild flowers within 10 km of the hives. Clothianidin was the most frequently detected (56% of bee samples), presumably from past canola treatments. Maximum measured concentrations of clothianidin were 0.079 ppm (reported as 7.1 ng/bee for a 90 mg bee).

Botias *et al.* (2017) collected foraging bumblebees in mixed agricultural lands of southeast England with canola, winter wheat, barley and pasture being the dominant crops. Others were collected from nearby urban environments. Levels of neonicotinoid insecticides were lower than those reported by Hladik *et al.* (2016). The maximum residue for any pesticide detected was 0.054 ppm – the fungicide boscalid from an urban site. Combined residues did not exceed 0.1 ppm in any of the species sampled.

4.4.5 Choosing Reasonable Residue Values for Risk Assessment Purposes

For acute exposure scenarios following a spray or seeding event, we propose to use the RUD value of 105 ppm for spray applications in keeping with US EPA procedures. We will use 409 ppm for seeding operations following the work of Tapparo *et al.* (2012).

For sub-chronic exposures, (weeks to a few months with repeated exposure to treated areas) we propose to use the EFSA recommendation of 21 ppm. This value will be used for both foliar and seed-treatment applications. This is predicated on the fact that the dust produced at seeding is likely to be washed off/rubbed off fairly quickly so that the very high insect levels expected immediately after seeding are probably short-lived.

Finally, to assess season-long risk in bats not necessarily associated with agricultural crops, or at least not associated with any recently-treated crops, we believe that a value of one ppm is appropriate. There is not much data here on which to base a reasonable value. However, even

with limited sampling, Hladik *et al.* (2016) reported a sample maximum of 0.31 pm in bees from grasslands in agricultural landscapes. This level of contamination might be expected in bats that have a short or even a negligible exposure to agricultural fields. It will clearly be under-protective in the case of bats that spend any part of their active season in proximity to crops subject to treatment.

4.5 Estimating the Daily Consumption of Insects by Bats

Based on the review by Kunz *et al.* (2011), insectivorous bat species consume approximately 25% of their body weight in insects for subsistence in captivity. However, field metabolic rates in times of peak energy demand (lactation in females) have given values of 70% of body mass (12 g Brazilian Free-tailed Bat – Tadarida brasiliensis) and 125% of body mass (7.9 g Little Brown Myotis – Myotis lucifugus). This value is likely to be higher still for the smaller species.

Because bats will not continuously be at peak energy demands, we relied on the recent review of existing doubly-labeled water studies by Bullen (2017). A combined regression for active bats including both sexes as well as non-pregnant, pregnant and lactating females gave the following regression for FMR (Field Metabolic Rate):

FMR (KJ/day): 5.7981 * (mass in g)0.7791

Based on the compendium of bat body masses compiled by Norberg and Rayner (1987), we chose a body mass of 4.2g given for the California Myotis. Most of the myotis species (a large component of the bat fauna in Canada) range between four and eight grams. Given the nature of metabolic rates in mammals, it can be expected that food consumption as a proportion of body weight will be highest for the smallest species. It is therefore important to choose a species at the low end of the bodyweight range in order to be protective of other species (given everything else equal; we know nothing of differing toxicological susceptibility). In addition, small-bodied species are probably more at risk by virtue of the fact they have fewer reserves to rely on in times of pesticide-induced illness or food shortage or both.

For a 4.2 g California Myotis, this equals to an average energy expenditure of 17.7 KJ/day. Based on Smit (2009) we can expect insect food to contain 21.9 KJ/g (dw) or 6.46 KJ/g (wet weight) assuming a 70.5% moisture content. The average assimilation efficiency for shrews and bats is given as 88% by Smit (2009). Based on these estimates, the daily average intake of the bat will be: (17.7 kJ/day / 6.46 kJ/g) * (1/0.88) = 3.12 g of insects per day. This represents about 74% of its body mass.

We therefore propose to use documented peak daily energy demand at 125% of bodyweight for the acute scenario (despite the fact that this will underestimate peak demand in the smaller myotis species we are modeling), and 74% for the sub-chronic (summer-long) estimate. For a chronic (i.e. one year) intake, we estimated an average daily intake of 41% of body mass. This

assumes 6 months of activity and 6 months of torpor with metabolic needs approximately 1/10th of active (Speakman and Rowland 1999). This is because temperate insectivorous bats store fat for hibernation not so much from hyperphagy (since insect levels tend to be lower at that time of the year) but through control of their energy demands through torpor.

4.6 Effect Levels from Toxicological Studies

Toxicity endpoints are used to establish toxicity thresholds, which are levels of a compound below which adverse effects are not observed. In order to identify likely effect levels based on toxicological studies, we relied primarily on regulatory reviews of the pesticides carried out by the European Union (European Commission (EC)), the European Food Safety Authority (EFSA), the US Environmental Protection Agency (USEPA), the Canadian Pest Management Regulatory Authority (PMRA) and California Department of Pesticide Regulation (CaDPR). Those reviews were obtained opportunistically up to the end of 2017. Regulatory bodies can be inconsistent in how much they report and when, following their review of submitted industry studies; this makes it useful to track down multiple reviews of industry data given that the full studies are not made public. When reviews of the same toxicological studies by different regulatory bodies are in agreement, this provides added confidence in the endpoints reported (although the increasing harmonization of US and Canadian reviews reduces the value of comparing those two reviews). In addition to regulatory reviews, we relied on our original literature search of Gibbons *et al.* (2015) and the recently published update (Pisa *et al.* 2017) but re-accessed the original publications in order to review effect levels and present them in a clearer fashion. The recent reviews by Cimino *et al.* (2016), Han *et al.* (2018) as well as Mikolić and Karačonji (2018) also proved useful to see whether key references might have been missed. Newer references were added where relevant. Where different values were derived for males and females, the lower of the two was chosen. We favoured oral dosing studies because intraperitoneal exposures are typically difficult to compare to gavage or dietary intakes without a detailed knowledge of pharmacokinetics in the test species. We decided to ignore dermal exposure for the time being although we recognize this may be an important route of exposure in some cases – e.g. contact with foliage in the course of foraging, rub-off during prey handling etc. We restricted our review primarily to in vivo dosing studies although in vitro studies are mentioned where relevant. In studying the potential for immune or endocrine effects of chemicals, in vitro systems are commonly used. For example, concerns have recently been raised with respect to the endocrine effects of thiamethoxam, thiacloprid and imidacloprid (Caron-Beaudoin and Sanderson 2016; Caron-Beaudoin *et al.* 2017) as well as the immunosuppressive effects of clothianidin (DiPrisco *et al.* 2017) using cell lines. In vitro methods have also shown the potential for effects on different mammalian cholinergic subtypes and have raised questions about the adequacy of in vivo testing to date. However, results from in vitro tests are seldom if ever used directly in human or wildlife risk assessments. Rather, they are used as triggers for more realistic in vivo studies or for directing epidemiological investigations.

Given the mode of action of neonicotinoids, it isn't surprising that neurotoxicity has been a focus of several investigations. Abreu-Villaça and Levin (2017) provide a recent review of the neurotoxic effects of neonicotinoids (as well as other insecticide classes). They warn that most of the studies have been on imidacloprid and that few studies have looked at the neurotoxicity of neonicotinoid metabolites despite the fact that some of those metabolites are known to have a higher affinity than the parent chemical to mammalian neural receptors. EU regulators (EFSA 2013) have expressed concerns based on in vitro receptor work (e.g. Kimura-Kuroda *et al.* 2012, 2016) that excitation or desensitization of specific nicotine acetylcholine receptors – as occurs with exposure to nicotine – could cause developmental neurological effects. They believe that studies of delayed neurotoxicity effects that were submitted for both imidacloprid and acetamiprid indicate a risk. An industry-led review (Sheets *et al.* 2016), following a strict protocol that caused the rejection of a number of independent studies, disputed those concerns and concluded that neonicotinoid insecticides, despite their known affinity to different cholinergic receptor types, 'do not selectively affect the developing nervous system'.

NOAELs or LOAELs (whether mortality or signs of toxicity) are often not provided in summaries of acute toxicity – e.g. LD50 determinations. Ideally, the original industry studies should be reviewed in order to derive dose levels at which clinical intoxication was observed, assuming the observation protocol is adequate. Such intoxication is expected to be biologically relevant in a wild organism and, as discussed above, a credible assessment of wildlife impact should try to predict debilitation as well as death. In the case of pesticides with a neurotoxic mode of action, a very useful study to assess the possibility of debilitation in exposed wildlife is the acute neurotoxicity screen. In theory, this should provide a more consistent measure of functional impairment than simple observation of individuals in the acute lethality studies.

NOAELs have serious limitations as endpoints – largely because they are sample size dependant. This is not a new problem but some useful recent guidance is provided by USEPA (2012b). For example, with an acute dosing study employing six animals per dose group, and a dichotomous response such as whether tremors or ataxia are or are not observed in dosed animals, the 95% upper confidence limit of the NOAEL approaches the EC50. In other words, the NOAEL may actually be the dose at which half of the subjects could be showing an adverse effect had sample sizes been higher. In developmental toxicity studies with 20 litters per dose, it has been shown that the typical response levels at the NOAEL is in the range of 5-20% on average – not 0% as the 'N' in NOAEL would imply. For that reason, statistically-based curve fitting exercises such as the 'Benchmark dose' (BMD) have been proposed (USEPA 2012b). These are levels calculated based on curve-fitting of the data and a defined effect threshold, typically of 5 - 10%. Where calculated, the BMD will be reported rather than the NOAEL. Unfortunately, this was a rare occurrence. The mammalian toxicology literature remains overwhelmingly dominated by the NOAEL concept.

Eidels *et al.* (2016) made use of this concept when acutely dosing Big Brown Bats with the OP neurotoxicant chlorpyrifos. They calculated a lower 95% limit of a BMD10 (the 'benchmark dose'

calculated to give rise to a 10% increase in response – akin to a LOEAL) to be 3.5mg/kg, 5.3mg/kg and 6.6mg/kg for impaired flight, appearance of tremors and impaired movement respectively. The LD50 was estimated to be higher than 60 mg/kg (4 out of 20 deaths at that dose). This suggests that the spread between functionally-important impairment and median lethality is well over a factor of 10, at least in the case of this cholinesterase-inhibiting insecticide.

4.6.1 Imidacloprid

Acute, sub-chronic and chronic effect levels based on regulatory reviews of imidacloprid are summarized in Appendix 1. In some cases, the same studies were interpreted differently by different jurisdictions. Notable is the concern by California EPA (CaDPR 2006) that, because brain morphological changes were seen in the developmental neurotoxicity study at the higher dose of 55 mg/kg but not looked for at lower doses, the reported NOAEL cannot be accepted. In their review of the delayed neurotoxicity study that produced those histopathological changes in the brains of females exposed in utero to 55 mg/kg/day, EFSA similarly expressed concern and recommended that a conservative NOAEL for human protection should be set at 5.5 mg/kg/day (EFSA 2013). This issue is not raised by USEPA in their latest draft evaluation (USEPA 2017). Also absent from discussion is the finding of significantly skewed sex ratios indicating possible endocrine toxicity after 10 days of dosing in one of the rat reproductive studies. Both of these findings are summarized in PMRA (2016) but apparently not used in setting a reference dose.

Other Published Toxicological Studies on Imidacloprid

A number of sub-chronic studies have been carried out by independent researchers but these studies have not lead us to change reference doses established through the regulatory studies. They are nevertheless reported here for the sake of completeness.

Lonare *et al.* (2014) documented locomotor changes and oxidative damage to nerve tissues of rats given 45 mg/kg/day for 28 days. In appearance, the rats were said to show 'dull movements'. No NOAEL was established from these results. In their 90 day feeding study in female rats, Bhardwaj *et al.* (2010) identified several toxicological effects at 20 mg/kg/day, placing a NOAEL at 10 mg/kg/day. Kapoor *et al.* (2011) similarly showed effects on female rat reproductive physiology at 20 mg/kg/day for 90 days with a NOAEL of 10 mg/kg/day. Toor *et al.* (2013) documented liver histopathological effects on mature female rats dosed with 45 mg/kg/day (using formulated material) for four weeks but no such effects at 9 mg/kg/day. Arfat *et al.* (2014) showed effects on bodyweight, as well as kidney and liver effects in mice given 15 mg/kg/day for 15 days. The NOAEL was 10 mg/kg/day. Neonatal rats dosed with 1.1 mg/kg for seven days showed disruptions in thyroid hormone titers (Ibrahim *et al.* 2015).

Bal *et al.* (2012a, b) documented several effects on the male reproductive system of mice starting at 0.5 mg/kg/day for 90 days. Testosterone levels as well as reproductive organ weights were affected

at this low level. DNA breakage and sperm deformities and were also seen at 2 mg/kg/day and 8 mg/kg/day. However, these effects mirrored bodyweight loss which may be a confounding factor. Hafez *et al.* (2016) found effects on sperm motility, vitality and morphology in rats given doses of 45 mg/kg per day or higher for 15 days while Abdel-Rahman Mohamed *et al.* (2017) documented a number of testicular, sperm and related gene expression effects in male rats dosed with 1 mg/kg/day for 65 days. Loss of bodyweight was also recorded. A NOAEL was not derived in either study. Incidentally, similar effects on sperm quality have been seen in agricultural workers and the effects appeared to correlate with plasma concentrations of imidacloprid (Hafez *et al.* 2016). However, no other pesticides were measured in that study.

Mikolić and Karačonji (2018) reviewed additional reproduction studies in rodents but these did not change any of the effect levels already established.

Kara *et al.* (2015) dosed adult and infant rats by daily gavage with 0.5, 2 and 8 mg/kg/day doses for a three month period. Using a standard water maze test, they documented deficits at 2 mg/kg/day in the infants and adults – although the effects were more pronounced in the infants. NOAEL dose levels were 0.5 in both cases although the data appear to show a clear dose-response (but NS) between control and 0.5 mg/kg/day. Khalil *et al.* (2017) gavaged adult rats with 0.5 and 1.0 mg/kg/day for 60 days. Behavioural changes, locomotor and swimming abilities were detected at both dose levels and a LOAEL was therefore <0.5 mg/kg/day.

A highly relevant study – because the only one that we know of actually carried out in a bat species –was that of Hsiao *et al.* (2016). They studied the effects of imidacloprid on the spatial memory of Formosan Leaf-nosed Bats. Three bats were dosed with 20 mg/kg/day for a five day period. After the second dose, echolocation ability was compromised. By the end of the experiment, increased apoptosis of neural cells in different parts of the hippocampus were described. No NOAEL was derived in this experiment.

Bats that go into hibernation are highly dependent on lipogenesis. An interesting study in this regard is the recent one by Sun *et al.* (2016). They fed low levels of imidacloprid to mice concomitantly given a low or high fat diet for a 12 week period. Mice at the lowest feeding levels of imidacloprid (measured at 0.08 mg/kg/day) and given a high fat diet had significantly higher bodyweight gain and a clear trend to higher individual adipose tissue weights (with the exception of mesenteric fat); the other adipose tissue weights were significantly higher at the next higher dose of 0.8 mg/kg/day. Histological analysis revealed that the lowest 0.08 mg/kg/day exposure did result in enlarged adipocyte size in at least one adipose tissue in the mice on a high fat diet. The authors also documented changes in blood leptin levels associated with the lowest dose levels as well as insulin resistance and various changes in mRNA expression involved in metabolic processes – in most cases showing a trend at the lowest dose level and significant changes at 0.8 mg/kg/day.

Weight increases were seen in rats given 0.5 mg/kg/day (but not 1.0 mg/kg/day) for 60 days and there was evidence of hyperglycemia, reduced insulin levels and impaired glycogenesis at both 0.5 and 1.0 mg/kg/day (Khalil *et al.* 2017).

Reference Doses for an Imidacloprid Bat Assessment

Based on the studies summarized above and in Appendix 1, we propose the following starting points for a bat risk assessment:

- For acute exposure scenarios, there is a strong convergence of NOAEL values for three species between 8 to 10 mg/kg. A starting point (reference dose) of 8 mg/kg is therefore reasonable. The neurotoxicity screen shows impairment in the rat at levels that may be as low as 9.3 mg/kg – yet simple observational data suggest the much higher NOAEL of 50 mg/kg. Given that the rat is clearly less sensitive than either the mouse or dog, it is likely that a thorough neurotoxicity screen on either the mouse or dog would uncover deficits much below the reported observed NOAELs of 10 and 7.8 mg/kg respectively. Tremors and other neurotoxic manifestations as were observed in the dog study would clearly be relevant to the survival ability of bats exposed to residues in their food supply. Given that neural cell apoptosis was seen in a bat species at just over twice that dose level, it does not seem overly conservative. There may be a slight margin of safety (approximately 2-7X depending on the study) based on the fact that these endpoints are NOAELs rather than actual effect levels. However, the idea that the true NOAEL has been found may be illusory as described above. In order to account for interspecies differences in sensitivity, we submit that a factor of 10 is amply justified. That is the usual interspecific factor retained in risk assessments. This would place our putative bat acute reference dose at 0.8 mg/kg.
- There is a multiplicity of possible endpoints for sub-chronic exposure scenarios. Based on the regulatory studies summarized above, the lowest NOAEL would be 7.3 mg/kg. In independent studies, there have been repeated findings of effects on sperm quality, motility or vitality in dosed rats. These have been variously reported at dose levels as low as 1 mg/kg/day for 65 days, 2 mg/kg/day for 90 days, 8 mg/kg/day for 90 days and 45 mg/kg/day for 65 days. However, reductions in sperm quality – clearly a concern in a human risk assessment – did not appear to translate into functional deficits in the chronic reproduction study. NOAELs in most independent studies have hovered around 10 mg/kg/day with a few notable exceptions. Notable is the claim by Khalil *et al.* (2017) that they detected and measured reduced locomotor activity and swimming ability in adult male rats and documented behavioural changes at a dose as low as 0.5 mg/kg/day for 60 days. This appears to be somewhat of an outlier and notwithstanding, we propose on the strength of evidence to use the value of 7.3 mg/kg/day for the dog NOAEL. This would give us a bat reference dose of 0.7 mg/kg/day for bats – not substantially different than the acute reference dose. It is difficult to know the real world significance of the metabolic research by Sun *et al.* (2016). Effects on lipogenesis in mice were

documented at dose levels 10 fold lower than our proposed reference dose. Given the critical nature of such metabolic processes for a hibernating species, this is a much needed area of research and will remain a large uncertainty in this risk assessment. Bats and, presumably, other hibernating species may have a very high sensitivity to some environmental contaminants for this reason.

- For chronic exposure scenarios, the value of 5.7 mg/kg/day chosen by most regulatory authorities and based on thyroid toxicity in the rat seems appropriate. This would place a chronic bat reference dose at approximately 0.6 mg/kg/day.

4.6.2 Thiamethoxam

Given that clothianidin is a major environmental metabolite of thiamethoxam, it is debatable whether separate endpoints should be generated for the two insecticides. Nevertheless, endpoints summarized in Appendix 2 were gleaned from a number of regulatory reviews. Differences of opinion between different regulatory bodies are noted in the appendix. It can be illustrative to look at successive reviews by the same agency. For example, PMRA (2001a) proposed to calculate an allowable dietary intake based on a chronic multi-generation rat NOAEL of 0.6 mg/kg/day. Following receipt of a second multi-generation study with a higher NOAEL of 1.6 mg/kg/day, a combined NOAEL of 1.2 mg/kg was derived (method not given); effects on F1 testes were seen at 1.8 mg/kg in the first study and 3.0 mg/kg in the second. USEPA (2011) also proposed this endpoint for the derivation of chronic dietary intakes in humans. PMRA (2007) argued that this endpoint would be relevant to the calculation of reference doses for occupational risk assessments even with short exposures.

Endpoints for Thiamethoxam Chosen for a Bat Risk Assessment

- Very little information exists to allow extraction of useful data (e.g. signs of distress or debilitation) from the acute lethality studies. PMRA and USEPA both use the developmental NOAEL of 34 mg/kg as the departure point for derivation of acute reference doses. Effects seen in laboratory animals (i.e. reduced brain size) are serious enough to be applied to a wildlife risk scenario. PMRA (2007) stress that these effects could happen following a short acute dose. The large spread between NOAEL and LOAEL suggests that this may provide a level of conservatism. On the other hand, the acute NOAEL of 25 mg/kg for clothianidin, a major metabolite of thiamethoxam suggests that a NOAEL of 34 mg/kg for thiamethoxam is reasonable. Applying the usual interspecific extrapolation factor gives a bat reference dose of 3.4 mg/kg.

- Organ toxicity in rats and mice were seen at NOAELs of 1.7 and 1.4 mg/kg respectively in 90 day sub-chronic dosing industry studies (Appendix 2). With shorter (28 day) dosing in rats, the NOAEL for kidney toxicity increased to 8.0 mg/kg. This also corresponds to haematological

effects as well as ovary and testis toxicity in the dog. This value seems to be a more reasonable fit (not as overly conservative) for a wildlife scenario. This would mean a bat reference dose of 0.8 mg/kg.

- The chronic reference dose of 1.2 mg/kg for testicular and sperm effects in male offspring is a reasonable endpoint for a wildlife assessment – especially since effects were seen at an exposure level not much higher than this – 1.8 mg/kg/day. This would suggest a chronic reference dose of 0.12 mg/kg/day. As suggested by the PMRA, this endpoint might be relevant to shorter exposure duration also.

4.6.3 Clothianidin

Available regulatory studies (Appendix 3) suggest that the rat is somewhat insensitive relative to other usual mammal test species. The regulatory reviews of the industry submission for Clothianidin were not as divergent as they were in the case of imidacloprid and thiamethoxam.

Other Published Toxicological Studies on Clothianidin

Hirano *et al.* (2015) administered purified clothianidin (extracted from formulated material) to mice and found that it enhanced anxiety behaviours at the lowest dose tested – a measured estimate of 7.5 mg/kg/day for a four week period. In subsequent research (Hirano *et al.* 2018), they documented increased levels of anxiety following a single oral administration of 5 mg/kg. At the higher dosing level of 50 mg/kg, the mice emitted a high number of distress calls when subjected to the same test environment.

Bal (2012c) documented an increased proportion of abnormal sperm cells in mice given 8 mg/kg/day for 90 days but with a clear but non-significant trend observed at 2 mg/kg/day. A subsequent study (Bal *et al.* 2013 reviewed in Han *et al.* 2018) found decreased epididymis weights and other testicular effects at 2 mg/kg/day for 90 days. Ozdemir (2014) using a similar protocol to that of Kara *et al.* (2014 – cited above in the imidacloprid section) administered 2, 8 and 24 mg/kg/day to infant and adult rats for 3 months, after which they were tested in a water maze. Infant rats appeared to be affected at the highest dose (NOAEL therefore 8 mg/kg/day) in a recall test only. Adults did not appear to have any consistent memory effects at any of the doses tested.

Based on his own reproductive study in mice, Tanaka (2012) noted (mostly) accelerated development of pup behavior at low and mid doses (15 and 30 mg/kg/day intake during lactation) but saw no effects at the higher dose.

Endpoints for Clothianidin Chosen for a Bat Assessment

- For acute dosing scenarios, the endpoint of 25 mg/kg was the one retained by both USEPA and PMRA. At the higher 50 mg/kg dose, mice exhibit decreased motor activity, tremors and

respiratory difficulties – all clearly relevant to a wildlife exposure scenario. This would place a bat reference dose at 2.5 mg/kg. This does not seem overprotective in light of the work by Hirano *et al.* (2018) who showed neurobehavioural deficits in mice given a single dose of 5 mg/kg. Clearly the type of impairment documented by Hirano *et al.* (2015) is much more subtle than what has been measured in industry studies.

- For the purpose of a bat risk assessment, we submit that a NOAEL of circa 7.5 – 8 mg/kg/day is defensible for sub-chronic exposure. This corresponds to effect levels on sperm quality as well as behavioural effects in mice. It is slightly more protective than the 9.8 - 11.5 mg/kg NOAEL retained by North American regulators based on the rat chronic study but higher than the one time 5 mg/kg effect level documented by Hirano *et al.* (2018) or the testicular changes seen at 2 mg/kg/day (see review by Han *et al.* 2018). With the usual 10X factor for interspecies differences, this would put the reference toxicity value at 0.75 mg/kg/day, very much in line with the value we derived for imidacloprid and thiamethoxam.
- For the purpose of a chronic risk assessment, we propose to use the value as for the short-term exposure, 0.75 mg/kg, given the slightly higher NOAEL retained by registration authorities.

4.6.4 Acetamiprid

Data from regulatory summaries are tabulated in Appendix 4.

Other Published Toxicological Studies on Acetamiprid

In a very well described and executed study, Sano *et al.* (2016) administered 1 and 10 mg/kg/day in pregnant mice from day 6 of gestation through to weaning after 21 days of lactation. After the offspring reached sexual maturity, they were put through a series of tests to measure their sexual, aggressive and anxiety behaviours. The lower 1 mg/kg/day dose (but not the higher dose) enhanced aggression and sexual response in the male offspring; this was not due to increased testosterone levels. Both dose levels made the male mice more 'foolhardy' (our choice of words) in that it reduced their open field anxiety and possibly made them hyperactive. Such behaviours in wild mice could increase risk of predation. The authors reviewed many studies that showed nicotine can similarly affect anxiety in a non-monotonic fashion.

Endpoints for Acetamiprid Chosen for a Bat Assessment

- The clear (and only) choice of endpoint for an acute exposure scenario is the NOAEL of 10 mg/kg based on locomotor ability. This places a bat reference dose at 1 mg/kg.
- For sub-chronic exposure, we concur with the PMRA and EFSA that the neurological effects seen in rat pups at 10 mg/kg/day are relevant endpoints. The NOAEL for that study was 2.5 mg/kg/day and the bat reference dose would be 0.25 mg/kg/day.

- The standard chronic NOAELs are higher (lowest NOAEL is 6.5 mg/kg/day rather than 2.5 mg/kg/day for sub-chronic exposure; we will therefore defer to the latter for chronic exposures also given that any derived sub-chronic benchmark doses logically apply to chronic exposures also.
- The 1 mg/kg/day effect level obtained by Sano *et al.* (2016) brings uncertainty to these proposed NOAEL values. These data have not been incorporated into regulatory assessments as of yet. Because of the unique methodology used by Sano *et al.* (2016), it isn't clear whether this effect is unique to acetamiprid or is common to all neonicotinoids. The lack of a clear dose-response (these effects were not seen at the higher 10 mg/kg/day dose) will undoubtedly prove a challenge from a regulatory point of view.

4.6.5 Thiacloprid

Data from regulatory reviews are summarized in Appendix 5. The EU (2004b) did not appear to require neurotoxicity studies which seems surprising in light of the insecticide's mode of action.

Other Published Toxicological Studies on Thiacloprid

In a recent study (Babecová *et al.* 2017), 60 ovulating female mice were mated and dosed with either 0.03 or 3 mg/kg during the pre-implantation period (days 1-3 of pregnancy). At that point, the mice were euthanized and blastocysts flushed out of their reproductive tracts. The authors report finding retardation in blastocyst development at both dose levels relative to control. There was no effect, however, on the incidence of dead blastocysts. Unfortunately, the results are difficult to interpret because the statistical analysis of the data appears to be faulty, there being no consideration of individual effects (i.e. all blastocysts considered independent regardless of donors).

Han *et al.* in their 2018 review were able to find a few other studies on genotoxic endpoints for both thiacloprid as well as imidacloprid. However, the dose levels were higher than effect levels already reported for other endpoints.

Endpoints for Thiacloprid Chosen for a Bat Assessment

- For acute exposure scenarios, the NOAEL of 3.1 mg/kg based on locomotor ability is the obvious and most relevant choice for a bat risk assessment. The bat reference value for acute exposure scenarios would be 0.31 mg/kg.
- Sub-chronic effects in both the rat and rabbit are reported with NOAEL values around 2 mg/kg/day. This is probably not overprotective; effects on liver, testes, and prostate were shown to occur at 8.5 mg/kg/day (three months) in dogs with no NOAEL reported for that study. The bat reference value for sub-chronic exposure scenarios would be 0.20 mg/kg with the 10X interspecies extrapolation factor.

- For chronic intake, the NOAEL of 1.2 mg/kg/day is the logical endpoint; liver and thyroid toxicity as well as nervous system degeneration are serious deficits relevant in a wildlife risk assessment. The bat reference value for chronic exposure scenarios would be 0.12 mg/kg. The very small spacing between the NOAEL and LOAEL (2X) leaves a very small margin of safety.

4.7 Summary of Proposed Toxicity Reference Values for Bat Risk Assessment Purposes

Based on the information reviewed above and summarized in Appendices 1-5, here are the proposed values in order to assess the potential direct risks of neonic insecticides to bats through the dietary route.

Table 4.4 Summary of the toxicity reference values derived for acute, sub-chronic and chronic bat assessments.

Active ingredient	Acute reference dose (mg/kg)	Sub-chronic reference dose (mg/kg/day)	Chronic reference dose (mg/kg/day)
Imidacloprid	0.8	0.70	0.6
Thiamethoxam	3.4	0.80	0.12
Clothianidin	2.5	0.75	0.75
Acetamiprid	1.0	0.25	0.25
Thiacloprid	0.31	0.20	0.12

4.7.1 Immune System Effects

Because of the current issue of white-nose syndrome and claims that have been made with respect to a temporal overlap between several wildlife diseases and the advent of systemic insecticides, it is particularly relevant to look at potential effects of neonicotinoids on immune function to see whether the usual effect levels documented in industry data submissions and other independent studies based on neurotoxicity or reproduction may not be protective enough. The field of immunotoxicology, especially the impact of xenobiotics on the developing immune system is in its infancy (Kreitinger *et al.* 2016). The immune system is complex and there are a plethora of different tests and measures that are carried out. We will not attempt to prioritise one type of effect over another but just report results as found by different investigators. Evidence was reviewed earlier that immune disruption may be contributing to impacts on pollinators; there is also some indication that imidacloprid could be immunotoxic in birds (Lopez-Antia *et al.* 2015).

However, the situation in bats is also much more complex than in standard mammalian laboratory models. Bats appear to downregulate some parts of their immune systems during hibernation (Meteyer *et al.* 2012). Indeed, it has been suggested by these authors that the reason white-nosed syndrome is so devastating in North American bat species is that the pathogen is allowed to

proliferate largely unchecked during the bat's torpor; upon post-hibernal emergence, bats show a massive inflammatory response and consequent immune-mediated tissue destruction called the "immune reconstitution inflammatory syndrome" or IRIS.

Disruption of the immune response in any living organism can have devastating consequences; heterothermic bats appear to already be maintaining a fine balance in their immune system.

Imidacloprid

Mohany *et al.* (2012) documented changes in a number of cellular immunological function markers as well as spleen and thymus histopathology in adult rats receiving 0.21 mg a.i./kg/day for 28 days. However, imidacloprid was administered as the 20% Confidor Bayer formulation and the authors could not control for any possible effects of the formulants. Most of these effects were modulated/reversed by concurrent administration of the antioxidant thymoquinone which fits a frequent pattern with neonicotinoid insecticides – cellular damage through the formation of reactive oxygen species.

Badgujar *et al.* (2013) showed that daily administration (for 28 days) of 5 mg/kg or higher in mice had immunosuppressive effects as a result of cytotoxic effects of imidacloprid on T cells. The NOAEL effect in this study was 2.5 mg/kg/day. Both bodyweight and spleen weights were decreased at the higher dose of 10 mg/kg/day.

Clearly, after any interspecies uncertainty factor is applied, the fact that effects on the immune system of rats were seen at 0.21 mg/kg/day places a reference dose much lower than what has been proposed in Table 4.4. At this juncture, however, we do not believe that sufficient studies have been carried out to allow the use of an immune system endpoint in the formal risk assessment being presented here. Rather, it will be appropriate, in reviewing the results of our assessment to consider that effects on the immune system (as seen with imidacloprid for example) could be occurring at dose levels an order of magnitude below more 'standard' toxicity endpoints being modeled.

Acetamiprid

Mondal *et al.* (2009) found that dose levels of 25 mg/kg/day for 28 days affected humoral-mediated immunocompetency in rats. Cellular immune response was affected only at higher doses. The material was administered as a 20% formulation and it isn't clear whether the dose level was expressed as product or active ingredient. (However, a later study – Mondal *et al.* 2011 – describes similar signs of toxicity at the same dosage levels of the active ingredient.) The 25 mg/kg/day dose produced mild salivation but no other visible signs. Tremors, excessive salivation and hyperaesthesia were observed at the highest dose level of 200 mg/kg/day.

Shakthi Devan *et al.* (2014) documented effects of formulated (a 20% SP-soluble powder, Nagarjuna Agrichem Ltd., Hyderabad, India) acetamiprid on immune function (lymphoproliferative response towards B cell mitogen and nitrite production of macrophages) following 90 days of dosing with 22 mg a.i./kg/day. The NOAEL for this study was 11 mg/kg/day. No effects were seen on spleen, thyroid or mesenteric lymph node weights or histopathology. Unfortunately, there were no controls for a potential effect of the formulants on the response seen.

In the case of acetamiprid, it appears that the dose levels that have been shown to cause immune toxicity are in fact higher than those derived from more 'standard' endpoints in the corpus of mammalian toxicology studies.

Caption: Northern long-eared Myotis foraging. Photo credit: Sherri and Brock Fenton.

4.8 Summarizing the Elements of the Direct Risk Assessment

A common measure of risk assessment is the extent to which the various derived reference doses have been exceeded once exposure has been estimated. This is best conveyed as a risk ratio, for example, the ETR (Exposure Toxicity Ratio). It is simply the expected exposure divided by the reference dose. Any ratio over one implies a concern; the higher the ratio, the more reduced any margin of safety brought about by any conservative assumptions made in assessing exposure or toxicity. Unlike a human risk assessment, we have not made this assessment particularly conservative. For example, the only uncertainty factor that was introduced was a factor of 10 to account for possible interspecies differences in susceptibility. Although the reference doses were based on NOAELs, the literature is clear about the fact that, with the small number of animals used in most toxicology studies, the NOAEL is not a true no-effect level but denotes an incipient effect in anywhere between 5 - 50% of the population at large.

Results of the acute exposure scenario are outlined in the tables that follow:

Table 4.5. Results of an acute risk assessment for a small myotis species weighing 4.2 g and feeding at peak FMR equivalent to FW food intake of 125% of mass per day.

Active ingredient	Application type	Max application rate (g a.i./ha)[a]	Insect RUD value (ppm/ kg a.i. application)[b]	Insect residue values (ppm)[c]	Residue intake in mg/kg[d]	Bat acute reference dose (mg/kg)[e]	ETR (Exposure/ Toxicity ratio)[f]
Clothianidin	Airblast	210	105	22.1	28	2.5	11
Clothianidin	Foliar	350	105	36.8	46	2.5	18
Clothianidin	Seed treatment	99	409	40.5	51	2.5	20
Acetamiprid	Airblast	168	105	17.6	22	1	22
Acetamiprid	Foliar	84	105	8.8	11	1	11
Acetamiprid	Seed treatment	45	409	18.4	23	1	23
Thiacloprid	Airblast	210	105	22.1	28	0.31	89
Imidacloprid	Airblast	91	105	9.6	12	0.8	15
Imidacloprid	Foliar	330	105	34.7	43	0.8	54
Imidacloprid	Seed treatment	196	409	80.2	100	0.8	125
Thiamethoxam	Airblast	96	105	10.1	13	3.4	4
Thiamethoxam	Foliar	700	105	73.5	92	3.4	27
Thiamethoxam	Seed treatment	41.7	409	17.1	21	3.4	6

[a] From Table 4.1; [b] From section 4.4.5 and the preceding sections; [c] Product of columns a and b
[d] Calculated for the scenario given: A small myotis species weighing 4.2g and feeding at peak FMR equivalent to FW food intake of 125% of mass per day, [e] Based on the relevant sections pertaining to each active ingredient; [f] Column d / column e.

Table 4.6. Results for the sub-chronic assessment. In that scenario, the same myotis species (4.2g) is foraging to 74% of its bodyweight daily over the course of the summer period (approximately three months).

Active ingredient	Application type	Max application rate (g a.i./ ha)	Insect RUD value (ppm/ kg a.i. application)	Insect residue values (ppm)	Residue intake in mg/kg	Bat sub-chronic reference dose (mg/kg)	ETR (Exposure/ Toxicity ratio)
Clothianidin	Airblast	210	21	4.41	3.26	0.75	4.4
Clothianidin	Foliar	350	21	7.35	5.44	0.75	7.3
Clothianidin	Seed treatment	99	21	2.08	1.54	0.75	2.1
Acetamiprid	Airblast	168	21	3.53	2.61	0.25	10.4
Acetamiprid	Foliar	84	21	1.76	1.31	0.25	5.2
Acetamiprid	Seed treatment	45	21	0.95	0.70	0.25	2.8
Thiacloprid	Airblast	210	21	4.41	3.26	0.2	16.3
Imidacloprid	Airblast	91	21	1.91	1.41	0.7	2.0
Imidacloprid	Foliar	330	21	6.93	5.13	0.7	7.3
Imidacloprid	Seed treatment	196	21	4.12	3.05	0.7	4.4
Thiamethoxam	Airblast	96	21	2.02	1.49	0.8	1.9
Thiamethoxam	Foliar	700	21	14.70	10.88	0.8	13.6
Thiamethoxam	Seed treatment	41.7	21	0.88	0.65	0.8	0.8

Table 4.7. Results from a chronic (year-long) exposure. In this scenario, bats are taking 41% of their body mass per day. This is meant to be an 'amortised' value that accounts for six months of activity and six months of torpor.

Active ingredient	Application type	Max application rate (g a.i./ha)	Insect RUD value (ppm/ kg a.i. application)	Insect residue values (ppm)	Residue intake in mg/kg	Bat chronic reference dose (mg/ kg)	ETR (Exposure/ Toxicity ratio)
Clothianidin	Airblast	210	1	0.21	0.09	0.75	0.11
Clothianidin	Foliar	350	1	0.35	0.14	0.75	0.19
Clothianidin	Seed treatment	99	1	0.10	0.04	0.75	0.05
Acetamiprid	Airblast	168	1	0.17	0.07	0.25	0.28
Acetamiprid	Foliar	84	1	0.08	0.03	0.25	0.14
Acetamiprid	Seed treatment	45	1	0.05	0.02	0.25	0.07
Thiacloprid	Airblast	210	1	0.21	0.09	0.12	0.72
Imidacloprid	Airblast	91	1	0.09	0.04	0.12	0.31
Imidacloprid	Foliar	330	1	0.33	0.14	0.6	0.23
Imidacloprid	Seed treatment	196	1	0.20	0.08	0.6	0.13
Thiamethoxam	Airblast	96	1	0.10	0.04	0.12	0.33
Thiamethoxam	Foliar	700	1	0.70	0.29	0.12	2.39
Thiamethoxam	Seed treatment	41.7	1	0.04	0.02	0.12	0.14

Based on these tabulated assessments, the risk is largely driven by insect residue values and is therefore highest when/if bats forage in or near recently treated crops. In the case of the acute scenario, the defined risk threshold is exceeded in every pesticide and application type; up to 100 fold in one case and more than 20 fold in several other cases. The acute reference dose is meant to be the dose at which motor ability of an exposed bat will not be impaired. Because this is based on crude testing in rats, it is likely that even a slight impairment may have much more serious consequences in a flying mammal that relies on complex echolocation ability and intricate flying manoeuvres to capture its prey and avoid obstacles. It is noteworthy that, in most of the exposure scenarios tabulated, the 20 mg/kg dose rate shown to impair echolocation ability in Formosan Leaf-nosed Bats (Hsiao *et al.* 2016) is exceeded. In that work, impairment in memorizing a regular flight pattern appeared as early as on the second day of dosing. Our calculations therefore support these researchers' opinion that: "… agricultural pesticides may pose severe threats to the survival of echolocation bats."

Some of the rat toxicological data on imidacloprid hint at the margin of safety between a NOAEL derived from the standard motor activity screen and more serious impairment (Appendix 1). Based on the NOAEL and LOAEL, significantly impaired motor activity starts between 12 and 42 mg/kg. Tremors, indicative of much more serious motor impairment start between 50 and 100 mg/kg –

again based on the NOAEL and LOAEL. In the rat, therefore, progressing from a significantly altered motor activity score to outright debilitation such as tremors represents an approximate threefold increase in dose. Given that the NOAEL for motor ability is exceeded by factors often higher than 20 fold and up to 100 fold, it is clear that we should be concerned. Risk quotients of that magnitude will remove any 'safety margin' we have built in to the assessment to account for the fact we use a NOAEL value and the interspecies extrapolation factor. The risk of acute intoxications of foraging bats is highly plausible – as is the risk of sub-chronic intoxications in the course of a summer.

Because the chronic toxicity reference levels are not that much lower than the sub-chronic ones but the average level of contamination of invertebrates 'amortised' over an entire year is much lower, we find that the chronic risk to bats is less of an issue – at least based on the standard toxicity endpoints. Clearly, effects on immune function or effects on lipogenesis and metabolism hinted at in some of the studies reviewed above could be game changers if they occurred in exposed bats and made them more susceptible to white-nose syndrome or other pathogens.

4.9. Uncertainties Associated with a Bat-centric Assessment

Some of the uncertainties associated with a bat-specific pesticide assessment are well recognised and have been summarized in previous work, notably the assessment of the two organophosphorous and carbamate orchard insecticides by Stahlschmidt and Brühl. (2012). As these authors indicated, there is no evidence that bats are necessarily more toxicologically sensitive than murine species to pesticides as judged by a comparison of a very few acute toxicity endpoints. A notable exception here would be an increased susceptibility of bats to bio-accumulating substances because of the potential for sudden release during hibernation.

However, as discussed above, bats are likely to be more at risk (functionally sensitive) than the standard laboratory model species given equivalent doses or exposed to equivalent concentrations in food. As several other researchers interested in risk from chemicals (e.g. Clark and Shore 2001, Stahlschmidt and Brühl 2012, Secord *et al.* 2015b) have already discussed, bats have high energetic needs, a long life-span and low reproductive output. They are highly dependent on torpor, diurnally and seasonally – a process under fine hormonal control that can easily be disrupted. Their gregarious habits make them hugely susceptible to pathogens emphasizing the critical role of immune function, a system very much complicated by their seasonal hibernation.

Secord *et al.* (2015b) also postulate another mechanism by which bats (and other hibernating species) could be uniquely affected: disruption of torpor, either through effects on the thyroid or prostaglandin systems. Regulatory toxicology testing has shown that imidacloprid, thiamethoxam and thiacloprid have shown thyroid toxicity (appendices 1, 2 and 5). Kugathas *et al.* (2015)

identified that imidacloprid (amongst several other pesticides) show prostaglandin antagonism – at a level just below that of aspirin. As pointed out by Secord *et al.* (2015a), the relevance of this possible pathway needs to be researched fully.

As highlighted above, possible low level effects of neonicotinoids on lipogenesis and sexual aggression and behaviour are interesting and need to be researched further. Given the considerable exposure to neonicotinoids in the human population, we trust that some of these aspects will indeed be investigated further.

Finally, bats (much like humans) are not exposed to neonicotinoid insecticides in isolation but, rather show exposure to a number of other contaminants such as industrial chemicals, pharmaceuticals and other personal care products (Secord *et al.* 2015b); in the case of bats, this likely happens when they forage over heavily-contaminated areas such as sewage treatment plants, outfalls and constructed wetlands.

5. Conclusions

Based on the science available to date, there is evidence to support the claim that bats are being negatively affected by neonicotinoid insecticides in several different ways, indirectly through reduction in insect abundance and directly through impairment.

Despite being generalists, bats are highly dependent on an abundant supply of insects in the right size class. Their foraging expenditures are such that they require high insect densities in order to provide a net energetic balance and their echolocation system limits the size range of insects available to them. Reduced prey availability will cause bats to stop foraging altogether and wait for better conditions. There is current evidence for reduced insect densities associated with agricultural intensification. Although this intensification predated the neonicotinoid class of insecticides, there is increasing evidence that neonicotinoids are worsening the situation and hastening insect declines. This is because of their persistence, mobility and systemic nature, their very high invertebrate toxicity as well as their indiscriminate use in prophylactic treatments. Most of the evidence to date for a neonicotinoid-accelerated decline is for terrestrial insects (e.g. pollinators, butterflies, predators and parasitoids). However the extent and level of aquatic contamination from neonicotinoid insecticides as well as the heavy use of surface water by many of our bat species suggest that a reduction in aquatic emerging insects is a key aspect of the bat vs. neonicotinoid insecticide question – indeed as has been shown to be the case with insectivorous bird species (Hallman *et al.* 2014). The impact of neonicotinoid insecticides is very much extended both in time and space compared to the aquatic impacts from older products such as organophosphorous or pyrethroid insecticides. There is evidence that entire watersheds are being contaminated at damaging levels on a year-long basis.

In addition, the best available evidence strongly suggests that neonicotinoid insecticides are affecting bats directly. Despite the fact that these insecticides tend to be of lower toxicity than some of their predecessors (e.g. many organophosphorous insecticides previously registered), bats can be exposed to toxic levels, especially on the short and medium term (i.e. acute and summer-long exposures). There is a real potential for bats to be acutely affected if they forage in or on the edges of treated fields or tree crops. Levels of residues expected, whether from foliar, air blast or seed treatment uses are high enough to put bats at risk of motor impairment and death. It is somewhat counter-intuitive that risk can be as high as or higher from seed treatments than from sprays applications. This is because of the high amount of dust generated in the seeding process. Because seed treatments are currently used prophylactically on a large proportion of the total crop area for our major field crops (e.g. corn, soy, cereals, oilseeds), there will be a risk to bats on most of our agricultural crop area.

Hoary Bat. Photo by Sherri and Brock Fenton.

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Appendix I

Summary of industry mammalian studies with imidacloprid.

Endpoint classification	Species	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Source and notes
Acute (1 dose)	rat	Apathy, ataxia, tremors etc.	50	100	CaDPR (2006) (Note that LD50 values range from 379 to 642 mg/kg based on sex in 3 different studies); PMRA (2016b)
Acute (1 dose)	mouse	Apathy, labored breathing, tremors etc.	10	71	CaDPR (2006) (LD50=131 mg/kg; EFSA (2008))
Acute (1 dose) Neurotoxicity	rat	Decreased motor activity, neurotox. score	9.3-12*	42	CaDPR (2006); NOAEL of 42 according to EFSA (2008); No NOAEL according to USEPA (2017) or PMRA (2016b)
Acute (Effects seen within hours of first exposure of 90 day study)	dog	Tremors	7.8	22-24	CaDPR (2006); EFSA (2008); USEPA (2017)
Sub-chronic Developmental study (10 days during gestation)	rat	Body weight in dams. Terata in pups with endocrine effect noted (higher proportion of male foetuses)	30	100	CaDPR (2006); PMRA (2016b) and Cox (2001) give NOAEL of 10 mg/kg/day for maternal effects
Sub-chronic (98 days)	rat	BW reduction	14	57	CaDPR (2006); NOAEL of 57 according to EFSA (2008).
Sub-chronic (96 days)	rat	BW reduction, liver toxicity	14	61	CaDPR (2006); PMRA (2016b); NOAEL of 61 according to EFSA (2008)
Sub-chronic (91-98 days?) Developmental neurotoxicity	Rat (Fischer)	BW reduction, reduced grip strength	9.3	63	CaDPR (2006); EFSA (2008); PMRA (2016b) (Account of study duration varies)

Developmental neurotoxicity (91 days)	rat (Wistar)	Body W reduction in pups, decreased motor activity in dams	19	55	Cal EPA (2006) (Because brain morphology changes at LOEL but not investigated in NOAEL, recommend NOAEL of 5.5 based on 10X factor); NOAEL of 30 mg/kg according to EFSA (2008); Pup NOAEL of 20 mg/kg according to USEPA (2017) and PMRA (2016b).
Sub-chronic (107 days)	mouse	labored breathing, decreased motility, staggering gait and trembling	86	427	CaDPR (2006) (re-calculated because of unrealistic food consumption data; disparity with acute toxicity noted)
Sub-chronic (28 days)	dog	Food consumption, thyroid and liver toxicity	7.3	31	CaDPR (2006); EFSA (2008); no NOAEL according to PMRA (2016b)
Sub-chronic (90 days)	dog	Tremors	7.8	22-24	CaDPR (2006); EFSA (2008); PMRA (2016b); USEPA (2017) and PMRA (2016b) use this NOAEL as departure point for most of their assessments
Sub-chronic (13 days)	rabbit	Mortality and weight loss in dams, post implantation losses, decreased BW in foetuses	24	72	CaDPR (2006)
Chronic (2 years)	rat	Thyroid toxicity	5.7	17	CaDPR (2006) (but NS trend seen at lowest dose of 5.7); EFSA (2008); PMRA (2016b)
Chronic (2 generations)	rat	Decrease in pup weight	13	38	CaDPR (2006); according to EFSA (2008), parental and pup NOAEL of 20 mg/kg
Chronic (2 years)	mouse	Body weight	47	143	CaDPR (2006) (re-calculated because of unrealistic food consumption data; disparity with acute toxicity noted); NOAEL noted as 208 mg/kg by EFSA (2008)

Chronic (1 year)	dog	Liver toxicity	15	41	CaDPR (2006) (Disparity with shorter dosing studies noted); NOAEL of 41 mg/kg according to EFSA (2008) and PMRA (2016b).

* Effective dose of 12 mg/kg derived from 'benchmark dose analysis' with 5% effect. The 95% confidence bound is 9.3 mg/kg.

Appendix II

Summary of industry mammalian studies with thiamethoxam.

Endpoint classification	Species	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Source and notes
Acute (Neurotoxicity)	rat	Reduced locomotor activity, neurotox panel including drooped palpebral closure, rectal temperature, forelimb grip strength	100	500	EU (2006), PMRA (2001a), CaDPR (2008) (Note: The LD50 is reported as 1563 mg/kg)
Developmental neurotoxicity (brain effects noted as possible following a single exposure)	rat	Maternal BW and food consumption	34	298	PMRA (2007a) (BW, food consumption in dams, BW, delayed sexual maturation and brain size in pups; concludes that the behavioural assessment (water maze) was not refined enough to be useful.) USEPA (2011). CaDPR (2008) considers 298 to be NOAEL.
Sub-chronic Developmental study	rat	Maternal BW gain, 'transient' skeletal variations in pups	30	200	PMRA (2001a, 2007a)
Sub-chronic (90 days)	rat	Kidney toxicity, lesions with clear dose-response	1.7	17.6	PMRA (2001a; 2007a). CaDPR (2008).
Sub-chronic Neurotoxicity (90 days)	rat	No effects seen	95	>95	EU (2006), PMRA (2001a), CaDPR (2008). No indication of neurotoxicity
Sub-chronic (28 days)	rat	Kidney toxicity (but effects may be reduced at higher dose)	8.0	81.7	PMRA (2001a; 2007a). CaDPR (2008).
Sub-chronic (90 days)	mouse	Liver toxicity in males	1.4	14.3	EU (2006), PMRA (2001a; 2007a), CaDPR (2008)
Sub-chronic Developmental study	rabbit	Reduced fetal weight, delayed ossification and increased post-implantation loss	50	150	EU (2006). Effects seen at maternally-toxic doses only. Used as departure point in EU acute reference dose (EFSA 2009b). PMRA (2001a) notes maternal NOAEL of 10 mg/kg/day in range-finding study; CaDPR (2008) gives maternal NOAEL of 15 mg/kg/day

Sub-chronic (90 days)	dog	Haematology, ovary and testis effects	8.2	32	EU (2006), PMRA (2001a; 2007a), USEPA (2011). CaDPR (2008) considers 32 to be NOAEL.
Sub-chronic (28 days)	dog	Food consumption, BW, haematology, thymus, thyroid, brain, liver and spleen changes	31.6	43	PMRA (2001a; 2007a), CaDPR (2008)
Chronic (2 years)	rat	Kidney toxicity	21	63	PMRA (2001a), CaDPR (2008)
Chronic (2 generations)	rat	Testicular atrophy in F1	0.6	1.8	PMRA (2001a; 2007a) reports F1 testicular effect; EU (2006) reports 62 as NOAEL.
Chronic (2 generations)	rat	Sperm number, testis weight in F1	1.6 (1.2)	3.0	PMRA (2007a) (repeat study). PMRA (2007a) proposes combined NOAEL of 1.2 mg/kg/day (given 1.8 and 3.0 LOAELs) as departure point to calculate allowable daily intakes. CaDPR (2008) had initially dismissed testicular findings but nevertheless had derived a similar NOAEL of 1.3 mg/kg/day based on reduced pup weight.
Chronic (18 months)	mouse	Liver toxicity	2.6	64	EU (2006) uses this endpoint in the calculation of allowable daily intake, PMRA (2001a: 2007a).
Chronic (1 year)	dog	Effects on testes, haematology	4.0	21	EU (2006), PMRA (2001a; 2007a), CaDPR (2008).

Appendix III

Summary of industry mammalian studies with clothianidin.

Endpoint classification	Species	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Source and notes
Acute (1 dose) Neurotoxicity	mouse	Decreased motor activity, tremors, deep respirations	25	50	USEPA (2003; 2011). PMRA (2004). Retained as endpoint for acute risk assessment by those regulators
Acute (Neurotoxicity)	rat	Neurotox panel, decreased motor and locomotor activity	60	100	USEPA (2003), PMRA (2004). NOAEL from separate study. Note that the LD50 is reported to be >5000 mg/kg.
Sub-chronic Developmental study	rat	Maternal decreased BW gain and food consumption	10	40	USEPA (2003), PMRA (2004), EU (2005). Developmental NOAEL >125 mg/kg/day
Sub-chronic (90 days)	rat	Decreased BW and BW gain	27.9	202	USEPA (2003), Tanaka (2012)
Sub-chronic Neurotoxicity	rat	Food consumption, BW and BW gain	60	177	USEPA (2003), PMRA (2004). No evidence of neurotoxicity.
Developmental neurotoxicity	rat	Decreased BW and BW gain in pups, startle habituation, motor activity, surface righting, brain histometry findings	12.9	42.9	USEPA (2003), PMRA (2004), EU (2005), Tanaka (2012)
Sub-chronic (28 days)	rat	Food consumption and BW gain	120	228	PMRA (2004)
Sub-chronic (90 days)	rat	BW and BW gain; Liver enzymes	27.9	202	PMRA (2004)
Sub-chronic (28 days)	mouse	Food consumption, lung toxicity	<90	90	PMRA (2004)
Sub-chronic Developmental study	rabbit	Mortality, BW, abortion etc… in dams, lung terata in pups	25	75	USEPA (2003), PMRA (2004).
Sub-chronic (90 days)	dog	Thinness, decreased body weight, body weight gain and anemia (one male); decreased white blood cells, albumin, and total protein (female).	19.3	40.9	USEPA (2003), Tanaka (2012)
Sub-chronic (28 days)	dog	Mortality; organ and blood toxicity	34.3	36.9	PMRA (2004)
Chronic (2 years)	rat	BW and food consumption, liver and kidney toxicity,	27	82	PMRA (2004)

Chronic (2 generations)	rat	Decreased BW gain, delayed sexual maturation, decreased thymus weight in F1 and increased stillbirths.	9.8	31.2	USEPA (2003; 2011). PMRA (2004). EU (2005). Retained as endpoint for chronic assessments. Noted as NAOEL 10.7 mg/kg/day by Tanaka 2012 for Japanese regulatory review and amended to NOAEL of 11.5 mg/kg/day and LOAEL of 36.8 by PMRA (2016a).
Chronic (78 weeks)	mouse	Behaviour (vocalisations), BW and BW gain	47	171	PMRA (2004)
Chronic (1 year)	dog	Anemia	40.1	52.9	USEPA (2003), PMRA (2004)

Appendix IV

Summary of industry mammalian studies with acetamiprid.

Endpoint classification	Species	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Source and notes
Acute (1 dose) (Neurotoxicity)	Rat	Reduced locomotor activity	10	30	EU (2004a) (Note: LD50 given as 314 mg/kg). USEPA (2002) notes that range-finding study noted drop in body temperature at NOAEL. PMRA (2002) gives LD50 for female as 146 mg/kg with clinical signs at 80 mg/kg. Reduced motor activity persists for 14 days.
Sub-chronic Developmental study	rat	BW and BW gain reductions, liver toxicity in dams; skeletal terata (rib shortening) in pups.	16	50	USEPA (2002), PMRA (2002)
Sub-chronic (90 days)	rat	Liver toxicity, BW and BW gain, food consumption	12.4	50.8	EU (2004a), EFSA (2013), USEPA (2002). PMRA (2002).
Sub-chronic Neurotoxicity	rat	Reduced BW and food consumption, efficiency (No neuropathology shown – surprising in light of acute results)	14.8	59.7	EU (2004a), USEPA (2002), PMRA (2002)
Developmental neurotoxicity	rat	Reduced startle response in pups. Effects at higher doses (45 mg/kg) include reduced viability and changes in brain morphology	2.5	10	PMRA (2010) (Notes that maternal NOAEL is 10 mg/kg/day). The pup NOAEL is used as departure endpoint for risk assessments. EFSA (2013) concurs.
Sub-chronic (90 days)	mouse	Reduced BW and BW gain, reduced organ weight, blood chemistry	106	211	USEPA (2002), PMRA (2002)
Sub-chronic Developmental study	rabbit	Maternal BW and food consumption; no developmental toxicity seen (NOAEL of 30 mg/kg/day)	15	30	USEPA (2002), PMRA (2002)
Sub-chronic (28 days)	dog	BW gain	16.7	28.0	PMRA (2002)
Sub-chronic (90 days)	dog	BW gain, food consumption	13	32	USEPA (2002), PMRA (2002)
Chronic (2 years)	rat	Liver and kidney toxicity, BW and BW gain	7.1	17.5	EU (2004a), EFSA (2013), USEPA (2002), PMRA (2002)

Chronic (2 generations)	rat	Reduced postnatal survival and decreased pup weight at parental toxic doses	6.5	18	EU (2004a). USEPA (2002) notes that foetal deaths at 17.9 mg/kg/day might be treatment-related but opt for this value as the NOAEL. Record makes note of possible endocrine effects (vaginal opening, preputial separation) at higher dose of 51 mg/kg. PMRA (2002) also opt for higher NOAEL of 18 mg/kg/day.
Chronic (78 weeks)	mouse	BW and BW gain, amyloidosis in numerous organs (males)	20	66	USEPA (2002). PMRA (2002) gives NOAEL as 66 mg/kg/day.
Chronic (1 year)	dog	BW and BW gain, organ weight changes attributed to reduced food consumption	20	55	USEPA (2002), PMRA (2002)

Appendix V

Summary of industry mammalian studies with thiacloprid.

Endpoint classification	Species	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Source and notes
Acute (Neurotoxicity)	rat	Decreased motor and locomotor activity (females)	3.1	11	(Note: LD50 reported in EU 2004b to range between 396-836 mg/kg depending on study and sex). 11 mg/kg reported as NOAEL for males with LOAEL of 21 mg/kg. PMRA (2007b) uses this value as point of departure for acute reference doses
Acute (4 daily doses during gestation	rat	BW, increased stillbirths	<35	35	PMRA (2007b)
Sub-chronic Developmental study	rat	BW, uterus wt., resorption and abortion in dams; multiple terata in pups	10	50	PMRA (2007b)
Sub-chronic (90 days)	rat	Liver and thyroid histopathology	7.3	28.6	EU (2004b), PMRA (2007b)
Sub-chronic Neurotoxicity	rat	Food consumption, grip strength	24	101	
Developmental neurotoxicity	rat	Decreased BW and BW gain in dams, decreased weight and delayed sexual maturation in male pups.	4.4	26	PMRA (2007b)
Sub-chronic (14 days)	rat	Chavvvnges to liver surface (lobulation), enzyme induction, BW gain (Special study to look at liver and thyroid toxicity)	2.3	9.6	PMRA (2007b) (Effects noted at NOEL but considered non-adverse)
Sub-chronic (14 days)	rat	BW gain, liver histopathology (Special gavage study to look at liver and thyroid toxicity)	20	60	PMRA (2007b)
Sub-chronic (21 days)	rat	Thyroid and liver toxicity (Special study to look at liver and thyroid toxicity)	9.0	36.9	PMRA (2007b)
Sub-chronic (21 days)	mouse	Increased liver weight and enzyme induction	30	368	PMRA (2007b)
Sub-chronic (90 days)	mouse	Increased severity of fatty vacuolation of the adrenal X-zone (in females)	18	27	PMRA (2007b) (NOAEL obtained from separate study)
Sub-chronic Developmental study	rabbit	BW, abnormal urination and defecation in parent; decreased BW of female foetuses	2.0	10	PMRA (2007b)

Sub-chronic (35 days)	dog	Decreased food consumption, BW (females), liver, prostate, thyroid hormone titer.	9.6	66	PMRA (2007b)
Sub-chronic (105 days)	dog	Liver, testes and prostate affected	<8.5	8.5	PMRA (2007b)
Chronic (2 years)	rat	Liver and thyroid histopathology, nervous system degeneration, carcinogenicity	1.2	2.5	EU (2004b), PMRA (2007b). Both regulators propose endpoint for chronic intake calculations.
Chronic (1 generation)	rat	clinical signs, BW gain, liver and thyroid weights in dams; reduced viability and number of pups	20	69	
Chronic (2 generations)	rat	Difficult births (dystocia), liver and thyroid toxicity in dams; reduced pup weight and viability.	2.7 (3.5)	21	EU (2004b), PMRA (2007b) had slightly different calculated NOAEL of 3.5 mg/kg/day
Chronic (78 weeks)	mouse	Liver and lymph node histology, ovary tumors	5.7	234	PMRA (2007b)
Chronic (1 year)	dog	Liver, prostate, kidney histology	8.3	33.8	PMRA (2007b)

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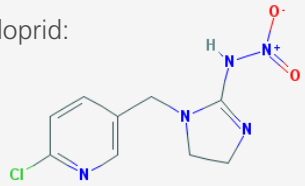

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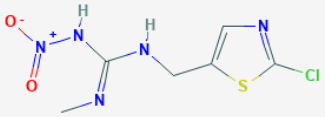

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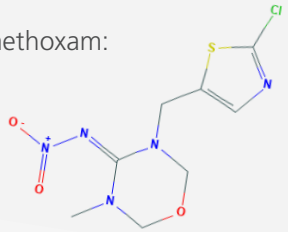

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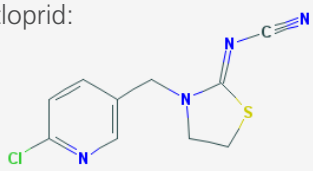

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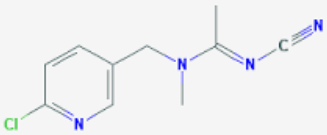

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