THE PRODUCER-POLLINATOR DILEMMA:

NEONICOTINOIDS AND HONEYBEE COLONY COLLAPSE

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DEDICATION

To my mom for her brilliance, guidance, inspiration, and love. Thank You.

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ABSTRACT

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Neonicotinoid insecticides are the most important new insecticide class introduced in the past 40 years. They are the number one selling insecticide in the world, and are used on over 90% of the corn produced in the U.S. However, neonicotinoids could very likely be causing widespread and severe impairment to bee colonies, and possibly contributing to Colony Collapse Disorder (CCD). This is problematic since bees, and honey bees in particular, are the single most important pollinator for global agriculture. Pollination services contribute to one of every three mouthfuls of food consumed (Xerces Society, 2011). Direct pollination services were recently valued in a Cornell University study to be worth 16 billion dollars a year in U.S. farm income (Calderone, 2012). As more is learned about the nature of systemic neonicotinoids and their adverse effects on beneficial pollinators, a potential conflict between crop protection and pollinator conservation becomes clear, posing a dilemma between food production required to feed a growing global population and the risk of widespread colony collapses.

The scientific community has been examining the phenomenon of CCD, and anecdotal links between the bee losses and the application of neonicotinoid insecticides, since it was first noticed by French beekeepers in 1994 and then in the U.S. in 2006. While previous studies failed to demonstrate links to CCD, a new generation of field-realistic studies has chronicled the synergistic and sublethal effects of neonicotinoids on individual bees and colonies over longer-term exposure using real-world foraging conditions. Recent studies strongly support the link between neonicotinoids and CCD (Henry et al., 2012; Whitehorn et al., 2012; Gill, Ramos-Rodriguez, and Raine, 2012; Lu et al., 2012; Tapparo et al., 2012; Krupke et al., 2012). However, independent researchers such as James Cresswell, Jim Frazier, and USDA scientist Jeffrey Pettis (Cresswell, 2011; Cresswell, Desneux, and vanEngelsdorp, 2012; Frazier et al., 2011; Frazier 2012; Grist.org) along with farming and crop protection interests and the producers of the neonicotinoid products all caution that there is not yet enough evidence to draw definitive conclusions, and that there are a variety of causal factors behind CCD. Can these pesticides continue to be used safely in the U.S. or do their risks to pollinators outweigh their benefits to humans and animals?

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INTRODUCTION

Neonicotinoid pesticides have been shown, in multiple independent studies conducted in the U.S. and Europe, to have negative impacts on both wild bees and managed honey bees (Henry et al., 2012; Whitehorn et al., 2012; Gill, Ramos-Rodriguez, and Raine, 2012; Lu et al, 2012; Tapparo et al., 2012; Krupke et al., 2012). France has banned such systemic pesticides based upon the precautionary principle, while in the U.S. neonicotinoids are used on over 90 percent of the U.S. corn crop (Bayer Crop Science Online Resource). The debate over continued approval of this potentially harmful class of pesticides has reached the U.S. EPA, where petitions for review have been raised by an alliance of beekeepers, concerned lawmakers, and environmental defense groups. Proponents of neonicotinoids, those in both agricultural and chemical industries, insist that these chemicals are safe for controlled use in the field and that recent studies used flawed assumptions on actual field dosage and faulty bee-colony-reproduction statistics. Despite the claims to the contrary, there does seem to be accumulating evidence that neonicotinoids, the number one selling class of insecticide in the world, are indeed detrimental to bees, but the question is--at what concentration, and are these realistic exposure rates in nature? Can these pesticides continue to be used safely in the U.S., or do their risks to pollinators outweigh their benefits to humans and animals? Can environmental scientists prove their point(s)? Studies have been published on both sides of this debate; this capstone project will seek to answer the question: are neonicotinoids harmful to pollinators, and if so, to what extent can they be safely used?

This capstone project will provide an overview of the crop protection industry, a summary of the insecticide market prior to neonicotinoid introduction, a history of the development, spread, and necessity of neonicotinoids, an examination of the multiple recent scientific studies conducted on neonicotinoids and their potential links to colony collapse disorder (CCD), an overview of the changing regulatory environment, and a concluding synopsis of the convergent research, along with recommendations for the safe use of neonicotinoids.

A GREEN REVOLUTION

Food is a paramount necessity--everyone has to eat. Hunger has driven population migrations and extinctions, forged hunter/gatherer and farming lifestyles, and, once met, fueled the development of higher culture, education, and fine arts. Nobel Prize winner Norman Borlaug--a groundbreaking agricultural scientist/plant geneticist/agronomist/humanitarian, and a pioneer in the "Green Revolution," whose work in developing "high-yield agriculture" in multiple nations during the mid-twentieth century is estimated to have saved over 1 billion people from starvation--worked from a motivation to curb hunger (Borlaug Nobel Lecture 1970) (see Figure 1).

Figure 1. Dr. Norman Borlaug, (Encyclopædia Britannica Online)

> "The Green Revolution has won a temporary success in man's war against hunger and deprivation; it has given man a breathing space. If fully implemented, the revolution can provide sufficient food for sustenance during the next three decades. But the frightening power of human reproduction must also be curbed; otherwise the success of the Green Revolution will be ephemeral only" (Borlaug, 1970 Nobel Lecture).

Dr. Borlaug achieved dramatic results by working with the International Maize and Wheat Improvement Center to introduce geographically specialized, high-yielding, faster-growing, and disease-resistant crop varieties to farmers in developing countries--essentially teaching them how to grow more food more efficiently. "Wheat production in Mexico multiplied threefold in the time that Borlaug worked with the Mexican government. In addition, dwarf wheat imported in the mid-1960s was responsible for a 60 percent increase in harvests in Pakistan and India" (Norman Borlaug, Encyclopædia Britannica Online). Yields for all developing countries rose 208% for wheat, 109% for rice, 157% for maize, 78% for potatoes, and 36% for cassava between 1960 and 2000 (Pingali, 2012). Combined fertilizer use (Nitrogen, Phosphate, and Potash) increased at an annual rate of 5.5% from 1960 to 1990, from 27 million nutrient tons to 143 million nutrient tons (Bumb and Baanante, 1996).

The production of cereal crops has tripled over the past 50 years with only a 30 percent increase in the land area under cultivation, though these global aggregations mask geographical disparities (Pingali, 2012). China, for example, has planted 82 percent of its arable land in modern crop varieties compared to 27 percent in Africa (Pingali, 2012). The spread of science-based agriculture and shared best practices championed by Dr. Borlaug brought with it an increased reliance on pesticides, fertilizers, and irrigation to keep the specialized crops growing vigorously. It will require continued investment and increasing agricultural inputs to sustain the advances made in the Green Revolution and to enable future increases in food production.

AN AGRICULTURAL NECESSITY FOR FEEDING THE PLANET

Even with the agricultural gains produced by the Green Revolution, too many people, mostly in developing nations, continue to grapple with hunger. "Hunger is the world's number one health risk, killing more people in a year than AIDS, Malaria, and TB combined" (United Nations World Food Programme). According to the United Nations World Food Programme (WFP):

> "925 million people do not have enough to eat and 98 percent of them live in developing countries. 65 percent of the world's hungry live in only seven countries: India, China, the Democratic Republic of Congo, Bangladesh, Indonesia, Pakistan and Ethiopia" (United Nations WFP).

Feeding a growing global population will perpetually challenge individuals and nations alike. The population on Earth is expected to grow from roughly 7 billion present day inhabitants to over 9.1 billion by the year 2050, requiring a food production increase of 70 percent--"involving an additional quantity of nearly 1 billion tonnes of cereals and 200 million tons of meat" (United Nations Food and Agriculture Organization, How to Feed the World 2050). More frequent extreme weather events are expected as the global mean surface temperature continues to rise, resulting in more droughts, heat waves, floods, and severe storms. Additionally, a rising demand for farm land, an increase in affluence and consumption patterns, and increased demand for biofuels from a shared food stock will all be contributing factors to increasing food pressures.

Food shortages and distribution problems presently exist at distressing rates in pockets of hunger around the world. The International Food Policy Research Institute releases an annual report on the global state of hunger. The

2012 Global Hunger Index report (see Figure 2) identified 20 countries with "alarming, or extremely alarming" hunger levels, as described by:

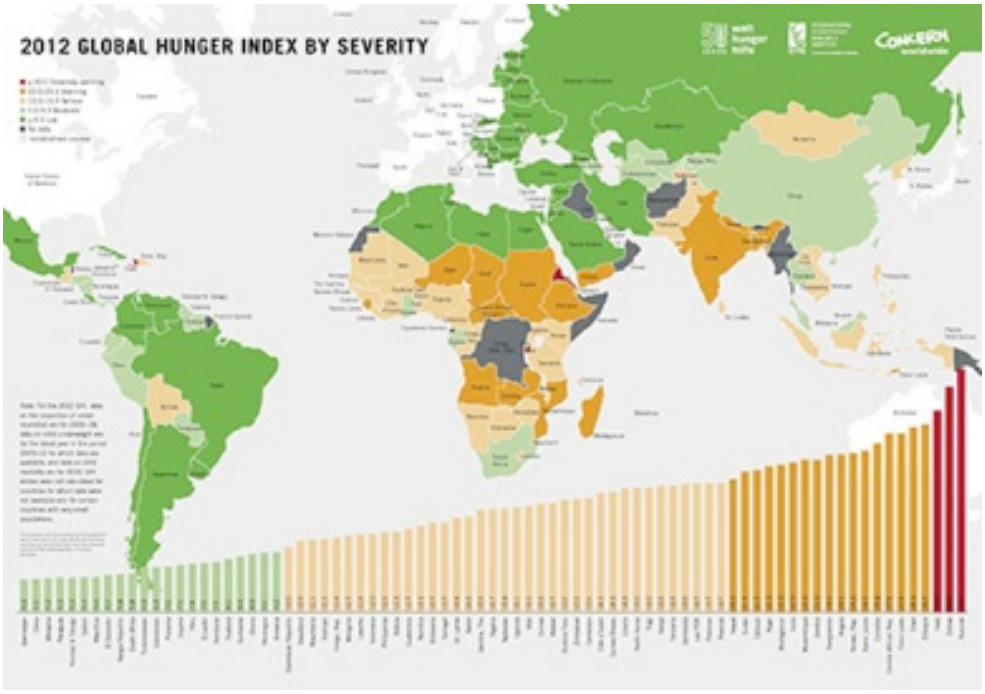


Figure 2. 2012 Global Hunger Index (International Food and Policy Research Institute)

> "The proportion of undernourished as a percentage of the population (reflects share of population with insufficient dietary energy intake); the prevalence of underweight in children under the age of five (indicating proportion of children suffering from low weight for their age); the under-five mortality rate (partially reflects fatal synergy between inadequate dietary intake and unhealthy environments)"
> (INTL Food and Policy Research Institute, 2012 Global Hunger Index).

In 2009 the United Nations Food and Agriculture Programme convened the High Level Experts Forum in Rome, Italy to address the question of "How to Feed the World in 2050?" Three main drivers for increased food pressure were identified as: *population growth, increased urbanization, and increasing incomes*. "In developing countries, 80 percent of the necessary production increases would come from increases in yields and cropping intensity and only 20 percent from expansion of arable land" (United Nations, How to Feed the World 2050). The

Green Revolution has shown that it will require specialized seeds, adequate fertilizer, and adaptive pest control in order to meet global food demand.

PESTICIDE OVERVIEW

It is estimated that chemical insecticides preserve twenty percent of annual crop yield (Blacquiere et al., 2012), making them crucial for sustaining global food supplies. EPA market estimates indicate $12.5 billion is spent on pesticides in the U.S., amounting to nearly one third of the roughly $40 billion dollar aggregate worldwide pesticide expenditure (see Table 1) (EPA Pesticide Industry Sales and Usage 2007). “The use of synthetic pesticides in agriculture is the most widespread method for pest control, with farmers justifying this high cost by a direct dollar return ranging from $3 to $5 for every $1 spent on pesticides" (EPA.com Agricultural Pesticides).

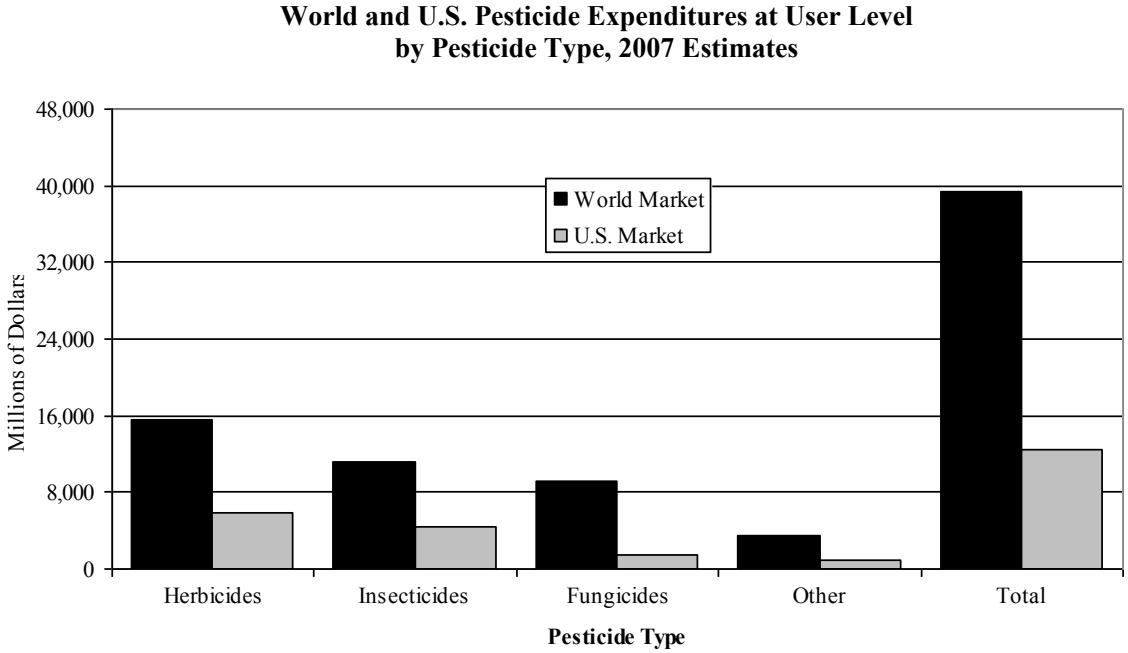


Table 1. 2007 EPA Market Estimates for Pesticide Industry Sales and Usage (EPA.com)

Pesticides are formally defined as:

> “Any toxic substance used to kill animals or plants that cause economic damage to crop or ornamental plants or are hazardous to the health of domestic animals or humans. All pesticides interfere with normal metabolic processes in the pest organism and often are classified according to the type of organism they are intended to control” (Encyclopædia Britannica Online).

The term pesticide has many subcategories. The common name for each may include the pest for which it is targeted (Briggs, 1992). Pesticide categories include: Acaricides, Algacides, Antibiotics, Avicides, Desiccants, Fungicides, Herbicides, Insecticides, Molluscicides, Nematocides, Piscicides, Plant Regulators, Repellents, Rodenticides, Sterilants, and sometimes wood preservatives.

Agrochemicals (which are synonymous with pesticides and plant-protection products) are subdivided into three primary categories; herbicides, insecticides, and fungicides. Herbicides comprise about 45-50% of world pesticide expenditure, followed by insecticides at 25-30%, then fungicides at 20-25%. Nematocides, rodenticides, and fumigants account for the remaining 10% (Pollack, 2011) (see Figure 3). In 2012 there exist over 900 “structurally diverse compounds” that act by roughly 100 mechanisms to control insects, weeds, and fungi (Casida, 2012). In addition, genetically modified crops engineered with Bacillus thuringiensis (Bt) were introduced for the control of pests in 1995, introducing a new era of biological pest control (Casida, 2005). All five major groups of insecticides, detailed briefly in the following pages, act as neurotoxins to insect pests (Walker, 2012).

The history of insect pest control can be divided into three phases: 1) the first, the period before 1870, when natural pesticides were used; 2) the second period, from 1870-1945, characterized by the use of inorganic synthetic pesticides consisting of both natural materials and inorganic compounds; and 3) the contemporary period since 1945 when organic synthetic pesticides were first

synthesized (Zhang et al., 2011). “Insect pest control has evolved from botanicals and inorganics, to chlorinated hydrocarbons, to organophosphorus compounds and methylcarbamates, then synthetic pyrethroids and most recently synthetic nicotinoids as the major classes” (Yamamoto and Casida, 1999, p. v).

What is considered a safe pesticide changes with our scientific comprehension, public awareness, and perception of risk. Some of the pesticides used prior to 1945, and sold with limited restriction, included: lead arsenate, mercury salts and other organic mercury compounds, zinc arsenate, cyanide salts, nicotine, nitrocresol, and sodium chlorate--few, if any of which, are now considered safe (Stenersen, 2004). At the time of its inception, DDT was the first efficient synthetic pesticide that possessed all the desirable properties for an insecticide in that era (Stenersen, 2004). Unfortunately, many pesticides have proven to have unintended consequences for ecosystems and human health. The environmental movement was spurred by Rachel Carson’s *Silent Spring*, and her book’s investigation of DDT insecticide and its associated health and environmental consequences.

There is a level of risk analysis inherent with the use of all pesticides. The goals of green chemistry are to produce pesticides which are safe, effective, and biodegradable with minimal environmental disruption (Casida, 2012).

> “Understanding and optimizing pesticidal activity requires knowledge of structure-activity relationships at the primary target site coupled with structure-biodegradability relationships at the organismal level. Substituents are introduced or replaced to enhance target fit and control rates of bioactivation or detoxification. As a result, pesticides are becoming more potent and selective and generally more complex in structure” (Casida, 2011, p. 2768).

The perfect pesticide--one that is completely non-toxic and non-disruptive to non-target species, immune from pest resistance, and minimally persistent in ecosystems--is still in development. Pesticides have been engineered to be safer to humans and ecosystems while also being less toxic and less persistent in nature, but continue to pose problems for non-target species.

"Pesticide-environment interactions are bidirectional. The environment alters pesticides by metabolism and photo-degradation, and pesticides in turn change the environment through non-target or secondary effects" (Casida, 2012, p. 487). As our knowledge of insects, weeds, genetically modified crops, pesticides, and chemistry has advanced, we have gained the ability to create tailor-made compounds in order to protect our food supply, but have created a feedback loop in the process.

The discovery and application of new forms of crop protection induces a response in individual species and throughout ecosystems. Insects and plants continually evolve and adapt to agrochemicals, and each generation of crop pest develops a greater resistance to the previously effective pesticide, until the chemical compounds in the insecticides and herbicides are no longer effective. New pesticide classes with different modes of action must then be developed to stay ahead of the pest resistance.

Before reviewing the specific properties of the newest major insecticide class, the neonicotinoids, a brief general discussion of the previous major insecticide groups is in order. The following pages note the history, mode of action, and reasons for use and development in the major insecticide groups

used throughout history. See Appendix Table 13 for a listing of some of the major pesticides used and their ecotoxicology.

MAJOR PESTICIDE CLASSES OVER TIME

Botanical Pesticides

The first botanical pesticides included such naturally occurring compounds as nicotine, rotenone, and sabadilla (Silva-Aguayo). Most botanical pesticides work by deterring insects rather than killing them directly. Such compounds "inhibit the normal development of insects" by disrupting the metamorphosis of the insect, by creating a feeding deterrent which will cause the insect to stop feeding and starve, by repellant properties which serve to irritate the insect, or by confusing the insects so they cannot find the specific food source they seek (Silva-Aguayo). 2,500 plants in 247 families exhibit some sort of toxic property against insects (Silva-Aguayo).

> "It has been noted that the Romans first used plant extracts and powdered plant parts as insecticides. There are reports that in 400 B.C. during Persian King Xerxes' reign, the delousing procedure for children was with a powder obtained from the dry flowers of a plant known as pyrethrum (Tanacetum cinerariaefolium, Compositae). The first botanical insecticide, used as such, dates back to the XVII Century when it was shown that nicotine, obtained from tobacco leaves, would kill plum beetles" (Silva-Aguayo).

Tobacco was introduced to Portugal and Spain from the Americas in 1859 by the Indians. It reached France and Italy where it was used mostly for smoking but "since the late seventeenth century has been used as an important insecticide or insect repellent" (Yamamoto and Casida, 1999). Various organic insecticidal preparations such as ground tobacco, the Pyrethrum flower, and organic plant materials containing rotenone continued to be widely used in the

19th century before giving way to the next class of manufactured, inorganic pesticides (Gillis, 1993).

Nicotine is a “non-persistent contact insecticide and its mode of action consists in mimicking acetylcholine when binding to its receptor at the post-synaptic membrane of the muscular union” (Silva Aguayo).

> “The acetylcholinic receptor is a site of action of the postsynaptic membrane which reacts with acetylcholine and alters the membrane permeability. Nicotine activity causes the production of new nerve impulses which cause convulsions, and death” (Silva Aguayo).

Xenobiotics. The compounds that botanical pesticides are derived from can be considered organic pollutants, or xenobiotics, in the sense that a chemical that is normal to one organism may be foreign to another if the xenobiotic compound does not play a role in the organism’s normal biochemistry (Walker, 2012). These poisonous compounds evolved in plants as a defense against predators and insects. Botanical pesticides such as pyrethrins, nicotine, and various mycotoxins are examples of naturally occurring xenobiotics (Walker, 2012).

As plants have evolved compounds to protect themselves, the animals that feed off them have also evolved. Some have referred to this evolutionary process as a “coevolutionary arms race,” and a form of “natural chemical warfare,” where certain grasses, for example, can synthesize secondary enzymes that are highly toxic to the animals that graze off them, thereby protecting this type of grass from grazing. The grazing animals then develop detoxification enzymes to protect against the plant toxins (Walker, 2012, p. 9).

These naturally occurring xenobiotic compounds later provided the conceptual framework for synthesized neonicotinoids and pyrethrin pesticides (Walker, 2012). An important new class of insecticides, commonly known as neonicotinoids, are “synthetic copies or derivatives of the nicotine structure” (Silva-Aguayo). A more in-depth focus on the neonicotinoid category will be provided later in this study, insofar as this is the core of the producer-pollinator dilemma.

Inorganic Pesticides

While natural pesticides derived from plants were considered to be the first class of insecticides, they acted more as benign repellents than as actual insect killers. Inorganic pesticides were more toxic and persistent due to their derivation from inorganic toxicants based on arsenic, copper, lead, mercury, sulfur, fluorine, and other compounds (Casida, 2012). The most popular inorganic pesticides of the mid-19th century, Paris Green and London Purple, belonged to a group of compounds called “arsenicals” (Gillis, 1993). These compounds would not be permitted for agricultural use today but at the time of their discovery, they proved more effective and popular than any other pesticide available. One such inorganic insecticide, London Purple, was a byproduct of the aniline dye industry, and was composed largely of calcium arsenite (Gillis, 1993). Another arsenical was Paris Green, which was the first mass-produced insecticide:

> “Paris Green--the common name for cupric acetoarsenite--is an emerald green powder containing 43% arsenic and was used from 1865 until the 1940s. It effectively controlled the Colorado potato beetle, chewing pests of cotton and many other crops, and mosquito larvae, with sustained U.S. use levels of 4,000,000 lb/yr.” (Casida, 2012, p. 487).

Non-target Species and Persistence. The inorganic class of insecticides provided “partial to adequate” pest control, but were toxic to a variety of non-target pests, and persistent in soils since metals are nonbiodegradable and don’t easily break down in nature. Once the metals enter soils and sediments they tend to stay there for years in the surface layers (Casida, 2012; Walker, 2012).

Organochlorines

Synthetic organic insecticides marked the third evolutionary stage in the battle with agricultural pests, following the botanical and inorganic classes of insecticides previously described. Organochlorine pesticides were developed in the 1940’s and 1950’s following Paul Muller’s Nobel Prize-winning discovery of DDT’s insecticidal properties in 1939 (Walker, 2012). This synthetic organic class of insecticides includes organochlorine insecticides, also referred to as “chlorinated hydrocarbons,” meaning insecticides containing at least one covalently bonded chlorine atom (Michigan.gov).

Aside from DDT, other notable organochlorine compounds derived and applied in this era include the following now heavily regulated or banned compounds: chlorinated cyclodiene insecticides (aldrin and dieldrin), and hexachlorocyclohexanes (HCHs) such as lindane (Walker, 2012), chlorinated benzene, chlorinated camphene, and chlorinated cyclodienes (Casida, 2012).

These new compounds were far more potent insecticidal weapons than were the botanical and inorganic insecticide classes which preceded them. Organochlorines were often used as emulsifiable concentrates applied to crops or insects as sprays, as was a primary application of DDT, but organochlorines were also used as seed dressing (Walker, 2012).

Organochlorines acted as a nerve poison for insects, impairing the passage of Sodium and Potassium, and thereby action potential along nerves and across nerve synapses, since “the passage of an action potential along a nerve depends on the flow of Sodium and Potassium across the nerve membrane” (Walker, 2012, p. 132). Despite being particularly effective with regard to mosquito control and malaria, DDT and other organochlorines created global controversy due to their toxic effects on non-target species such as birds, bees, and fish (Casida, 2012).

Bioaccumulation. Organochlorines, and DDT in particular, provided a case study on the adverse environmental effects of pesticides in nature due to bioaccumulation. DDT was transported by streams where it accumulated in lakes as a result of runoff and accumulation in sediments. Research conducted at Lake Michigan in 1966 found bioaccumulation upward from the plant kingdom through the animal pyramid (Walker, 2012). A key indicator species in the Lake Michigan region, the Herring Gull, a year-round resident, was found to have thinning egg shells directly attributable to DDT poisoning, causing the eggs to break and leading to reproductive failure (Walker, 2012). The same effects of DDT poisoning were demonstrated in other fish-eating birds such as the bald

eagle, cormorant, and the peregrine falcon (Walker, 2012). Mounting evidence indicting DDT as a biohazard led to a protracted legal battle in 1971 and 1972, pitting the U.S. EPA and the Environmental Defense Fund against the chemical industry. After 9000 pages of testimony, 125 expert witnesses, and four to six billion pounds of DDT applied in the field, DDT was highly restricted or banned in the U.S. in 1973 (although it is still presently used in some developing nations) (Walker, 2012; Casida, 2012).

Organophosphates

In an effort to counter the detrimental effects of organochlorines, development of the next major class of insecticides focused on the need to make the pesticides less persistent in nature. The discovery of the organophosphate insecticide class occurred during WWII in Germany as a serendipitous byproduct of nerve gas development (Organophosphate Fact Sheet). Organophosphates ("OPs") are organic esters of phosphorus acids which, because they are less stable than the organochlorines and more easily processed via chemical and biochemical agents, "are generally less stable than organochlorine insecticides...thus they tend to be relatively short-lived when free in the environment and the environmental hazards they present are largely associated with short-term (acute) toxicity" (Walker, 2012, p. 14).

Of the more than 100,000 OPs that have been tested, only a little over 100 of these have been found useful as commercial insecticides. These include products such as Dursban, Lorsban (chlorpyrifos), Sumithion (fenitrothion), and

Actellic (pirimiphos-methyl). The OPs have been found to have a variety of uses, including applications as herbicides and fungicides, as well as contact, systemic, and fumigant insecticides (Organophosphate Fact Sheet).

Mode of Action. Insecticides are developed to act at a particular receptor or "site of action." The mode of action, (or molecular mechanism by which a pharmacological substance produces an effect on an organism) of organophosphates differs from the organochlorine class. Organophosphate insecticides were designed to act as nerve poisons by inhibiting the enzyme acetylcholinesterase (AChE) (Walker, 2012) leading to tetanus, a condition where muscles remain in a fixed state, unable to contract or relax in response to nerve stimulation (Walker, 2012). Organochlorines inhibited nerves of insects differently--by acting on the sodium channel (Na channel) or the chlorine channel (GABA receptor) resulting in tremors, twitching, and convulsions (Walker, 2012). Interestingly, all five major classes of insecticides act as nerve agents. See Appendix Figure 16 for a diagram depicting how the most widespread insecticides disrupt neural transmissions.

Organophosphates can be formulated into granules for gradual release as a soil treatment, they may also be applied as seed dressings, or, since they are water soluble, they can act as a systemic pesticide when reaching high enough concentrations in the plant to poison insects (Walker, 2012). Insecticides become systemic once they are absorbed and translocated by plants, often from a seed dressing but sometimes from root drenching.

Carbamates

The carbamate class was introduced in the 1950‘s after further research into the anticholinesterase action mechanism of organophosphates. Both organophosphates and carbamates utilize the same mode of action (illustrated in Appendix Figure 16), by exerting an anticholinesterase action on the nervous system of insects, acting at the nerve synapses (Walker, 2012). Carbaryl, which goes by the brand name Sevin, was put on the market in 1956 as the first successful carbamate insecticide (Carbamate Fact Sheet). “Carbaryl is one of the most widely used broad-spectrum insecticides in agriculture, professional turf management and ornamental production, and residential pet, lawn, and garden markets” (EPA CARBARYL IRED FACTS). Carbamates are derivatives of carbamic acid, and are usually solids but may be liquids. They are also similar to organophosphates in that they are readily degradable by chemical and biochemical agents, therefore posing fewer risks of persistence. Carbamates are commonly used as surface sprays or baits in the control of household pests and have proved useful against insects that have developed resistance to organophosphates (Carbamate Fact Sheet). Some carbamates, such as aldicarb and carbofuran, can be used as systemic pesticides (Walker, 2012).

Cumulative Effects by Mode of Action. The EPA’s review of the cumulative effects of carbamate insecticides originated in 1996. Instead of looking at insecticides individually, the EPA began looking at all insecticides which they categorized as sharing a “common mechanism of toxicity.” The objective of this review was to determine the aggregate occupational and ecological risk levels

(such as being very highly toxic on an acute exposure basis to honey bees, estuarine/marine invertebrates, and other aquatic animals, including Atlantic salmon). With respect to the carbamate class, the EPA ultimately determined that:

> "Although all uses may not meet the current safety standard and some uses may pose unreasonable risks to human health and the environment, these effects can be mitigated by the measures identified in the Carbaryl interim reregistration eligibility decision." **(**EPA Interim Risk Assessment for Carbaryl).

For pesticide classes it is important to consider not just the effects of one specific compound, but rather the cumulative effects of all pesticides which use that particular mode of action, since all such compounds will have "aggregate effects and risks" once applied in the field.

Synthetic Pyrethroids

Synthetic pyrethroid insecticides were derived from the naturally occurring xenobiotic compounds, known as pyrethrum or pyrethrins, found in the Chrysanthemum plant species (Walker, 2012). The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids which are strongly lipophilic and rapidly penetrate many insects, paralyzing their nervous systems (Beyond Pesticides Online Resource). The synthetic pyrethroid Permethrin was introduced in 1973; there are currently over 30 pyrethroids on the market (Krieger, 2001). The mode of action for synthetic pyrethroids is the same as the organochlorine insecticides, acting as a

neurotoxin to impair the passage of action potential along nerves and across nerve synapses at the sodium channel (Walker, 2012).

Synthetic pyrethroids are to be preferred over organochlorine insecticides because they have been demonstrated to be less toxic to birds and mammals (EPA Online, Regulating pesticides), and “are readily biodegradable and have short biological half-lives,” (Walker, 2012, p. 16), breaking down in a matter of minutes or hours (Texas A&M Pyrethroids). The photo-stability of synthetic pyrethroids has been improved over time in the second generation of this insecticide class, making them more stable and effective insecticides, though differing vastly on the molecular level from the original pyrethroid compounds from which they were synthesized (Texas A&M Pyrethroids). Synthetic pyrethroids are used to help control West Nile virus when they are mixed with water and oil and applied in an ultra low-volume spray to kill mosquitoes (Illinois Dept. of Public Health). They are also used to control other insect vectors of disease such as tsetse flies in parts of Africa (Walker, 2012). Despite their benefits, however, pyrethroids are solids of very low water solubility that present some of the same problems of environmental persistence found with DDT. Synthetic pyrethroids are highly toxic to aquatic organisms and can bind to soils and sediments (Walker, 2012). The first generation of synthetic pyrethroids was produced by chemists following WWII when the insecticide Allethrin was introduced in 1949 (Texas A&M Pyrethroids). There has been some concern over pyrethroids being an endocrine disruptor since they contain manmade

xenoestrogens that can increase the amount of estrogen in the body, causing an increased cancer risk in humans (Beyond Pesticides Online).

RESISTANCE AND CROSS-RESISTANCE

Insects and weeds have steadily developed resistance to the various plant-protection products used and their respective modes of action:

> "Only a few years after DDT was introduced, resistant insect strains were selected for many pests often with cross-resistance to some pyrethroids due to a common low-sensitivity modified binding site in the voltage-activated sodium channel. Even the organophosphates and methyl-carbamates became ineffective for some pests as resistant strains were selected with a less-sensitive acetylcholinesterase and enhanced detoxification systems" (Tomizawa and Casida, 2009, p. 261).

Insects may be resistant to more than one pesticide, and often to insecticides in more than one class. When insects developed resistance to a class of insecticides it became likely that resistance to other insecticides (in other classes) with the same mode of action would occur (Krieger, 2001). "In a strain of insects, such resistance due to the same mechanism is termed cross-resistance, in contrast to multiple resistance, which is the resistance of a strain to different compounds and resulting from different mechanisms" (Krieger, 2001, p. 101). "When resistance to more than one insecticide is achieved by a single mechanism, this is true cross resistance, but when several resistance mechanisms are involved this is called multiple resistance" (Walker, 2012, p. 248).

Insects have evolved three main mechanisms of resistance. One method is by increasing detoxification (which increases the rate at which the insecticide is broken down). A second method used is to decrease sensitivity of the target site (as the result of a mutation and selection of the target protein). "Resistance Mechanisms are the consequences of genetic differences between susceptible

and resistant strains of the same pest species. Most commonly, a resistant strain possesses a highly active form (or forms) of a detoxifying enzyme or one or more genes encoding for an insensitive form of the target site" (Walker, 2012, p. 249). The third mechanism is behavioral, which includes increased sensitivity and avoidance after low-level dosage (Walker, 2012).

Pesticide Treadmill

Once one class of insect pest control has exhausted its effectiveness as a result of the pests' evolved resistance, then either greater quantities of the pesticide must be applied, or a new plant-protection product must be used, or a combination of the two must be employed. Charles Benbrook, a research professor for Washington State University's Center for Sustainable Agriculture and Natural Resources, and an advocate for reduced pesticide use, has referred to this cycle as "a chemical treadmill," in which new herbicides are needed for cross-resistant super weeds, genetically engineered seeds with Bacillus thuringiensis (Bt) are required to be able to tolerate the broad-spectrum herbicides, and different combinations of insecticides with systemic properties and novel molecular mechanisms of toxicity are required in order to keep the multi-resistant pests from destroying crops (Benbrook Interview) (NY Times Articles).

According to Benbrook's 2012 paper: "Impacts of genetically engineered crops on pesticide use in the U.S. - the first 16 years," the use of Bt in genetically engineered crops led to a 28% reduction in insecticide use from 1996-2011. This

is due to more herbicide use, 527 million pounds more in the U.S. in those 16 years examined, and mostly coming in the form of the world's #1 selling agrochemical, Monsanto's broad-spectrum glyphosate herbicide, Round-Up. Benbrook determined that ultimately "pesticide use on each acre planted with a genetically engineered crop was about 20 percent higher than on acres not planted with genetically engineered crops" (New York Times, Superweeds), and there are now over 26 "superweeds" and counting, for which Round-up has lost its effectiveness (Benbrook Interview). Insects are also becoming Bt resistant, which will result in the eventual use of more insecticides along with new combinations of insecticides and herbicides in order to keep up with the pests, or risk losing the crops (Benbrook Interview). "The expanding importance of crops expressing Bt endotoxin encourages neonicotinoid use, because the types of pests not controlled by the endotoxin are often those highly sensitive to neonicotinoids" (Casida, 2005, p. 250).

In order to counter insect resistance, a new class of compounds was required that acted on agricultural pests with a different mode of action than the previous classes. The new pesticide would also need to be safer to non-target species. "Future insecticides, as seen from the retreat of chlorinated hydrocarbons from the primary seat, are required to have not only high insecticidal potential, but also low toxicity to vertebrates and no damage to the environment" (Yamamoto and Casida, 1999, p. 91). The synthetic nicotinoid, or neonicotinoid pesticide class, was developed for these very reasons.

PESTICIDE SUMMARY AS OF 1990 (Pre-Neonicotinoids)

To briefly summarize, insecticides have continually transitioned from the botanical and inorganic classes before yielding to the organochlorines, which eventually shifted to organophosphates, carbamates, and synthetic pyrethroids. Each insecticide class had its relative merits, such as increased insecticidal properties, selectivity, and lessened persistence in ecosystems, as well as their respective drawbacks, most of which came in regard to non-target species toxicity, as well as ecological and human-health-related risks, and pests' evolved resistance to the harmful compounds. "In 1992, global organophosphate sales were US $2,880 million out of a total insecticide market of US $7,400 million, which made OPs the most widely used group of insecticides, worth nearly 40% of the market - at that time" (Organophosphate Fact Sheet). "In the cotton growing industry where 22.5% of all insecticide use occurs, synthetic pyrethroid use overtook organophosphate use in the early 1990s. By 1994, the synthetic pyrethroids accounted for 42.5% of the cotton insecticide market, with OP products still approaching 40%" (Organophosphate Fact Sheet). This is the pesticide snapshot before the neonicotinoids hit the market, where they quickly came to dominate the global insecticide market (see Figures 3 and 4).

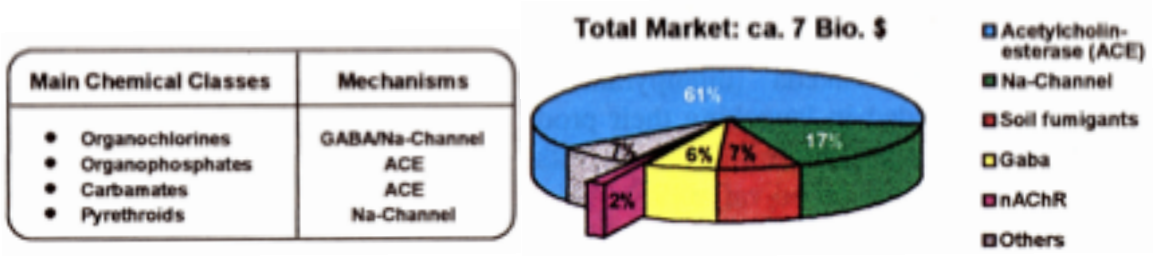


Figure 3. 1991 Insecticide Market by Mode of Action (Yamamoto and Casida, 1999)

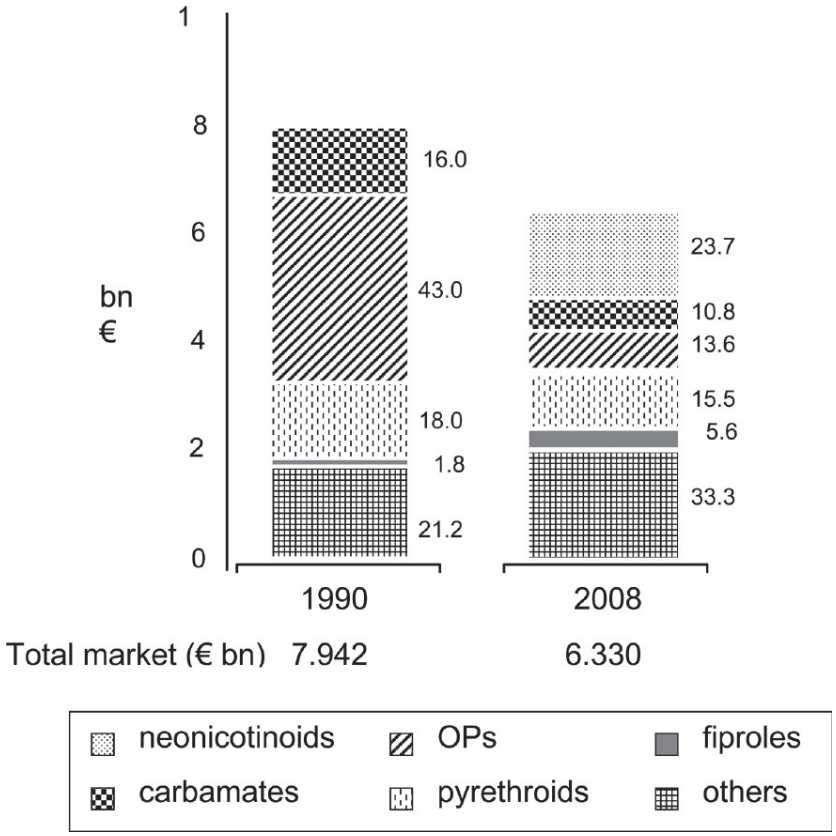


Figure 4. Development of insecticide classes in modern crop protection, 1990-2008, expressed as percentage of total (Jeschke et al., 2011)

Prior to the 1991 launch of the neonicotinoid imidacloprid, the nearly 8-billion-dollar agrochemical market was dominated by organophosphates (OPs) (43%), pyrethroids (18%), and carbamates (16%). By 2008, neonicotinoids had gained nearly a quarter share of a slightly decreased total market of 6.3 billion dollars, mainly at the expense of OPs (13.6%) and carbamates (10.8%) (Jeschke, 2011) (see Figure 4). The story behind the development and rapid ascent of the neonicotinoids to become the world's number one selling class of insecticide, and the only new major insecticide class developed in the past four decades, is described next.

DEVELOPMENT AND SPREAD OF NEONICOTINOIDS

A New Mode of Action Mimicking a Natural Compound

History. The first neonicotinoid compound, imidacloprid, was developed in 1984 by Nihon Tokushu Noyaku (now Nippon Bayer), drawing upon two previous generations of chemical research (Yamamoto and Casida, 1999). Imidacloprid is a result of research on nicotine, which, for more than 200 years, was the principal botanical insecticide for controlling sucking insect pests on plants (Casida, 2011). Throughout the 1970s, researchers at Shell Development Company's California labs conducted a screening and optimization program for new crop-protection products based on nicotine's insecticidal properties. In 1977 this program resulted in the patent of imidacloprid's lead compound, nithiazine (Casida, 2005; Yamamoto and Casida 1999; Krieger, 2001) (see Figure 5).

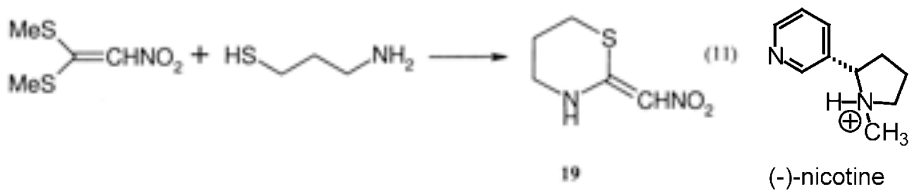


Figure 5. Synthesis of Nithiazine (L), and structural similarity to Nicotine (R)

(Yamamoto and Casida, 1999; Casida, 2005)

Nithiazine is therefore the first synthesized neonicotinoid structure, and the compound from which imidacloprid and all other neonicotinoid insecticides would be derived. The Shell research team revealed that nicotine, nithiazine, and imidacloprid all interact with the same site, the ACh recognition site of the nicotonic receptor or nAChR (Yamamoto and Casida, 1999) (see Figure 12). "Shell's work on nithiazine revealed a new structural class of insecticides and

also a new mode of action" (Yamamoto and Casida, 1999, p. 85). "Biological evaluation revealed a toxicity index of 1700 on corn earworm, an astounding 1000-fold increase in whole organism activity" (Yamamoto and Casida, 1999, p. 79). However, despite demonstrating "potency, selectivity, and systemic properties," nithiazine was not commercially successful due to its poor field persistence, and its lack of photo-stability in particular (Yamamoto and Casida, 1999, p. 81). Shell Labs had engineered a new compound with excellent insecticidal properties, but nithiazine's photo-instability severely limited its commercial potential (though a highly effective commercial fly trap was produced for fly abatement in livestock facilities) (Casida, 2005). Shell made many attempts to improve on nithiazine but, "in spite of the best intentions, plans, and syntheses of more than 1000 compounds, no more interesting ring systems or effective nitromethylene group replacements would be found" (Yamamoto and Casida, 1999, p. 80).

Imidacloprid would eventually be synthesized based on nithiazine, but only after chemical alterations applied in another lab operated by Bayer. Fourteen years after Professor Henry Feuer of Purdue University began his investigation of nitroalkyl heterocycles (Nitromethylene Compounds) in 1970 (Yamamoto and Casida, 1999), leading to Shell Lab's synthesis of nithiazine, it would be Bayer that made the ultimate breakthrough with imidacloprid. As a starting point for his investigation, Professor Shinzo Kagabu, the lead Bayer scientist, who received the 2010 American Chemical Society International Award for Research in Agrochemicals in recognition of his discovery of imidacloprid (IMI) and

thiacloprid, explains (Utrecht University): “Shell’s new insecticide nithiazine caught our attention because it acts at the same receptor as nicotine even though there is no apparent structural similarity between them...but more surprisingly and interesting for us was that nithiazine is active against insects but of low toxicity to mammals” (Yamamoto and Casida, 1999, p. 91). Dr. Kagabu and his team at Bayer embarked on an optimization program for nithiazine in which over 2000 compounds were prepared, screened, and assessed based on the various structure-activity relationships which resulted. They found dozens of compounds possessing high insecticidal activity in lab tests. Out of these compounds, 10 were selected for field testing, and of these, imidacloprid was selected for commercial development based on positive field results (Yamamoto and Casida, 1999). Bayer scientists would combine nithiazine with the unique (6-chloro-3-pyridinyl) methyl N-substituent when they created a neonicotinoid prototype, which became the intermediate compound to imidacloprid (Yamamoto and Casida, 1999) (see Figure 6).

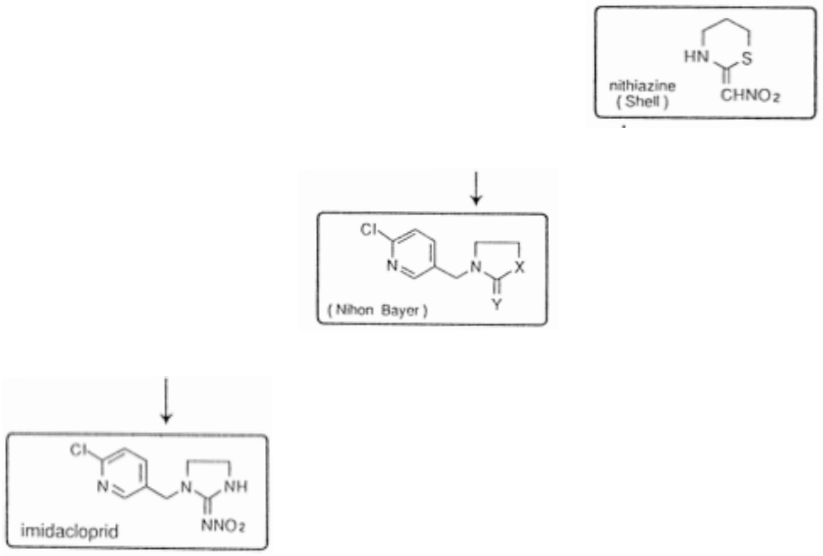


Figure 6. Synthesis of Nithiazine, Intermediate Prototype, then Imidacloprid (Yamamoto and Casida, 1999)

The Bayer scientists had created a novel new class of insecticides which met a "strong market demand for the new broad-spectrum insecticides with new modes of action and favorable toxicological and environmental properties" and after much investigation into the structure-activity relationship of this new class of compounds, finally figured out how to incorporate the desired photo-stability (Yamamoto and Casida, 1999, p. 110).

Chloropyridin Moiety. Nicotine, nithiazine, and imidacloprid all worked at the same action site because "nicotine and its analogues have a basic nitrogen that even at physiological pH picks up a proton to form a positive ion, whereas neonicotinoids contain a chlorinated pyridyl group, or another heterocyclic group, that withdraws electrons from an imido group and thus makes it partially positive without being protonized" (Stenersen, 2004, p. 134). "By introducing a 3-pyridylmethyl group as a substituent on the heterocyclic nitromethylenes, the insecticidal activity increased dramatically" (Yamamoto and Casida, 1999, p. 177). This breakthrough by Bayer is referred to as the "chloropyridin moiety," and it proved to be the key for the market launch of the neonicotinoid insecticide class (Yamamoto and Casida, 1999).

The neonicotinoids featured a new and distinct mode of action compared to other insecticides that were on the market. "In insects, the nicotonic receptor (nAChR) is present only in the central nervous system. Imidacloprid, once it has entered the body (by sucking or injection) is easily accessible to the target site while nicotine is not" (Yamamoto and Casida, 1999, p. 21). The net result is that imidacloprid, and other neonicotinoids, easily penetrate the nervous system of

insects (but not mammals) and bind selectively to the nicotonic acetylcholine receptors (Stenerson, 2004). “The superiority of imidacloprid resulted from nonionization, higher hydrophobicity, and thus penetrability into the target site,” and the factors affecting insecticidal and selective action for imidacloprid could be summed up “as the binding affinity to the nAChR, hydrophobicity (allowing for great insect penetration), and metabolism (or lack thereof in mammals and vertebrates)” (Yamamoto and Casida, 1999, p. 21).

In 1985 Bayer sparked a wave of patent activity in the agricultural chemical research industry with its publication of the first patent applications for imidacloprid. Novartis/Ciba/Syngenta, Takeda, Nippon Soda, and others soon entered this new research area, creating patents for thiacloprid (1985), nitenpyram (1988), acetamiprid (1989), clothianidin (1989), thiamethoxam (1992), and dinotefuran (1994) (Yamamoto and Casida, 1999; Tomizawa and Casida, 2005). Pharmaceutical firms realized that unique neonicotinoids could be made based on molecular substitutions, resulting in new insecticides with different properties (see Figure 7).

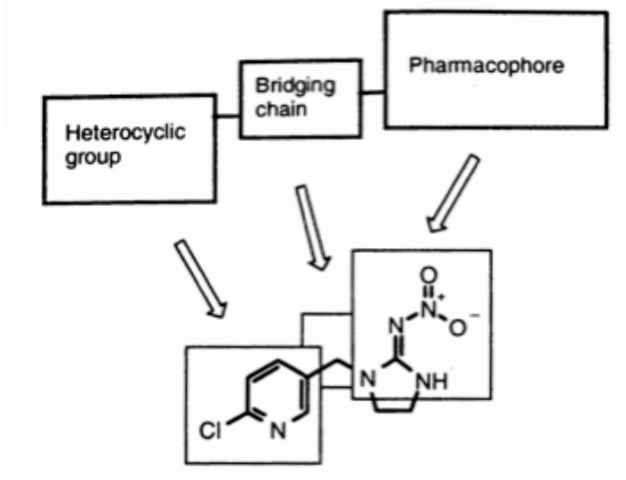


Figure 7. Structural Elements of Neonicotinoids (Yamamoto and Casida, 1999)

“The new chemistry showed a relatively broad variability and it is remarkable that the chloropyridine moiety (of the heterocyclic group) can be replaced by other aromatic and even saturated heterocyclic systems” (Yamamoto and Casida, 1999, p. 110). After further field testing to verify safety, imidacloprid was brought to market in 1991 by Bayer. Two other neonicotinoids soon followed when Takeda Pharmacueticals began selling nitenpyram in 1995 and Nippon Soda’s acetamiprid entered the market in 1996 (Yamamoto and Casida, 1999).

Neonicotinoid Types

The versatility and market demand for neonicotinoids led agrochemical companies to further investigate and expand the new market for neonicotinoid compounds and plant-protection products, resulting in seven presently available commercial neonicotinoids, six of which are commonly used on plants (Xerces Society Online Resource) (see Figure 9 and Table 2).

Generational Neonicotinoid Distinctions/Subclasses. First generation neonicotinoids: imidacloprid, thiacloprid, nitenpyram, and acetamiprid all demonstrated the “chloropyridin moiety,” which referred to the heterocyclic group of the neonicotinoid structure and were developed between 1984 and 1989 (Yamamoto and Casida, 1999; Krieger, 2001) (see Figure 7 and Table 4). Bayer proposed the widely adopted term “chloronicotinyl” to describe this subclass of neonicotinoids (Yamamoto and Casida, 1999).

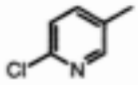
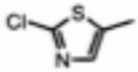
Neonicotinoid subclass	Heterocyclic Group	Example	Remark
Nitromethylene compounds	None	Nithiazin	Lead structures
Chloronicotinyl compounds		Imidacloprid Nitenpyram Acetamiprid	1st-generation neonicotinoids
Thianicotinyl compounds		CGA 293'343 TI-435 AKD-1022	2nd-generation neonicotinoids

Table 2. Neonicotinoid Heterocyclic Subclasses (Yamamoto and Casida, 1999)

A second generation of neonicotinoids including clothianidin (TI-435) and thiamethoxam (CGA 293'343) were subsequently developed between 1989 and 1992, but instead of the chloropyridin moiety, this neonicotinoid subclass featured a "chlorothiazolyl moiety" on the heterocyclic group (Krieger, 2001) (Agrow online resource). "Thianicotinyl" compounds is the name for this subclass (Maienfisch et al., 2001). A third subclass was discovered when it was found that the chloropyridine or chlorothiazole rings could be replaced with (±)-tetrahydro-3-furylmethyl, referred to as the "furnanicotinyl moiety," resulting in Mitsui Chemicals 1994 synthesis of dinotefuran using acetylcholine as the lead compound (Wakita et al., 2003) (see Figure 9).

Structural Neonicotinoid Classification. Much more would be learned about the structure-activity relationships as various neonicotinoid research and optimization programs advanced. Pharmaceutical and agrochemical researchers discovered that different structural elements of neonicotinoids evoked a range of widely variable biological and chemical properties. Neonicotinoid activity could

be manipulated by modifying and making substitutions to the “pharmacophore” structure as well as the heterocyclic group (see Figures 7, 8, 9, and Table 3). The second generation neonicotinoid, thiamethoxam, which was first registered for use in New Zealand in 1997, illustrates the extent to which structural modifications of both the heterocycle and pharmocophore impact a neonicotinoid compound and its potential uses (see Figure 8):

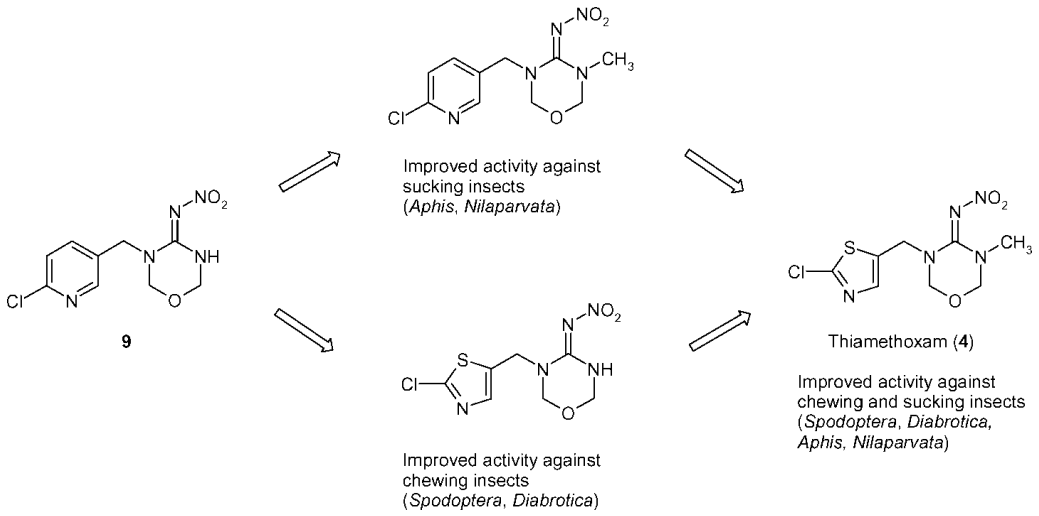


Figure 8. Substitutions leading to 2nd Generation Neonicotinoid Thiamethoxam

(Maienfisch et al., 2001)

> “A series of structural modifications on this lead structure revealed that replacement of the 6-chloro-3-pyridyl group by a 2-chloro-5-thiazolyl moiety resulted in a strong increase of activity against chewing insects, whereas the introduction of a methyl group as pharmacophore substituent increased activity against sucking pests. The combination of these two favorable modifications led to thiamethoxam” (Maienfisch et. al, 2001, p. 907).

The pharmacophore has a dramatic effect on the insecticidal activity, and is responsible for some of the specific properties “such as photolytical stability, degradation in soil, metabolism in plants, toxicity to bees and beneficials” (Yamamoto and Casida, 1999, p. 179). For instance, the other

second-generation neonicotinoid, clothianidin, with its non-cyclic pharmacophore structure, “has longer residual activity and less water solubility than other neonicotinoids, such as thiamethoxam which decreases the potential risk of leaching in the soil profile and also means it has superior rain-fastness along with faster movement within the leaf’s tissue” (Clutch FAQ) (See Appendix Figure 17).

In addition, the pharmacophore group is responsible for physiological reactions that “induce the expression of specific functional proteins involved in various stress defense mechanisms of the plant allowing it to better cope under tough growing conditions, such as: drought; low pH; high soil salinity; free radicals from UV radiation; heat stress leading to protein degradation; toxic levels of aluminum; wounding from pests, wind, hail, etc., and; virus attack (Agropages Neonicotinoid Story).

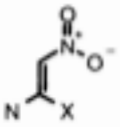
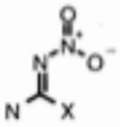
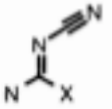
	Nitroenamine Pharmacophore	Nitroamidine Pharmacophore	Cyanoamidine Pharmacophore
Functional group			
Example	Nitenpyram Nithiazin	Imidacloprid CGA 293’343 TI-435 AKD-1022	Acetamiprid

Table 3. Neonicotinoid Pharmacophore Subclasses (Yamamoto and Casida, 1999)

Overall, N-nitroguanidines (imidacloprid, thiamethoxam, clothianidin, and dinotefuran) are the most commonly featured pharmacophore subclass, as they account for approximately 85% all neonicotinoid insecticide sales (Jeschke, 2011). It should be noted that, based on their pharmacophore-induced

physiochemical properties, nitroguanidines are considered acutely toxic to honey bees, whereas cyanoamidines (acetampiprid and thiacloprid) are considered much less so, and nitromethylenes, of which nitenpyram is the sole representative, are not commonly used in agriculture, but more so in flea control for pets and livestock (PAN Online Resource, Xerces Society Online Resource; Agropages). A further structural distinction in regard to pharmacophore type is based on whether a neonicotinoid pharmacophore possesses a “ring system” such as imidacloprid, thiacloprid, thiamethoxam, or if it is “non-cyclic,” as is the case with nitenpyram, acetamiprid, clothianidin, and dinotefuran (Jeschke, 2011) (see Table 3 and Figure 9).

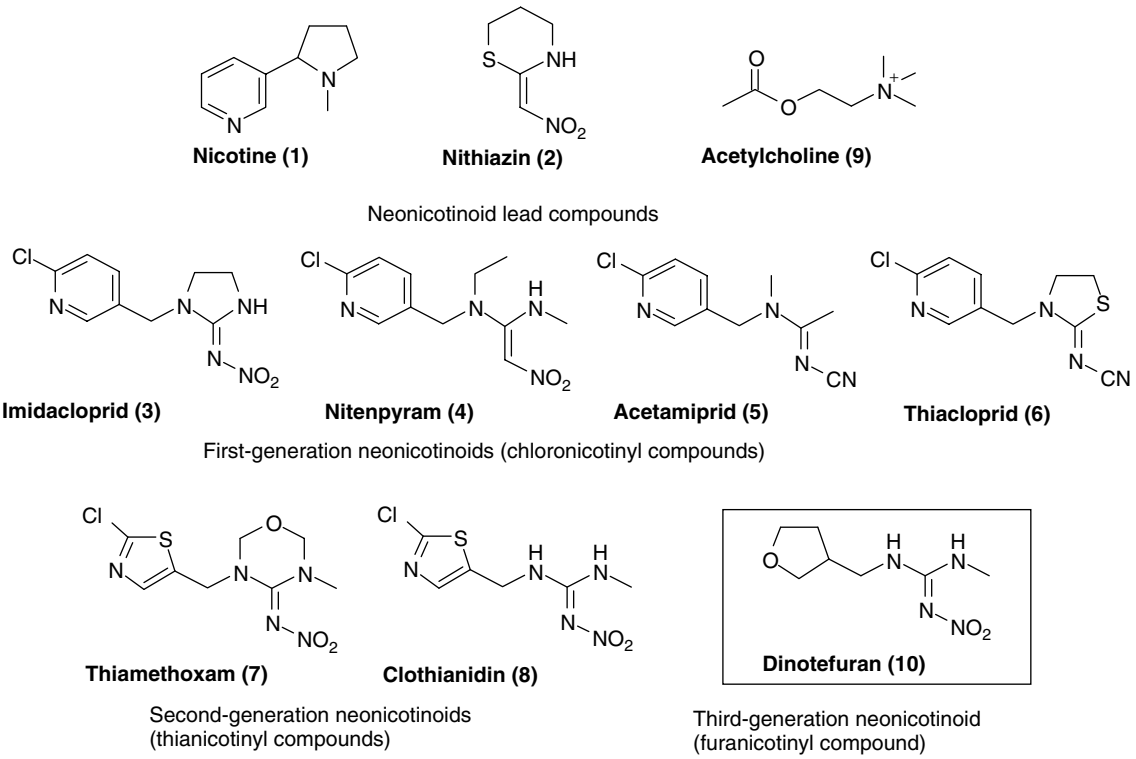


Figure 9. The 3 Generations of Neonicotinoids and their Lead Compounds (Wakida et al., 2003)

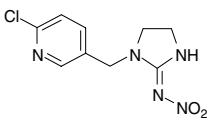
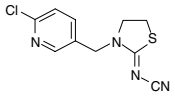
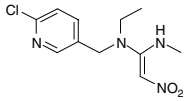
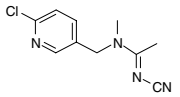
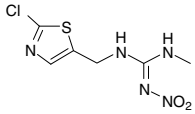
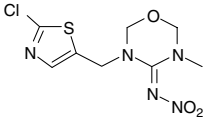
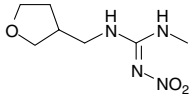
Neonicotinoid	Product Names	Patent Info	Commercial Use
Imidacloprid 	Gaucho, Admire, Hachikusan, Earth Garden, Merit, Imicide Provado Macho Malice, Sepresto Widow, Wrangler, Numerous Bayer Products, DIY Tree Care Products Multi-Insect Killer, Ferti-lome 2-N-1 Systemic, Hi-Yield Systemic Insect Spray, Hunter, Knockout Ready-To-Use Grub Killer Lesco Bandit, Marathon, Monterey Once a Year Insect Control II, Ortho Bug B Gon Year-Long Tree/Shrub Insect Control, Orhto MAX Tree/Shrub Insect Control, Surrender, GrubZ Out	Bayer 1985, commercial introduction 1991 Japan 1993 U.K. 1994 U.S	Seed dressing, soil drench, granules, injection, or spray to a wide range of field and tree crops, as well as ornamental plants, trees, and turf. Also, topical use on pets for flea control and application to buildings for termite control.
Thiacloprid 	Winbariard, Eco-one Froable, Calypso	Bayer 1985, commercial introduction 1999 Brazil	Foliar spray to cotton and pome fruit crops
Nitenpyram 	Bestguard, Pedan Best, Capstar	Sumitomo Chem. Co. 1988, commercial introduction 1995	*Not Commonly used on Plants in U.S. Topical use on pets and livestock for flea control. Used in Japan/China on rice, fruit.
Acetamiprid 	Mospilan, Matsu green, Kadan, Yielder SG, Assail, Tristar, Ortho Flower, Fruit and Vegetable Insect Killer, Ortho Rose and Flower Insect Killer	Nippon Soda 1989, commercial introduction 1995 Japan	Foliar spray for leafy vegetables, fruiting vegetables, cole crops, citrus fruits, pome fruits, grapes, cotton,ornamental plants and flowers.
Clothianidin 	Poncho, Dantotsu, Full Swing, Moriate, Hustler, Takelock, Arena, Clutch, Belay, Aloft, Bayer Advanced All-in-One Rose & Flower Care granules, Green Light Grub Control with Arena	Sumitomo Chem. Co. & Bayer 1989, commercial introduction 2002 Japan 2003 U.S	Seed treatment, foliar spray or soil drench for a variety of field and tree crops, also for turf and a variety of ornamental trees and flowers.
Thiamethoxam 	Actara, Cruiser FS30, Actara, Adage, Centric, Platinum, Flagship Maxide Dual Action Insect Killer, Meridian	Syngenta 1992, commercial introduction 1997 N. Zealand	Seed dressing, soil drench, injection, granules, foliar spray to a wide range of field crops, as well as ornamental plants and turf.
Dinotefuran 	Starkle, Albarin, Bonfram, Venom Scorpion, Green Light Tree & Shrub Insect Control with Safari 2 G, Safari, Transect, Zylam 20SG Systemic Turf Insecticide	Mitsui Chemicals 1994, commercial introduction 2002 Japan	Soil drench or foliar spray to leafy and fruiting vegetables, turf, and ornamental plants. Also used as bait or granules for cockroach control.

Table 4. Neonicotinoid Chart (AGROW Online, JEPA Online, Xerces Society Online, Iwasa et al., 2004; Jeschke et al., 2011; Tomizawa and Casida, 2005; Wakita et al., 2003; Tomlin, 2009)

The Versatility and Spread of Neonicotinoids

Neonicotinoids have made a major impact on pest control in a relatively short period of time. Imidacloprid has quickly become the number one selling insecticide in the world. Different waves of plant-protection products entered the market in the early 90s. "Expansion of neonicotinoid insecticides has been driven by growth of established products such as imidacloprid as well as newer entrants such as thiamethoxam and clothianidin" (Jeshke et al., 2011, p. 2898).

> "Imidacloprid currently accounts for approximately 41.5% of the whole neonicotinoid market (in 2009: U.S. $2632 million). At U.S. $1091 million imidacloprid is the largest selling insecticide in the world; its sales value growth is also being affected by generic material. Thiamethoxam is now the second biggest neonicotinoid (in 2009: U.S. $627 million) in terms of sales, and clothianidin has grown rapidly to U.S. $439 million. In 2009 the sales of other neonicotinoids such as acetamiprid (U.S. $276 million), thiacloprid (U.S. $112 million), dinotefuran (U.S. $79 million), and nitenpyram (U.S. $8 million) are expected to increase" (Jeshke et al., 2011, p. 2898).

Presently in the U.S., there are over 400 neonicotinoid products on the market. Residential, construction, backyard gardening, and veterinary uses cannot be overlooked as "products containing imidacloprid come in many forms, including liquids, granules, dusts, and packages that dissolve in water and imidacloprid products may be used on crops, houses, or used in flea products for pets" (National Pesticide Information Center Online Resource). (See Appendix Figures 18 and 19 for a sampling of products which use neonicotinoids and Appendix Table 15 for a list of key players in the neonicotinoid industry). Neonicotinoid pesticides are used in over 120 countries (Jeschke, 2011) and on

crops such as vegetables, pomes, nuts, citrus, rice, cotton, maize, potatoes, sugar beets, rapes and soybeans (Agrowpages Online Resource).

Neonicotinoids have an endless range of uses because their unique physiochemical properties and translocation rates, combined with residual activity, make them highly effective against sucking and chewing species, including aphids, whiteflies, leafhoppers, planthoppers, and the Colorado potato beetle (Jeshke et al., 2011). Seven different neonicotinoids are featured in a multitude of home and agricultural plant-protection products, for a wide variety of pests, using an assortment of application methods (see Table 5).

neonicotinoid insecticide	no. of crop uses	additional pest spectrum	foliar uses	soil uses	seed treatment
imidacloprid	140	thrips, mealybugs, leafminers, termites	++(+)	+++	++(+)
nitenpyram	12	−	++	+	−
acetamiprid	60	codling moth, diamondback moth	+++	+	−
thiamethoxam	115	mealybugs, plant bugs, leafminers, termites	+++	+++	++
thiacloprid	50	codling moth, pollen beetle	+++	+	+
clothianidin	40	wooly aphid, oriental fruit moth, corn rootworm	++(+)	++	+++
dinotefuran	35	soft scales, thrips, mealybugs	+++	++	−

[a] Modified after ref 5. Uses are defined as follows: +++, broad; ++, good; +, limited; −, not relevant.

Table 5. Neonicotinoid Applications (Jeschke et al., 2011)

The range of application methods includes foliar sprays; irrigation water in drip, drench systems, or in floating box systems; direct soil injection; trunk and bud injection; and also seed treatments (Agrowpages Online Resource). “The neonicotinoid insecticides have a high degree of versatility, not seen to the same extent in other chemical classes” (Jeshke et al., 2011, p. 2900). Since the insecticide is absorbed by all parts of the plant, it is considered highly systemic and toxic to pests throughout different phases of the plant’s lifecycle.

Seed Treatments. Seed treatments have proven to be a particularly efficient and effective application method which requires less overall insecticide

use as a result of neonicotinoid potency. Seed dressings, coatings, and soil treatments are also viewed as far safer to agricultural workers, and may eliminate the need for foliar spraying due to their systemic properties that are translocated throughout the plant, allowing for efficacy against pests from the outset of the growth cycle. "New opportunities have been opened up in modern crop protection. Today approximately 60% of all neonicotinoid applications are soil/ seed treatments, and most spray applications are especially targeted against pests attacking crops such as cereals, corn, rice, vegetables, sugar beet, potatoes, cotton, and others" (Jeshke et al., 2011, p. 2900) (see Figure 10).

> "Seed dressing, film coating, pelleting, and multilayer coating allow an environmentally safe and perfect protection of young plants against insect attack. With this method, application of the active ingredient is virtually independent of the weather and can be applied directly at the site of action. The application amount (g of active ingredient per hectare) used per unit area is thereby reduced remarkably" (Jeschke, 2011, p. 2900).

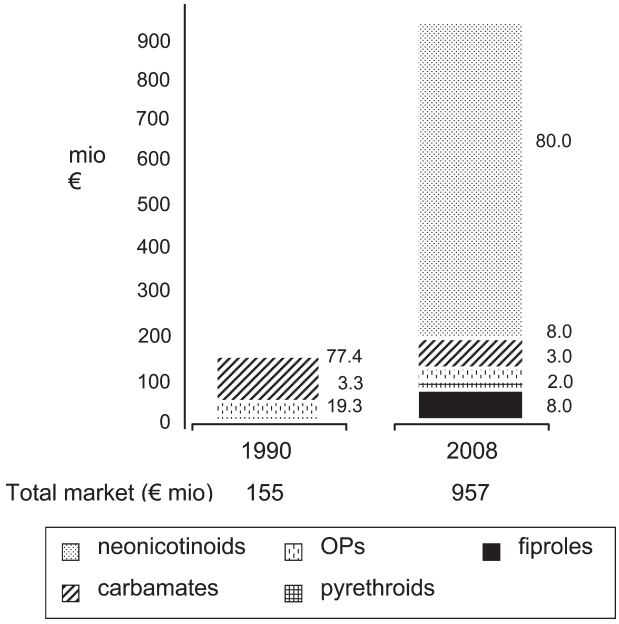


Figure 10. Development of seed treatment,1990- 2008, percentage of total (Jeschke et al., 2011)

As a result of the wide application of seed treatments, neonicotinoids are used on 94%, or 147 million acres, of all corn planted in the U.S., with historical data

showing a resulting 6 to 14 bushels per acre yield increase (Bayer CropScience Online Resource). (See Appendix Table 16 for U.S. Neonicotinoid Use).

Market and Economic Success

The launch of neonicotinoids was an immediate economic success. Some four years after its 1991 launch, imidacloprid became the second biggest selling insecticide in the world, with 1995 sales of $360 million (close behind the organophosphate chlorpyrifos) (Yamamoto and Casida, 1999). By 1997, sales of the active ingredient (including crop and animal health applications), reached $562 million, giving imidacloprid the distinction of being the top-selling insecticide in the world (Yamamoto and Casida, 1999). Global crop-protection-industry news, analysis, and data provider Cropnosis (formerly Wood Mackenzie), confirms imidacloprid's top-selling insecticide status. 2008 global sales of the Top Ten Agrochemicals appear in Figure 15.

Brand	Active Ingredient	Company	Application	Sales 2008	
				$ billion[a]	MT
Round-up	Glyphosate (**I**)	Monsanto	Herbicide	8.30	620,000
Admire, Gaucho	Imidacloprid (**II**)	Bayer CropScience.	Insecticide	1.28	5450
Heritage	Azoxystrobin (**III**)	Syngenta	Fungicide	1.16	7000
F 500	Pyraclostrobin (**IV**)	BASF	Herbicide	1.10	7200
Flagship	Thiemethoxam (**V**)	Syngenta	Insecticide	0.73	1895
Callisto	Mesotrione (**VI**)	Syngenta	Herbicide	0.62	2040
Grammoxone	Paraquat-dichloride (VII)	Syngenta	Herbicide	0.60	26,000
Flint	Trifloxystrobin (VIII)	Bayer CropScience.	Fungicide	0.60	3405
Horizon, Folicur	Tebuconazole (**IX**)	Bayer CropScience.	Fungicide	0.55	2860
Regent MG, Frontline	Fipronil (**X**)	BASF	Insecticide	0.53	1375

[a] Ex-factory.
11–20: (Figures in $ million/MT) clothienidin (509/546); chlorpyrifos (482/34,945); chlorothalonil (475/48,559); lambda-cyanhalothrin (454/1085); 2,4-D (453/64,725); prothioconazole (417/1550); mesosulfuron-methyl (414/530); kresoxym-methyl (409/3450); acetochlor (400/39,000); glufosinate-ammonium (399/3990).
Source: Cropnosis Ltd—Agranova.

Table 6. 2008 Top Ten Agrochemicals in terms of Global Sales (Pollack, 2011)

Unique Physiochemical Properties. The reasons for the success of neonicotinoids are their numerous unique chemical and biological properties, which are summarized as follows.

Neonicotinoids are highly potent and considered a “low-rate technology” that requires lower application rates when compared to other commercial pesticide classes. Table 6 illustrates this point in that, Round-Up, Monsantos’ herbicide, the number one selling agrochemical in 2008, requires a relatively high application rate of 500-4000 gallons/hectare (Pollack, 2011) versus imidacloprid’s application rate of .05-.125 pounds/acre (Extoxnet: Imidicloprid). Imidacloprid is priced accordingly with one of the most expensive per unit costs of all agrochemicals, at over $500/kg (Pollack, 2011).

Since neonicotinoids possess good water solubility, they are readily absorbed and translocated by root systems and leaves alike, making these compounds highly systemic, particularly when used as a seed dressing (Yamamoto 178). As a result of these systemic properties, neonicotinoids possess “excellent activity especially against homopteran (i.e. aphids and leafhoppers), coleopteran (i.e. beetle species), dipteran (i.e. flies), and lepidopteran (i.e. leafworms) pests” (Yamamoto and Casida, 1999 pgs. 178, 186) by penetrating not only the roots and leaves of a sapling, but also affecting the soil around the root zone (see Figure 11). This property makes neonicotinoids highly complementary to Bt seeds and crops, which take between 3-6 weeks to build up sufficient Bt levels in emerging seedlings to deter pests, whereas

neonicotinoid seed coatings provide immediate efficacy against devastating early-growth-stage pests such as corn rootworm species (Benbrook interview).

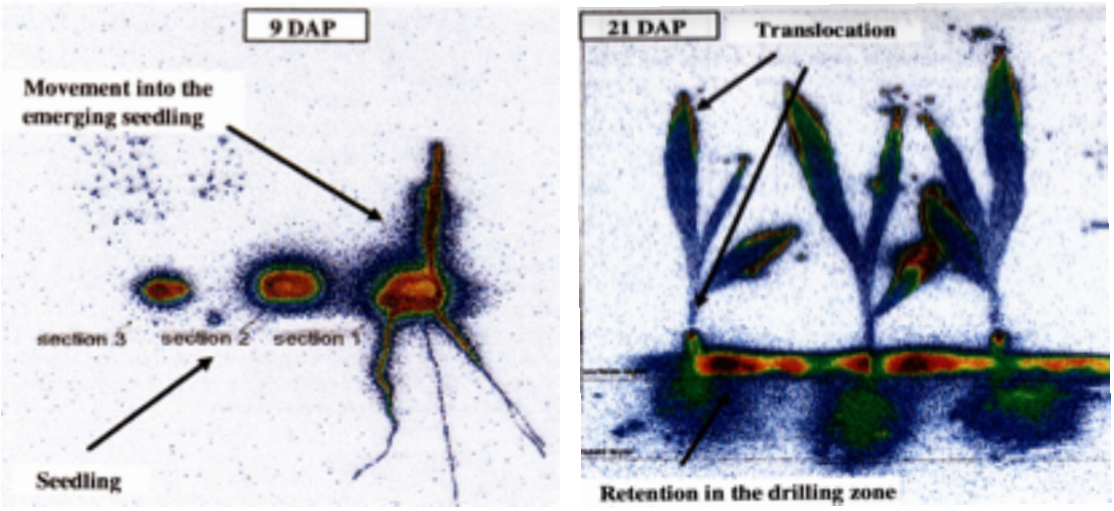


Figure 11. Neonicotinoid Treated Seed & Sapling 9 and 21 Days After Planting (DAP) (Yamamoto and Casida1999)

Due to the systemic penetration into all parts of the plant, some neonicotinoids, such as imidacloprid, thiamethoxam, and clothianidin, have been shown to have "strong preventative effects on some plant virus transmissions" (Maienfisch et al., 2001, p. 910; Jeschke, 2011). Neonicotinoids control not only pests, but also prevent the spread of viruses.

Yet another reason for this success is due to the lack of pest resistance to neonicotinoids because they possess a new mode of action: "Unlike other insecticides, the neonicotinoids bind at a specific site, the postsynaptic nicotinic acetylcholine receptor (NaChR), and there are no records of cross-resistance to the carbamate, organophosphate, or synthetic pyrethroid insecticides, thus making them important for management of insecticide resistance" (Agrowpages Neonicotinoid Insights). And, of course, the major strength of neonicotinoids

results from their low mammalian toxicity and favorable safety profile (Maienfisch et al., 2001; Yamamoto and Casida, 1999).

PERSISTENCE IN NATURE

Ecotoxicology

Whereas nicotine is quite toxic in mammals, imidacloprid and the neonicotinoids have been shown to be much less so in clinical lab studies of toxicology. Neonicotinoids exhibit a high 'No Observable Effects Level' and also high acceptable daily intake value for vertebrates (when determined)" (Steneren, 2004) (see Table 7).

Insecticide	LD_{50} (mg/kg)	
	Rat (oral)	Housefly (injection)
Nicotine	53	272
Imidacloprid	450	22

Table 7. Relative Toxicity of Nicotine and Imidacloprid (Yamamoto and Casida, 1999)

This finding is a result of the nicotinic receptor, or nAChR, the site where neonicotinoids selectively bind in insects, but fail to do so in mammals. The low vertebrate toxicity of neonicotinoids is due to the insensitivity of both brain and peripheral nAChRs in mammals as compared to insects, or put another way, "the partial positive charge in neonicotinoids can distinguish the insect nAChR from the vertebrate nAChR" (Yamamoto and Casida, 1999) (See Figure 12).

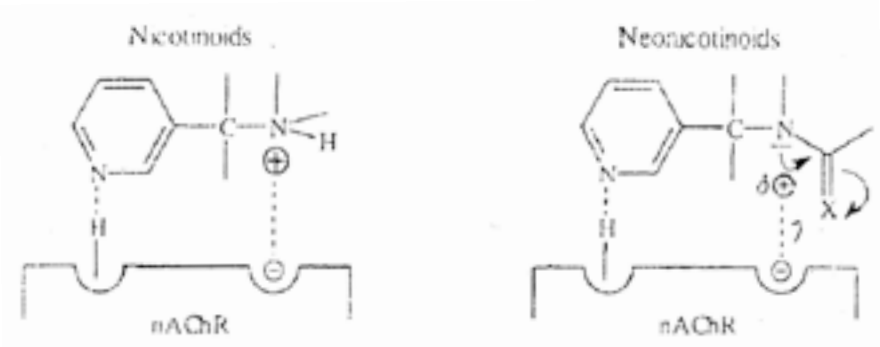


Figure 12. Partial Positive Charge of Nicotinoids and Neonicotinoids (Yamamoto and Casida, 1999)

Clinical tests on mammals confirm these findings with imidacloprid's producer, Bayer Crop Science, reporting (Yamamoto and Casida, 1999, p. 221):

- Imidacloprid is a specific nicotinergic receptor binder with very low mammalian binding potential
- Imidacloprid is rapidly absorbed, metabolized in the liver, and excreted mostly via urine
- Metabolism of imidacloprid is straightforward; no open ring structures with potential for other toxicological properties
- Imidacloprid does not penetrate the blood-brain barrier
- This lack of penetration leads to a low toxicity after acute oral, dermal, inhalatory, and long-term dietary exposure
- Symptoms of imidacloprid toxicity are non specific
- Imidacloprid shows specific neurotoxicological symptoms only at lethal doses (tremors)
- Imidacloprid is not oncogenic, not mutagenic, not a primary embryotoxin, not a reproductive toxin, and not a neurotoxin
- Imidacloprid has no worker exposure-related toxic potential
- Imidacloprid has no consumer-related toxic potential

Compared to the previous classes of insecticides, neonicotinoids were the environmentally friendly example of a new pesticide class that was highly selective and specific to insects while being relatively non-toxic to vertebrate species (Krieger, 2001). They were much safer not only for humans, but also for birds, fish, and crustaceans (but not aquatic insects) (Stenersen, 2004). "Due to its insecticidal potency and relatively low mammalian toxicity, imidacloprid has a very high margin on safety" (Krieger, 2001).

Soils

Neonicotinoids have differing persistence levels in soils and water based on a variety of factors including soil type, microbial and weathering conditions, concentration levels, etc. (see Table 8). In soils, acetamiprid and thiacloprid are on the low end of the spectrum with a half-life as low as 1 day, and clothianidin and imidacloprid can persist in soils for up to 1155 and 997 days, respectively, allowing for uptake by plants in subsequent seasons (Xerces Society).

Neonicotinoid	**Half-life in Soil (aerobic soil metabolism)**
Acetamiprid	1–8 days[1]
Clothianidin	148–1,155 days[2]
Dinotefuran	138 days[3]
Imidacloprid	40–997 days[4]
Thiacloprid	1–27 days[5]
Thiamethoxam (See note below)	25–100 days[6]

Note: Clothianidin is a primary metabolite of thiamethoxam.

Sources: 1. EPA 2002; 2. EPA 2003a; 3. EPA 2004; 4. NPIC 2010; 5. EPA 2003b; 6. Syngenta Group 2005

Table 8. Neonicotinoid Half-life in Soils (Xerces Society Online Resource)

Persistence data for imidacloprid in soils is rather inconsistent. “Some authors have reported that imidacloprid is relatively immobile in soil and that leaching below the topmost layer and into the groundwater is not likely to occur, while other authors have claimed the exact opposite” (van Dijk, 2010).

Leaching studies are important for determining a pesticide's capability to pollute ground water, particularly if the pesticide, such as the neonicotinoid imidacloprid, is highly soluble in water (van Dijk, 2010). One recent study from 2010 sought to determine the leaching potential and transport pattern of imidacloprid as it dispersed though different types of soil (see Table 9). "Results from soil columns indicated that imidacloprid insecticide is of moderate mobility in soils. After leaching of some 15 pore volumes of 0.005 M $CaCl_2$ solution, the amount of imidacloprid leached from each column varied among soils, ranging from 27% to 79% of that applied" (Selim et al., 2010, p. 380).

	Mahan	Mhoon	Sharkey	Vacherie	Sand
Total applied, μg	24,274	28,542	20,320	19,563	19,266
Cumulative leaching, μg	16,733	7,807	8,491	7,427	18,633
% Leached	69	27	42	38	97
Amount extracted, μg					
Column depth, cm					
0–2	879	674	515	1296	7
2–4	1,405	458	714	1,807	6
4–6	1,338	518	1,156	2,488	6
6–8	1,920	691	1,930	1,744	5
8–10	2,085	1,018	772	1,265	5
Total extracted, μg	7,626	3,360	5,088	8,600	28
Total extracted, %	31	12	25	44	0.15
Total recovery, %	100	39	67	82	97

Table 9. Mass Balance of Imidacloprid in Miscible Displacement Column Experiments (Selim et al., 2010)

Water

In water, neonicotinoids have been described as moderately soluble (Walker, 2012) to highly soluble (van Dijk, 2010):

> "Imidacloprid is generally persistent in water, and not easily biodegradable and is likely to remain in the water column in aquatic systems, with an

> aerobic sediment and water DT50 of 30 to 162 days (time for 50% decline of the initial pesticide concentration, or half-life time). pH and formulation on the persistence of imidacloprid in water have also been studied, and it was found that a higher pH, meaning alkaline conditions, increases half-life time and thus persistence" (van Dijk, 2010).

Plants

Neonicotinoids can "remain in plant tissues for months or even more than a year" and are found in pollen and nectar (Xerces Society Online Resource).

Metabolism

In animals and humans imidacloprid is absorbed readily by the gastrointestinal tract, where 70-80% is excreted via urine, with 20-30% excreted via feces (Extoxnet Online Resource).

> "Owing to their relatively high water solubility and slow metabolism in mammals, some (IMI and thiacloprid) to almost all (clothianidin, dinotefuran, and nitenpyram) of an oral neonicotinoid dose is excreted unchanged in urine. The chemical fate of neonicotinoids in and on crops is governed both by metabolic and photochemical reactions. These processes may produce identical or different products depending on the mechanisms involved" (Tomizawa and Casida, 2005, p. 253).

Most of the degradation processes of imidacloprid are common, at least quantitatively, in plants, animals, soil, and water (van Dijk, 2010). "The most important metabolic steps include the degradation to 6-chloronicotinic acid. In humans and animals 6-chloronicotinic acid may be conjugated with glycine and eliminated, or reduced to guanidine" (Extoxnet Online Resource), however, in honeybees, "6-chloronicotinic acid has been found to be more toxic to honey bees than imidacloprid itself" (van Dijk, 2010).

Neonicotinoids can be expected to persist in nature for some time after their application since they accumulate throughout water, soils, and flora due to their solubility, leaching potential, and systemic properties.

UNINTENDED CONSEQUENCES FOR POLLINATORS

The very same unique physiochemical properties that have made neonicotinoids so successful against agricultural pests have also made them highly toxic to beneficial insects. For bees, neonicotinoids were shown to have negative effects at very low doses. These results are not unexpected, considering that the lab tests conducted by Bayer Crop Science on honey bees concluded: “Imidacloprid is harmful to bees and should not be applied during the flowering period” (Yamamoto and Casida, 1999, p. 117), and during ecotoxicology testing on thiamethoxam, Syngenta scientists found their new neonicotinoid compound to be “highly toxic to honeybees, requiring adequate risk management” (Yamamoto and Casida, 1999, p. 205).

Of the seven commercially produced neonicotinoids, six are commonly used in agriculture (Xerces Society Online Resource). “The commercial products differ considerably with respect to soil and seed treatment uses, as soil stability is limited for some of them such as nitenpyram, acetamiprid, and dinotefuran, respectively” (Jeschke, 2011, p. 2901). Nitenpyram exhibits poor photo-stability, much like its lead chemical nithiazine, rendering it unsatisfactory for most field applications, and better suited for topical flea-control pet and livestock products (Yamamoto, 147) instead of agriculture. Of the 6 neonicotinoids used in agrochemicals, four are considered highly toxic to bees: imidacloprid, dinotefuran, clothianidin, and thiamethoxam. Acetamiprid and thiacloprid are less toxic to bees (Iwasa et al., 2004).

Acute Toxicity. The toxicity of neonicotinoids was determined using the Federal Insecticide, Fungicide and Rodenticide Act and EPA "standard acute toxicity exposure scenarios," which focus on the amount of the chemical required (by contact or ingestion) to kill 50 percent of the population in a specified time period (usually 24-48 hours) (Blacquire, 2012) (see Table 10). Nitro-substituted compounds are the most toxic to the honey bee with LD50 values of 18 ng/bee for imidacloprid, 22 ng for clothianidin, 30 ng for thiamethoxam, 75 ng for dinotefuran, and 138 ng for nitenpyram. The cyano-substituted neonicotinoids exhibited a much lower toxicity with respective LD50 values for acetamiprid and thiacloprid of 7.1 and 14.6 ug/bee, respectively (Iwasa et al., 2004).

Neonicotinoid	**Known Toxicity to Honey Bees**[1]		
		Contact LD_{50}	**Oral LD_{50}**
Acetamiprid	M	7.1 µg/bee[2]–8.09 µg/bee[3]	8.85–14.52 µg/bee[3]
Clothianidin	H	0.022 µg/bee[2]–0.044 µg/bee[4]	0.00379 µg/bee[5]
Dinotefuran	H	0.024 µg/bee[2]–0.061 µg/bee[6]	0.0076–0.023 µg/ bee[6]
Imidacloprid	H	0.0179 µg/bee[4]– 0.243 µg /bee[7]	0.0037 µg/bee[7]– 0.081 µg/bee[8]
Thiacloprid	M	14.6 µg/bee[2]–38.83 µg/bee[9]	8.51–17.3 µg/bee[9]
Thiamethoxam	H	0.024 µg/bee[10]– 0.029 µg/bee[2]	0.005 µg/bee[10]

H = highly toxic; M = moderately toxic
Toxicity: Highly toxic: LD_{50} < 2 µg/bee; Moderately toxic: LD_{50} 2–10.99 µg/bee; Slightly toxic: LD_{50} 11–100 µg/bee; Practically non-toxic: LD_{50} >100 µg/bee.

Sources: 1. WSDA 2010; 2. Iwasa et al. 2004; 3. EC 2004b; 4. EPA 2003a; 5: EC 2005; 6. EPA 2004; 7. Schmuck et al. 2001; 8. Nauen et al. 2001 ; 9. EC 2004a; 10. Syngenta Group 2005.

Table 10. Neonicotinoid Toxicity to Bees (Xerces Society Online Resource)

> “Acute LD50s average 28 and 24 ng/bee, respectively, for imidacloprid and clothianidin, although sublethal effects have been reported at much lower levels. It is unlikely that doses of neonicotinoids from routine systemic seed treatments will attain the necessary > 100 ppb levels in pollen or nectar to acutely impair honey bees” (Frazier et al., 2011).

Frazier’s findings show neonicotinoids are unlikely to reach lethal levels in the pollen and nectar directly consumed by bees. However, the machines which plant neonicotinoid-coated seeds (drilling machines towed behind tractors) have been shown capable of producing neonicotinoid dust in concentrations lethal to bees (Tapparo et al., 2012). Another possible route of acute toxic neonicotinoid exposure comes from the exudates of corn sapling leaves, called guttation fluid, which can also contain concentrations of neonicotinoids great enough to kill bees (Reetz et al., 2011) (see Figures 13 and 14).

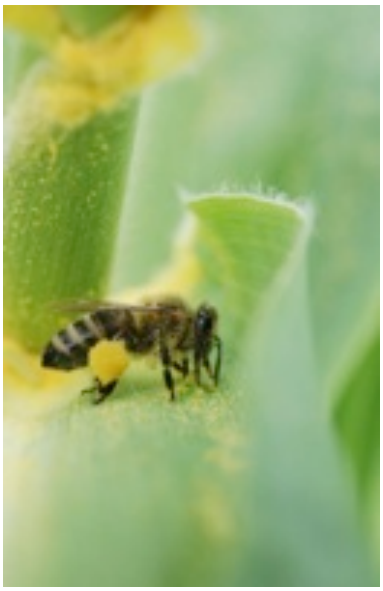


Figure 13. Corn Pollen Bee--left (Frazier et al., 2011)

Figure 14. Guttation drops--right (Reetz et al., 2011)

Secretion of guttation fluid is limited to the first three weeks after germination of a seed, but “during the first 3 weeks after emergence, imidacloprid concentrations

can be very high: From a seed treatment of 0.5 mg per seed (Gaucho 350 FS), the imidacloprid concentrations in the guttation fluid of plants grown in the laboratory ranged between 47 ± 9.9 and 83.8 ± 14.1 mg l-1" (Blacquiere et al., 2012).

Chronic/Sublethal Toxicity. Even at sublethal doses, neurotoxic insecticides such as the neonicotinoids have been noted to cause behavioral effects (Walker, 2012):

> "The effects of pollutants, and insecticides for that matter, on whole organisms fall into three main classes: neurophysiological, behavioral, and reproductive. These effects are sometimes interrelated in that neurological changes can affect behavior, and changes in behavior can impact reproduction" (Walker, 2012, p. 148).

In susceptible species, particularly flying insects, sublethal effects of neonicotinoids may be exhibited in reduced learning, signaling, navigation, foraging, and eventually starvation (van Dijk 2010; Gill, Ramos-Rodriguez, and Raine, 2012; Walker, 2012).

Since flying insects travel more than other insects, they have an increased likelihood of encountering more pesticide types than ground-dwelling insects. Honey Bees are known to forage over a radius of up to 10 miles: "The dynamic foraging of a typical honey bee colony includes a range of 3.73 miles radius 95% of the time, with a range up to 6.21 miles in times of limited sources, with the ability to detect the maximum rewarding nectar within a two hour period" (Frazier et al., 2011).

Synergism. Due to their widespread use combined with multiple application methods, neonicotinoids can easily combine with other agrochemicals

and pesticides used in the field. Such combinations could quite possibly occur in the U.S., where there are "over 1200 active ingredients distributed among some 18,000 pesticide products" (Frazier et al., 2011), and neonicotinoids are used on at least 147 million acres (Bayer CropScience Online Resource). "In some cases, toxicity may be substantially more than additive when organisms are exposed to two or more chemicals and potentiation, or synergism may occur" (Walker, 2012, p. 167).

> "When one compound (A) causes a change in the metabolism of another (B), two types of interaction are recognized which lead to potentiation of toxicity:
>
> 1. Compound A inhibits an enzyme system that detoxifies compound B. Thus the rate of detoxification of B is slowed down because of the action of A.
>
> 2. Compound A induces an enzyme system that activates compound B. Thus the rate of activation of B is speeded up because of the action of A" (Walker, 2012, p. 167).

Certain real-world mixtures of agrochemicals have proven synergistic effects when used together. Pyrethroid insecticides, for example, have been shown to become much more toxic to bees, exhibiting a synergistic ratio of 5 to 20 times, when applied in the presence of certain ergosterol biosynthesis-inhibitor (EBI) fungicides (Walker, 2012).

A 2004 study of the neonicotinoids imidacloprid, acetamiprid, and thiacloprid confirmed that fungicides widely used in the field have synergistic effects with neonicotinoids:

> "The DMI-fungicides are an important group of fungicides widely used in crop protection. Therefore, the fact that compounds like triflumizole can increase toxicity of the cyano-substituted neonicotinoids like acetamiprid

> against the honeybee, as much as 244-fold, is of some concern because of potential non- target effects when these compounds are used in combination. Colin and Belzunces (1992) and Pilling and Jepson (1993) found that the DMI-fungicides synergized pyrethroids at practical field rates" (Iwasa et al., 2004, p. 376) (See Appendix Table 14 for chart of synergism between neonicotinoids and DMI-fungicides).

Such chemicals can build up in beehives, contributing to additive, cumulative, and sometimes synergistic effects which could cripple a colony.

Chemical Cocktails. The potential result is a "chemical cocktail" that bees and other flying beneficial insects could become exposed to when they continually encounter multiple chemical combinations in sublethal doses (Walker, 51). A 2010 study by a Penn State research team turned up 121 different pesticides in a sampling of 887 hives managed by migratory and stationary beekeepers (Johnson et al., 2010).

> "These included 16 parent pyrethroids, 13 organophosphates, 4 carbamates, 4 neonicotinoids, 4 insect growth regulators, 3 chlorinated cyclodienes, 3 organochlorines, 1 formamindine, 8 miscellaneous miticides,/insecticides, 2 synergists, 30 fungicides, and 17 herbicides. Only one of the wax samples, 3 pollen samples, and 12 bee samples had no detectable pesticides" (Johnson et al., 2010, p. 13).

The study, titled "High levels of miticide and agrochemicals in North American apiaries: Implications for honey bee health," brought focus to a potential problem involving chronic and sublethal, cumulative, and possibly synergizing effects from multiple plant protection products (Mullin et al., 2010).

Bayer CropScience has developed new products based on mixtures of neonicotinoids with pyrethroid insecticides so as to "broaden the spectrum of neonicotinoid pesticides," while also protecting against cross-resistance by rotating different insecticide formulations (Jeschke, 2011). Some of the novel

imidacloprid/pyrethroids combinations developed and marketed by Bayer include (Jeschke, 2011, p. 2903):

- Muralla (imidacloprid and cyfluthrin) used on Central America vegetables and rice
- Confidor S (imidacloprid and cyfluthrin) used on S. America tobacco pests
- Leverage (imidacloprid and cyfluthrin) in the United States for broad-spectrum pest control in cotton
- Connect (imidacloprid and β-cyfluthrin) for stinkbugs and soybean pests
- Solomon and Thunder (imidacloprid and β-cyfluthrin) are cost-competitive solutions for African and Asian markets
- Confidor Energy (imidacloprid and deltamethrin) used in Europe for broad-spectrum insect control in vegetables, potato, tobacco, sugar beet, cereals

Cumulative Sublethal Exposure. Thus, it is very likely that managed honeybees, wild bees, and other beneficial pollinators are being exposed to multiple agrochemicals at sublethal levels from a variety of sources. Seed-treatment dust, foliar sprays, and guttation fluid have been proven to cause acute toxicity in bees, while pollen and nectars from neonicotinoid-treated plants can build up in hives, beeswax, and honey, sometimes with synergistic effects. A recent study from October 2012 sought to mimic real-world conditions by exposing bumblebees to both imidacloprid and the pyrethroid cyhalothrin, two commonly used agrochemicals that foraging insects could encounter. The study, discussed later, showed a link between cumulative sublethal neonicotinoid exposure and decreased individual foraging effectiveness with its associated

“knock-on” effects that impacted the health of the hive and colony (Gill, Ramos-Rodriguez, and Raine, 2012).

POSSIBLE LINKS TO COLONY COLLAPSE DISORDER

Concern for Beekeepers

In 2006 U.S. beekeepers began to notice 30-90% declines in the number of bees found in their hives (Kaplan, 2012). While certain winter losses are typical with honeybee colonies, unexplainably large numbers of managed honeybees were disappearing without explanation. Pollinator declines have now been noted in many parts of the world and on all continents except Antarctica (where there are no honeybees) (United Nations, Pollinators Status Report). The recognition of widespread pollinator losses, identified broadly as Colony Collapse Disorder, is now recognized in Europe and the U.S., as put forward in the U.N.'s 2008 Rapid Assessment of Pollinators' Status Report, which has helped generate consensus amongst numerous leading researchers that bee losses are real (Murray, Kuhlmann, and Potts, 2009). "The defining characteristic of CCD is the disappearance of most, if not all, of the adult honeybees in a colony, leaving behind honey and brood but no dead bee bodies" (Kaplan, 2012). Discovering the cause of the bee declines has proven to be a complicated question for the leading researchers.

Bees, both wild and managed, including honey bees, bumblebees, and solitary bees, are the most predominant pollinator group in most geographical regions and therefore are particularly important for global agriculture since they are directly or indirectly essential for an estimated 15-35% of food production (Kremen et al., 2007; Blacquiere et al., 2012). Furthermore, honey bees, mainly *Apis mellifera*, remain the most economically valuable pollinators of crops

worldwide (Klein et al., 2007) and are “estimated to be valued at 5-14 billion dollars per year in the U.S. alone,” (Kremen et al., 2002, p. 16812) with an estimated global annual value of 200 billion (Blaquiere, 2012).

Complex Interactions

Multiple factors are known to contribute to the bee losses, making it difficult to pinpoint just one cause. “While worldwide managed honey bee populations have increased over the past 50 years, colony populations in many European and North American Nations have decreased significantly” (Pettis et al., 2012, p. 153). At the U.S. Dept. of Agriculture Bee Research Lab, the honey bee loss factors are grouped into four types: pathogens, parasites, environmental stressors, and management stressors (list of factors compiled from Kaplan, 2012, and USDA Online Resource).

- **Pathogens:** scientists are considering Nosema (a pathogenic gut fungus), Israeli Acute Paralysis Virus, and possibly unknown pathogens as possible culprits in CCD. No one pathogen of any class directly correlates with the majority of CCD incidents. Rather, a higher total pathogen load of viruses and bacteria correlates more directly with CCD than any one specific pathogen.

- **Parasites:** Varroa mites are often found in honeybee colonies that are affected by CCD. It is not known if the Varroa mites are directly involved or if the viruses that Varroa mites transmit (similar to the way mosquitoes transmit the malaria virus) are a factor in causing CCD.

- **Management stressors:** Among the management stressors that are possible contributors to CCD are poor nutrition due to apiary overcrowding and increased migratory stress brought on by the practice of transporting honeybees to multiple locations across the country.

- **Environmental stressors:** Such stressors include the impact of pollen/ nectar scarcity, lack of diversity in nectar/pollen, availability of only pollen/ nectar with low nutritional value, and limited access to water or access

only to contaminated water. Stressors also include accidental or intentional exposure to pesticides at lethal or sublethal levels.

Habitat loss (and fragmentation as a result of agricultural intensification), invasive species, and global climate change all have the potential to impact bee populations as well (Brown and Paxton, 2009; Kremen et al., 2002). Bees awakening from hibernation have in the past been synchronized to the bloom of flower plants used as floral resources by bees, but now many plant species are blooming while the bees are still in hibernation (The Guardian, Bees Stung by Climate Change Link).

A factor that stresses bees may not be directly responsible for an untimely bee mortality, but the cumulative effects of multiple stressors combined with parasites and disease are proving to be more than bees and colonies can handle. Multiple studies have been conducted to determine the extent to which neonicotinoids are harmful to bees at both acute and sublethal levels. A convergence of research is zeroing in on sublethal, cumulative, and synergistic impacts (Frazier et al., 2011).

Imidacloprid was shown in a laboratory study over a 10-week sublethal-dose experiment to bring about significantly increased levels of the gut parasite Nosema (Pettis et al., 2012).

> "In the study, bees were first exposed to low levels of the pesticide imidacloprid in the field and then introduced to Nosema mites in the laboratory. Bees that had been exposed to the neonicotinoid had three to four times the level of Nosema spores 12 days later than bees in a control group that had not been exposed to the insecticide" (Crop Protection Association Online).

The authors of this noteworthy study, U.S. Department of Agriculture lead researcher and bee scientist Jeff Pettis, and coauthor, Penn State University entomologist Dennis van Engelsdorp, made the following remarks about their research in the documentary “The Strange Disappearance of the Bees,” when filmmaker Mark Daniels caught up with Pettis at the international conference of bee scientists, Apimondia, in Montpellier, France, in September 2009. Pettis and Dennis Van Engelsdorp spoke frankly about their findings for the film: (Grist.com; Beeuntoothers; Strange Disappearance of the Bees):

> **van Engelsdorp:** We’re finding that virus levels are much higher in CCD bees. But since we’re not finding a consistent virus, or a consistent pathogen, that implies that something else then is happening underneath it. Something is breaking down their immune system or somehow challenging them so that they’re more susceptible to disease.
>
> **Pettis:** I’ve done a recent study, actually in collaboration with Dennis and some others, where we exposed whole colonies to very low levels of neonicotinoids … and then challenged the bees from those colonies with Nosema, a gut pathogen, and we saw an increase. Even if we fed the pesticide at very low levels we saw an increase in Nosema levels in direct response to the low level feeding of neonicotinoids as compared to the ones who were fed normal protein.
>
> **van Engelsdorp:** … The only reason that we knew the bees had exposure is because we exposed them. Otherwise you would never have known they were exposed.
>
> **Pettis:** The take-home message is that interactions may be key. Bee health is very complex and these interactions are often … overlooked and/or hard to tease apart. In this case we’re manipulating one pesticide, and one pathogen, and we’re clearly seeing the interactions.

The dialogue created by USDA scientists and this possible link among Nosema, neonicotinoids, and CCD would lead to more studies to attempt to confirm this

connection. Doing so would require a different methodology than traditional toxicological testing.

Realistic Field Studies

Results from the lab do not always approximate real-world conditions, however. Pettis later went on to clarify his comments by stating: "This study did not look for nor establish any connection between either imidacloprid or Nosema and CCD, but the effect of the combination of imidacloprid and Nosema demonstrates that there are many complex interactions between stress factors that need to be considered in looking for a cause of CCD and high honeybee mortality in general" (Kaplan, 2012). More studies are needed that track bees in real-world environments where there are varied chemicals coming from multiple sources, and over longer times than the 24-48 hours which LD50 tests use.

A new era of research is now yielding data that enable researchers to track individual bees with radio frequency identification tags (RFID) as they enter and exit the hive to forage. A greater level of comprehension is possible when the aggregated behavior patterns of individual bees can be studied and compared to the overall hive's performance and well-being. This unique methodology for bee research began in early 2011 with experiments conducted by Decourtye et al. which aimed to show how the RFID device can be used to study pesticide effects on bees' behavioral traits and lifespan (Decourtye et al., 2011).

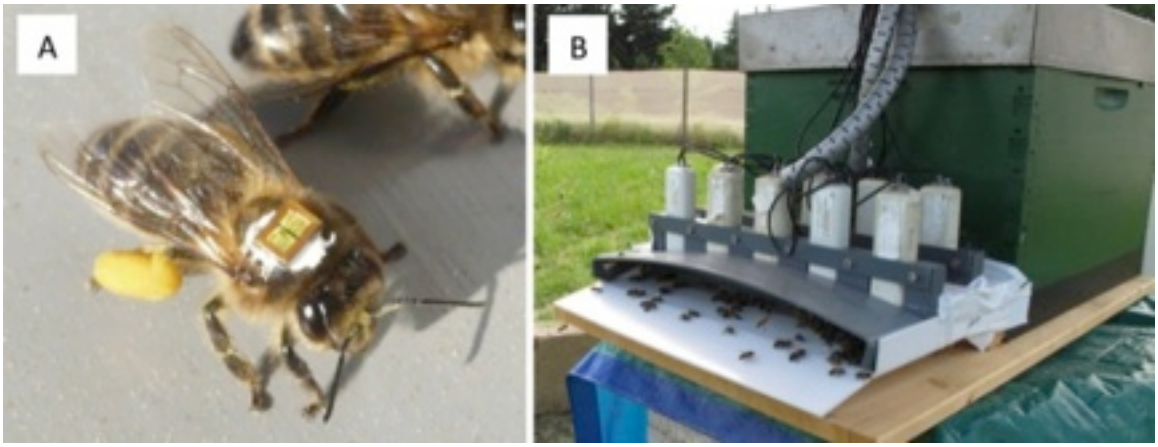


Figure 15. Honey bee RFID monitoring equipment: (A) A pollen-forager honey bee fitted with a 3-mg RFID tag, (B) A hive entrance equipped with RFID readers for detecting returning marked foragers (Henry et al, 2012)

Others soon expanded on RFID tracking experiments. The 2012 study conducted by Henry et al. tested the hypothesis that "a sublethal exposure to a neonicotinoid indirectly increases hive death rate through homing failure in foraging honey bees" (Henry et al., 2012) (see Figure 15).

Another 2012 study conducted by Gill, Ramos-Rodriguez, and Raine from Royal Holloway, University of London, provided additional insights into hive dynamics and complex foraging interactions. Using RFID radio tags, Gill's team was able to track different control groups of bees as they entered and exited their hives over a long-term, four-week exposure, to demonstrate cumulative, sublethal, and synergistic effects of neonicotinoids and pyrethroid fungicides. This study demonstrates how imidacloprid impairs the ability of individual bees to return to the hive, thereby reducing foraging efficiency and altering colony dynamics (Gill, Ramos-Rodriguez, and Raine, 2012) (see Table 11):

Effect level	Effect type	I	LC	M
Effects on individual behaviour	Number of foragers	+	ND	+
	Foraging bout frequency	ND	ND	−
	Amount of pollen collected	−	ND	−
	Duration of pollen foraging bouts	+	ND	+
Effects at colony level	Worker production	−	ND	−
	Brood number	−	ND	−
	Nest structure mass	ND	ND	ND
	Worker mortality	ND	+	+
	Worker loss	+	−	+
	Worker mortality & loss	ND	+	+
	Colony failure (*n* failed/*n* survived)	0/10	0/10	2/8

Significant decrease (−), significant increase (+) and no detected effect (ND) at the 5% significance level.

Table 11. Results of field realistic dose and long term exposure study (note that "I" refers to imidacloprid, "LC" to cyhalothrin, and "M" refers to imidacloprid/pyrethroid mix) (Gill, Ramos-Rodriguez, and Raine, 2012)

> "These findings show a mechanistic explanation to link recently reported effects on individual worker behavior and queen production as a result of neonicotinoid exposure. Moreover, exposure to a second pesticide l-cyhalothrin (pyrethroid) applied at label-guideline concentration for crop use caused additional worker mortality in this study, highlighting another potential risk. Bee colonies typically encounter several classes of pesticides when foraging in the field, potentially exposing them to a range of combinatorial effects.... that impairs natural foraging behavior and increases worker mortality leading to significant reductions in brood development and colony success. **We found that worker foraging performance, particularly pollen collecting efficiency, was significantly reduced with observed knock-on effects for forager recruitment, worker losses and overall worker productivity. Moreover, we provide evidence that combinatorial exposure to pesticides increases the propensity of colonies to fail**" (Gill, Ramos-Rodriguez, and Raine, 2012).

This study proved that a cascade of effects could impact the colony when the additive effects of long-term exposure to field-realistic combinations and doses of agrochemicals were examined.

Based on anecdotal evidence from their own hives, and due to studies like the one conducted by Gill, Ramos-Rodriguez, and Raine (2012), many beekeepers around the world suspect the neonicotinoids are a primary

contributing factor which has pushed their colonies to collapse as a result of impaired foraging combined with weakened immunity to disease.

NEONICOTINOID REGULATION IN THE U.S. AND EUROPE

Neonicotinoid insecticides have been a great success in pest control, having replaced previously used, more harmful pesticides, with a family of agrochemicals which are much more benign to humans and animals. Compared to DDT, organophosphates, and pyrethroids, the neonicotinoids are not only safer, but allow for more concentrated and targeted use, thereby requiring far less volume applied in order to be effective. As has been the case with previous pesticide classes, the secondary effects of a new chemical's use became known after the widespread use of the new product. There are many sides to the debate over safe neonicotinoid use.

Stakeholders. At one end of the debate over neonicotinoid use are the beekeepers, honey producers, pesticide safety advocates, and bee researchers who claim neonicotinoids harm bees and at the other end are the agrochemical and pharmaceutical firms that produce neonicotinoid products and claim they are safe when properly used. The agrochemical companies control a large portion of the genetically modified crop, plant, and seed markets (the top ten companies for 2008 sales: Syngenta, Bayer CropScience, Monsanto, BASF, Dow Agrosciences, Dupont, Makhteshim-Agan, Nufarm, Sumitomo Chemical, and Ayrsta LifeScience) (Pollack, 2011). Farmers and government agencies responsible for the public and environmental health have been in the middle of the debate, while the public to some extent has remained largely unaware and unconcerned over the use of neonicotinoids and their possible link to bee declines. Numerous stakeholders clearly have different objectives. At the

regulatory level, countries such as France, Italy, Germany, and Slovenia have imposed neonicotinoid restrictions (EPA clothianidin registration) and bans, while nations such as the U.S. continue to study the problem and weigh the evidence.

European Restrictions

Beginning in 1994, French beekeepers began to report “mad bee disease,” a condition synonymous with CCD (the term later coined in 2006 by U.S. beekeeper David Hackenberg), in which bees become disoriented and unable to return to the hive resulting in “melting away of the hive” (The Guardian Online Resource). In describing the 1994 and 1995 bee losses, an anecdotal link was made between the bee declines and the introduction of the systemic neonicotinoid seed coating, Gaucho, on Sunflower seeds in central France.

One team of French researchers, lead by Dr. Colin of the Institute National Researche Agricole (INRA), determined, after years of study, that “the problem has worsened with the increasing use of the seed-dressing formulation of imidacloprid on sunflower, maize, and rape, in west European countries. From this, imidacloprid has been suspected of having harmful effects on honeybees, whereas other factors such as Varroa infestations or viruses development had to be studied as well” (Bonmatin et al., 2005, p. 5336). Anecdotally, while the scientific studies were under way, French beekeepers continued to notice widespread hive collapses during the years when imidacloprid was introduced as a seed coating, with the National Union of French Beekeepers reporting that one third of their colonies had disappeared (resulting in a loss of over 90 billion

French bees in ten years), causing honey production to fall from 110 tons in 1996 to 50 tons in 1999 (API Services Online Resource; Soils Association Online). A government commissioned French science team concluded after three years of research:

> "The French researchers (led by Dr Colin at INRA) looked at doses of IMD down to <1 ppb (parts per billion) and found **that as little as 6ppb could impair the foraging behaviour of the bees – and their feeding behavior. This was of course completely at odds with the manufacturer, Bayer's, figure of IMD being safe at levels 50-100ppb"** (Soils Association Online Resource)**.**

French Ban. On Jan 22nd 1999 the French Minister of Agriculture acted on the scientific evidence and directed the suspension of imidacloprid on sunflowers, pending research which proved it safe (Soils Association Online Resource). France became the first country to apply the precautionary principle in banning imidacloprid in 1999 as a sunflower seed treatment, and then again renewed the ban in 2001 and 2004. In 2004, the imidacloprid ban was extended to corn seeds (Maxim and van der Sluijs, 2010). The precedent had been set that imidacloprid and the neonicotinoids could pose danger to pollinators and threaten food security. In July of 2012, the French Minister of Agriculture extended the scope of the neonicotinoid restriction when it banned Syngenta's thiamethoxam-based seed coating, Cruiser OSR (Beyond Pesticides Online Resource).

In Italy, Slovenia, and Germany various neonicotinoid restrictions and bans have been enacted while environmental scientists continued to study the effects of sublethal and field-realistic doses. In Italy, imidacloprid and other neonicotinoid seed treatments were suspended temporarily, but foliar uses were

still permitted (EPA clothianidin registration). In Germany temporary restrictions on seed treatments went into effect following a 2008 accident when 12,000 bee colonies were severely poisoned by contaminated neonicotinoid seed-treatment dust from the bags of seeds and also from the drilling machines used to plant the seeds (Forster, 2011). The accident occurred when:

> "The formulation of the pesticide clothianidin used to protect seed corn from corn root worm in Germany did not include a polymer seed coating known as a "sticker." This coating makes the pesticide product stick to the seed. Although the formulation used in the United States also does not require a "sticker" on corn seed, the major seed suppliers and distributors, agricultural industry groups, and clothianidin's registrant have confirmed that it is typical practice to use "stickers" on corn seed in the United States" (EPA clothianidin registration).

Following further investigation and after putting new best practices into place to mitigate factors contributing to seed-treatment dust (including better label warnings), Germany eventually lifted the suspensions, except for clothianidin, which remains suspended as a corn-seed treatment (EPA clothianidin registration). Slovenia took similar action regarding restriction of neonicotinoid seed treatments (EPA clothianidin Registration).

U.S. Conditional Registration and Regulation

Conditional Registration. Neonicotinoids have not escaped U.S. controversy. There has been much debate surrounding the use of the neonicotinoid clothianidin, produced by Bayer, sold under the brand name "Poncho," and used as a seed treatment and foliar application on corn, wheat, soy, sunflowers, and canola, amongst other U.S. crops. In 2003, after submitting and satisfying the basic study requirements of the EPA, Bayer was granted

conditional registration for clothianidin for use in U.S. agriculture. The EPA was not completely sure about the environmental fate of systemic neonicotinoids, and requested additional studies at that time: “to address uncertainties about potential long-term effects of clothianidin on honey bees. In 2007 (the EPA) reviewed the additional studies submitted by Bayer and determined that it satisfied the EPA’s field study guidelines. However, the agency’s assessment of the usefulness of this study has changed since the 2007 review, which is not unusual in the scientific field” (EPA.com clothianidin registration).

Confirming U.S. Bee Losses. In 2006 U.S. beekeepers began to see the same type of widespread bee declines that had been recently observed in Europe. A team lead by Dennis vanEngelsdorp, “In an attempt to quantify the degree and extent of losses experienced in beekeeping operations in the United States between September 2006 and March 2007, requested that all members of the Apiary Inspectors of America (AIA) survey beekeepers in their state, and in all, 396 beekeepers were surveyed, who managed a total of 160,526 colonies at the end of September 2006” (vanEngelsdorp et al., 2007, p. 1) :

> “In all, 349 of the surveyed beekeepers reported on how many of the colonies they lost died without any or with very few bees. While 127 respondents reported some losses with no or very few bees in dead colonies, only 80 met our specified definition threshold of 50% of the operation’s lost colonies being found without bees (vanEngelsdorp et al., 2007).”
>
> “Overall, the total losses in operations suffering from CCD were nearly twice as high (45.0%) as the total losses experienced in the non-CCD suffering group (25.4%)” (vanEngelsdorp et al., 2007, p. 3) (see Table 12 below).

	Operation Size	No. of Respondents	Colonies Managed (plus Increases) in September 2006	Total Loss % (95 % CI)
CCD	1 to 50	41	426	45.3(40.6- 50.0)
	51 to 500	20	3,501	47.3 (45.6-49.0)
	500 +	19	50,308	44.8 (44.4-45.2)
	Total	**80**	**54,235**	**45.0 (44.9-45.1)**
not CCD	1 to 50	194	1,865	32.5 (30.4-34.6)
	51 to 500	41	6,770	30.6 (29.5-31.7)
	500 +	21	49,018	24.4 (24.0-24.8)
	Total	**256**	**57,653**	**25.4 (25.0-25.8)**

Table 12. Total beekeeper losses experienced by beekeepers suffering from and not suffering from CCD (vanEngelsdorp et al., 2007)

The U.S. bee losses were a focusing event. Following the widely reported and scientifically surveyed and confirmed bee declines, a consensus was reached that colony collapse disorder was impacting U.S. colonies. The cause remained the larger question for those involved. One Harvard researcher recently hypothesized that the collapses in the US could be due to a new route of exposure for managed bees. Harvard School of Public Health Associate Professor of Environmental Exposure Biology, Chensheng Lu, and his co-authors explain:

> "The uptick in CCD resulted from the presence of imidacloprid, a neonicotinoid introduced in the early 1990s. Bees can be exposed in two ways: through nectar and pollen from plants or through high-fructose corn syrup beekeepers use to feed their bees. (Since most U.S.-grown corn has been treated with imidacloprid since 2005, it's also found in corn syrup)" (Harvard Online Resource).

Evidence is mounting that sublethal, chronic exposure to neonicotinoids is very likely a factor/contributor causing CCD.

EPA Internal Communication. It has been contended that the reports produced by Bayer were internally questioned within the EPA since their initial submission. “Leaked memos written by EPA scientists stated that what studies Bayer did submit were poorly run, and the scientists openly admitted that neonicotinoids pose harm to honeybees” (Stonebrook, 2012). A published internal EPA communication, **SUBJECT: Clothianidin Registration of Prosper T400 Seed Treatment on Mustard Seed (Oilseed and Condiment) and Poncho/Votivo Seed Treatment on Cotton**, written in November 2010 by the Environmental Risk and Environmental Fate and Effects Division, seems to confirm that the EPA harbors doubts regarding clothianidin use, noting: “**This compound is toxic to honey bees. The persistence of residues and potential residual toxicity of clothianidin in nectar and pollen suggests the possibility of chronic toxic risk to honey bee larvae and the eventual instability of the hive** (Grist.org EPA internal memo on clothianidin).

Lobbyists and Coalitions. The internal communication from the EPA was received with great interest by groups such as The National Honey Bee Advisory Board, The American Beekeeping Federation, The American Honey Producers Association, Beyond Pesticides, Pesticide Action Network of North America, and The Center for Biological Diversity, who were all critical of the EPA’s “conditional registration” program. These groups formed a coalition and requested a “stop use order” on clothianidin due to “imminent hazard,” stating in a December, 2010 letter to the EPA:

> “The conditional registration of clothianidin in 2003 with outstanding data critical to its safety assessment represents a failure that could and should

> have been avoided. Clearly, the impacts on pollinators were not adequately evaluated prior to the issuance of the conditional registration, despite knowledge of "chronic toxic risk to honey bee larvae and the eventual instability of the hive" (EPA.com clothianidin stop use letter).

These groups claimed: “because the hazards to honeybee health are present within registered use parameters, it is clear that label changes alone will not offer adequate protection. The issue is not one of application error, in other words” (EPA.com clothianidin stop use letter).

EPA Rationale. The EPA responded to the “stop use” letter by saying that it had reviewed its original stance on Bayer’s clothianidin field-testing reports and, though the initial testing submitted by Bayer that was used to grant conditional approval has since been called into question, the EPA contends clothianidin was assessed during the registration process using “hundreds of studies,” and still meets the Agency’s risk/benefit standards (EPA.com clothianidin registration).

> “As the EPA’s understanding of honeybee biology has improved, staff scientists have started to recognize the challenges associated with field-pollinator study designs.
>
> It is clear that field-pollinator studies cannot be viewed in the same context as laboratory studies where experimental conditions can be strictly manipulated. Recognizing the complexity of conducting field studies, the EPA is endeavoring to make the best use of existing data to address uncertainties. Although the EPA noted deficiencies in the clothianidin pollinator field study, including some cross-contamination between treated and non-treated (control) experimental plots and inadequate separation between treated and control portions of the study, there was information that could be used to qualitatively describe hive survival following exposure to clothianidin.

> The re-evaluation of the study in question does not change the agency's conclusion that the registered uses of clothianidin meet the FIFRA risk/ benefit standard for registration" (EPA.com clothianidin registration)

The EPA went on to further clarify its rationale for the acceptable use of clothianidin and other neonicotinoids:

> "The Agency bases pesticide risk characterizations on the entire body of information submitted by the pesticide registrant and the open scientific literature data. For clothianidin, the weight-of-evidence risk characterization was based on 34 studies and not on the findings of a single, specific field study. Therefore, the reevaluation of the study in question does not change the Agency's conclusion that the registered uses of clothianidin meet the FIFRA risk/benefit standard for registration. **Clothianidin generally poses less risk to agricultural workers and fish and wildlife when compared to the organophosphate insecticide alternatives. While acute laboratory data show that clothianidin is toxic to honey bees, as are most insecticides, current labels for clothianidin products used as foliar treatments include bee hazard statements that prohibit applications when plants are flowering and bees are in the area. At this time, we are not aware of any data that reasonably demonstrates that bee colonies are subject to elevated losses due to chronic exposure to this pesticide.** Based on EPA's thorough review of scientific information, EPA does not intend at this time to initiate suspension or cancellation actions against the registered uses of clothianidin" (EPA.com clothianidin response letter).

The EPA continues to investigate neonicotinoid safety. A re-evaluation of all neonicotinoids is currently being conducted in coordination with Canada's Pest Management Regulatory Authority, along with the California Department of Pesticide Regulation. The EPA registration review began in December of 2011. "This extensive review will determine if any restrictions are necessary to protect people, the environment, or pollinators" (EPA.com clothianidin registration).

Emergency Citizen Petition. While the EPA continued to investigate and gather data, a group of 27 petitioners, including beekeepers and honey

producers, Beyond Pesticides, Center for Food Safety, International Center for Technology Assessment, and Pesticide Action Network of North America submitted a second petition to the EPA--this time “an emergency citizen petition” for the suspension of registration for clothianidin in March of 2012. The EPA responded in July 2012 with a response, and, while agreeing with petitioners in some regards, and straddling the fence on many others, the EPA ultimately failed to suspend, opting to continue study. Some of the summary findings of the EPA decision on clothianidin (from EPA.com July 2012 clothianidin petition response):

- Clothianidin use was widespread and common (neonicotinoids used on 90% of U.S. corn) with multiple routes of exposure for bees
- Clothianidin was persistent and stable across multiple soil, aquatic, and under conditions of reduced or low sunlight (EPA not sure if it binds longer than a one year and accumulates in successive growing seasons)
- EPA recognizes that clothianidin is acutely toxic--but questioned exposure levels (EPA not certain if clothianidin is generally available in the environment at levels that can cause serious, imminent danger to bee populations)
- EPA was aware of over 134 adverse incidents reported in 2012 involving clothianidin and bee losses (EPA also aware of German incidents of confirmed bee poisonings involving seed dust residues, but cited user error as the cause)
- Synergistic with Nosema and other pesticides (EPA agrees with USDA scientist Dr. Pettis, that concurrent exposure to insecticides at sublethal

levels is associated with some increased sensitivity to Nosema infestations, but EPA remains uncertain how to interpret this data)

- EPA ultimately could not make the link to CCD (EPA agrees that studies appear to show links between imidacloprid and sublethal effects on mobility, feeding activity and memory and associative learning capabilities, but stating that the studies cited failed to indicate if these effects are permanent or transitory or whether such effects would be likely for other neonicotinoids)

This petition and the response from the EPA sum up many of the points of contention in the neonicotinoid debate and emphasize the need for future studies testing synergistic combinations of sublethal neonicotinoids over longer time periods. "The EPA agrees with the scientific community that additional research in necessary to address CCD. However, the existence of uncertainty as to these questions is not sufficient to satisfy the high probability standard necessary to support a finding on imminent hazard" (from EPA.com July 2012 clothianidin petition response).

The EPA, recognizing the limitations of its current pesticide-testing procedures in the age of systemic neonicotinoids and bee declines, is actively seeking to update its methodologies. Working with the California Department of Pesticide Management and Canada's Pest Management Regulatory Agency, the U.S. EPA released its "Draft Pollinator Risk Assessment Framework" in August 2012 (Western Farm Press). "In September 2012, the agency will seek an independent scientific peer review on how to better assess the risks of pesticides

to pollinators. This effort will improve our understanding and strengthen the scientific and regulatory process to protect honey bees and other pollinators" (EPA clothianidin registration). The review is not expected to be completed until 2018 (Senator Gillibrand Online Resource).

U.S. Legislators. Such findings have not escaped concerned U.S. legislators such as Senator Kirsten Gillibrand (D) from New York, and a member of the Senate Agriculture Committee, who called in July 2012 for the EPA to expedite their review of pesticides which "could be inadvertently decimating honey bee populations." "Senator Gillibrand urged a quicker timeframe, asking that the neonicotinoid review be completed by the end of 2013, instead of 2018" (Senator Gillibrand Online Resource).

Congressman Richard Markley, (D) of Massachusetts, is also urging for the EPA to take greater action in light of growing evidence linking clothianidin and other neonicotinoids to CCD (Chemical and Engineering News). A month after the EPA responded to the emergency petition, Congressman Markley cited two recent noteworthy studies linked to bee impairment in an August 2012 letter to the EPA:

> "Two recent scientific studies offer evidence that neonicotinoids may cause Colony Collapse Disorder. In a study published in the journal Science on April 20, 2012, (by French researcher Dr. Mickael Henry et al.) scientists reported that honeybees treated with a nonlethal dose of thiamethoxam, a type of neonicotinoid, failed to return to their hive. In a related study published in the same issue of Science, (by Dr. Penelope Whitehorn et al.) researchers treated colonies of bumblebees with a low dose or high dose of imidacloprid, another type of neonicotinoid. They observed that bees exposed to imidacloprid had a lower body weight than non-exposed bees. Moreover, colonies exposed to imidacloprid produced fewer queens than non-exposed colonies. Many other studies show that neonicotinoids harm bees, as reviewed in the March 2012 petition and in

> the EPA's technical support document for the July 17, 2012 response" (Markley Letter to EPA, p. 2).

Markley contends, along with many others, that there is sufficient evidence linking neonicotinoids to CCD for the EPA to act now. As some concerned environmentalists have noted "Germany, France, and Slovenia have banned use of the controversial pesticides or limited it pending further study, and the U.K. is considering such a move. Why isn't the EPA more cautious when it comes to using chemicals? Why isn't it standard to wait until a chemical is proven to be safe to approve it, rather than wait until a chemical is proven to do harm to remove it?" (Grist.com Online Resource).

Counter Evidence and Study Bias. Whereas France banned Syngenta's Cruiser due to studies like the one conducted by Henry et al. (2012), other nations are taking a more measured approach, as claims of bias and sloppy science have been made about the Henry study. The British Food and Environmental Agency, in coordination with University of Exeter researcher Dr. James Cresswell, raised concerns about Henry's methodology, questioning the reproduction rates of the bees used in the study as well as the dose administered (Cresswell and Thompson, 2012).

> "They modeled a colony that isn't increasing in size and what we know is that in springtime when oilseed rape is blossoming, they increase rapidly," Cresswell told Reuters. And, in regard to the way bees were given the nectar laced with the insecticide, the dosage given was equivalent to a full day's intake. "We know that neonicotinoids affect honeybees, but there is no evidence that they could cause colony collapse." When we repeated the previous calculation with a realistic birth rate, the risk of colony collapse under pesticide exposure disappeared" (Center For Regulatory Effectiveness Online Resource).

Cruiser manufacturer Syngenta also believed such studies were incomplete. Mark Titterington, Syngenta's head of European, African, and Middle East affairs, said: "There are bee health declines in certain upland areas of Switzerland where there are no neonicotinoids used. In contrast, there is no significant decline in bee health in Australia but neonicotinoids are widely used." (Farmers Weekly).

> "Based on previous statements, we believe this committee is in danger of pinpointing the bee colony decline on a single pesticide when there are other important factors at play, such as climate change, habitat, and the Varroa mite (a serious honeybee colony pest)" (Farmers Weekly).

Syngenta makes these statements as Britain's Department for Environment, Food, and Rural Affairs (DEFRA), due to increasing pressure from conservation groups, asked government officials in Parliament's Environmental Audit Committee to "examine the practical consequences of a ban or restriction over the use of neonicotinoid pesticides" (Farmers Weekly).

While the governing agencies sift through the evidence and decide which agrochemicals the multinationals should be allowed to sell, the environmental advocates, conservation groups, and beekeepers will continue sounding the alarm, independent researchers will continue to look for links to CCD, and the public will likely grow more aware over time of the impact of neonicotinoids.

> "The contradictions between the different expert views have a triple origin: (1) the lack of shared definition and quantification of the signs observed in colonies; (2) the lack of specialist knowledge on honeybees; and (3) the strategic discursive practices associated with the lack of trust between experts representing stakeholders having diverging stakes in the case" (Maxim and van der Sluijs, 2010, p. 9).

The goal is that proper regulation can be implemented in order to avert a potential pollinator crisis. More studies using standardized methodologies, and time, will tell.

CONCLUSIONS: WEIGHING EVIDENCE AND MEASURING RISKS

The controversy over neonicotinoids is one about managing risks amidst uncertainty. The debate encompasses a crisis of hunger for far too many citizens of our planet while population growth, global climate change, increasing consumption patterns, and the evolved resistance of insect pests to crop protection products all culminate to exert amplified pressure on the global food supply. Pesticides have come a long way in the quest for control over our food supply, but bees are also important in the same regard, since roughly one third of all food consumed results from the pollination work of bees. Green chemistry has advanced to the point that we can now create tailor-made compounds to protect our crops by selectively targeting pests from the inside-out via translocated seed coatings, all with a very high margin of safety for humans and animals. The same could not be said before the arrival of the neonicotinoid insecticides. These advances have arisen in the past 50 years as the result of the search for the *perfect pesticide--one that is completely non-toxic and non-disruptive to non-target species, immune from pest resistance, and minimally persistent in ecosystems*. Pesticides have been engineered to be much safer to humans and ecosystems than previous crop-protection products, but continue to pose problems for non-target species, such as beneficial pollinators. The dilemma presently faced involves the risk that managed and wild bee colonies, along with solitary bees, could ultimately collapse as the result of the widespread use of neonicotinoids. This is problematic because neonicotinoids have proven

far superior to previous insecticide classes, and many commercial crops are now reliant on neonicotinoid seed coatings, despite their risk to pollinators.

Uncertainty over the safe use of neonicotinoids is a main result of the lack of understanding about how the neonicotinoid insecticide class impacts bees, but also is a result of how we value ecosystem services provided by bees. Whether neonicotinoids can be considered "safe," depends on how we quantify the effects of neonicotinoids, and also how we measure the value of bees. The developers and producers of neonicotinoids state that their products should be applied with care around bees, and restricted when bees are present, and have recognized that neonicotinoid compounds are synergistic with other agrochemicals. Independent researchers are now beginning to produce field-realistic studies (using radio-tagged individual bees) documenting sublethal doses that bees are exposed to, and these findings are beginning to shed light on the issue. The uncertainty over field-realistic quantities from cumulative, additive, and sometimes synergistic exposure routes of neonicotinoids to bees is beginning to be resolved with data from longer term, and better designed studies. Uncertainty remains within the scientific community over links of neonicotinoids to CCD, which, combined with the advocacy of environmental groups and beekeepers, is causing uncertainty within government environmental agencies. The variety of regulations and restrictions, and lack thereof, on neonicotinoids reflects this division of opinion at the regulatory level.

There would seem to be a convergence of evidence through 2012, as a new generation of studies has been published, and the increased focus on bee

losses around the world have led to a greater call for neonicotinoid bans. Brazil, Japan, and Britain are currently considering bans, and the U.S. is currently reviewing neonicotinoids. This convergence is based on the facts that are emerging. What multiple researchers are confirming is that sublethal doses of neonicotinoid insecticides, through cumulative and multiple routes of exposure, are hindering bees' cognitive abilities (such as memory, navigation of mazes, foraging, communication skills), causing chronic mortality, and *possibly* weakening individual and colony immunity and ability to fight disease. Many of these effects were reported at very low levels of dosage, far below the LD 50, and lower than the recommended application rates (in some cases at rates which would have been undetectable using most equipment had those bees not been part of the control group). Moreover, the chronic effects of the neonicotinoids very often take longer than 48 hours to create observable effects, more often requiring weeks of sublethal exposure before a tipping point is reached within individual bees which then impacts, and possibly collapses, the entire hive. There is little dispute that the neonicotinoid class of insecticides is highly toxic to bees--this is a fact reported by the manufacturers throughout the testing process. The key issue for the agrochemical companies, which have invested many R&D hours combined with massive monetary resources in creating a new and safer pesticide, is their assertion that the neonicotinoids are safe to bees for field use at the prescribed rates. The acute toxicity testing protocol required by the EPA has failed to assess the long-term sublethal effects. What the EPA and agrochemical companies alike have failed to do thus far is to conduct long-term

exposure studies, like those researchers who have conducted studies that are more field-realistic. What these researchers have concluded is that neonicotinoids cause sublethal effects in bees, even at fractions of the recommended use rates. As a person well versed in the neonicotinoids discourse and the competing studies, Dr. James Frazier, Professor of Entomology at Pennsylvania State University, states regarding the EPA conditional registration of clothianidin for use in the US: "For me this raises real concerns that the neonicotinoids that are currently being used in the market place were registered by a risk assessment process that was seriously flawed in its capacity to evaluate systemic pesticides" (Frazier Critique Letter, 2012, p. 6).

There is presently enough evidence to make the case that neonicotinoids represent tremendous risk to bees, however, neonicotinoids offer so many benefits over the alternative pesticides, that even with the added bee risks, the overall benefits to human and animals may outweigh the danger to bees. Furthermore, the neonicotinoids are the only new major pesticide class developed in the past 40 years, and it will undoubtedly take time to create the next generation of pesticides. Safe-use standards will need further exploration. It may be determined that seed treatments ultimately pose an unacceptable risk level to bees due to the translocation of neonicotinoids into pollen and nectar, whereas other neonicotinoid applications, such as foliar sprays, formulated with acetamiprid or thiacloprid (the neonicotinoids considered of less toxicity to bees because they degrade quickly), may be approved for safe use when flowers are not in bloom, and when applied during the evening hours when bees are less

likely to be exposed (Beecharmers Online Resource). While they are considered safe to humans and animals, the evidence has been building that neonicotinoids are highly toxic to bees and likely contribute to CCD. As a growing number of government environmental agencies investigate neonicotinoids to either justify their ban or continued use, more studies will continue to emerge which will only add to the evidence. Gaps in knowledge have been addressed and now testing methodologies are being refined in order to paint a clearer picture of the complex factors involved in CCD.

Many people are completely unaware of the predicament currently faced by bees. Worsening bee declines, in excess of natural rates, are a relatively new phenomena in the US and have only been noted since 2006. Pollinators, and bees in particular, are a proven bioindicator and their decline should be viewed as a warning that something may be amiss. "Individuals and populations can be used to monitor the environmental stress brought about by increased competitors, diseases, parasites, predators, as well as by chemical and physical factors, particularly pesticides and habitat modification" (Kevan, 1999, p. 373). Bees can provide valuable lessons about the environment if we know how to interpret the signals. "Entombing," like CCD, is a relatively new term used to describe beehives where poisonous pollen cells have been capped off, or entombed beneath a layer of propolis (a natural sticky resin with natural anti-bacterial and anti-fungal qualities collected by bees from plants), in order to protect the colony from the toxic contents of the foraged pollen (The Guardian, Honeybees Entomb). Entombing behavior was first observed and reported by

Dr. Pettis and Dr. vanEngelsdorp et al. in 2009 (vanEngelsdorp et al., 2009). Dr. Pettis, a USDA entomologist, notes that entombing is an ominous signal, and more often than not, a bees' last-ditch efforts to save themselves as the entombing behavior is found in many hives that subsequently die off (Grist.org, Should Some Pesticides Be Banned?). Pettis states in describing entombing behavior, that:

> "This is a novel finding, and very striking. The implication is that the bees are sensing [pesticides] and actually sealing it off. They are recognizing that something is wrong with the pollen and encapsulating it … Bees would not normally seal off pollen...The presence of entombing is the biggest single predictor of colony loss. It's a defense mechanism that has failed" (Grist.org, Should Some Pesticides Be Banned?)

Increasing rates of decline, colony collapse, and observed "entombing" should raise alarms about pesticide use considering the converging studies showing sublethal, cumulative, and synergistic effects of neonicotinoids on bees. This topic area, like the breakthrough of imidacloprid from nithiazine, can be difficult to synthesize, and even more complicated to cohesively report. The scientific findings can sometimes be contradictory, with claims that the studies and methodology were flawed. Further complicating the issue are competing interests between farmers, producers and manufacturers, regulators, beekeepers, environmental advocates, with the public largely in the middle to wade through conflicting claims about safe pesticide use. While there is little consensus on the cause of colony collapse, and scientists on both sides of the debate make valid points about neonicotinoids and colony collapse, the realization that bees are in trouble and in decline is apparent. While the science

and regulation are sorted out it would be prudent to raise awareness about the plight of bees and why they are so important for the ecosystem services they provide and also for our food supply. More needs to be done to educate the public on the importance of pollinators so we can better protect and conserve their vital pollination services.

Regulatory agencies in Europe seem to be ahead of the US in terms of recognition of pollinator decline and in creating a pollinator conservation framework. This is recently evidenced by the work of the European Food Safety Authority (EFSA). The December 2012 "Inventory of EFSA's Activities on Bees," was published by an EFSA internal task force created to "collect, collate and analyze data related to bee risk assessment, risk mitigation and monitoring" (EFSA Inventory on Bees, 2012). This task force is creating "specific protection goals" for actively reforming the regulation of pesticides and the scientific standards underpinning the risk assessment used in their approval. EFSA is also enhancing and expanding conservation frameworks for pollinators based on the value of bees ecosystem services such as; food (honey and other bee hive products), pollination, genetic resources, education and inspiration; and aesthetic values (EFSA Inventory on Bees, 2012). "For the development of robust and efficient environmental risk assessment procedures it is crucial to know what to protect, where to protect it and over what time period" (EFSA Inventory on Bees, 2012).

> "Given the importance of bees in the ecosystem and the food chain and given the multiple services they provide to humans, their protection is essential. With its mandate to improve EU food safety and to ensure a high level of consumer protection, the European Food Safety Authority

> (EFSA) has the responsibility to protect bees and the ecosystem services they provide to humans" (EFSA Inventory on Bees, 2012, p. 5).

The EFSA is predicting that a better approval process for plant-protection products combined with a comprehensive valuation of bees' ecosystem services will provide stakeholders and risk managers the quantitative tools they need to make informed decisions on food safety, plant-protection products, and pollinator conservation. "The final decision on protection goals needs to be taken by risk managers. There is a trade-off between plant protection and the protection of bees. The effects on pollinators need to be weighed against increase in crop yields due to better protection of crops against pests" (EFSA Inventory on Bees, 2012).

Pollinator conservation in the US will be addressed by the EPA and also in the newest version of the US Farm Bill, an omnibus bill which expired in October 2012, but was recently extended another year, and is expected to be voted on in the House of Representatives at some point in 2013. This important piece of legislation governs wide-ranging food related programs including food stamps, conservation, commodities, crop insurance, energy, and exports (Farmbillfacts.org). The Audubon Society reports that over 500 billion dollars are expected to be appropriated in the next version of the Farm Bill (Audubon.com). Land use incentives to farmers will be one of many economic incentives offered in the bill which could help protect bees. Restoration of pollination services in areas with the greatest agricultural intensification will require a decrease in insecticide use and an increase in the nesting habitat and floral resources used by bees when they are not using crops (Kremen et al., 2002). Conservation

biologist Dr. Claire Kremen advocates creating patches of stepping stone habitats within large-scale agriculture where both native bees and honeybees could find the required floral resources and nesting habitat. Farmers could also utilize smaller field sizes, mixed crop types within fields, and create patches of non-crop vegetation, such as hedgerows, fallow fields, meadows, and seminatural habitats (Kremen et al., 2007). This type of land use, leading to more habitat heterogeneity within the foraging range of bees, could be incentivized through the newest Farm Bill (Kremen et al., 2002).

The emerging pollinator conservation framework shares certain features with organic and sustainable agriculture best practices. Sustainable agriculture and organic farming are synonymous in many ways, and these farming systems differ from large-scale commercial agriculture in regard to (Organic Farming Research Foundation, pg. 3):

- Crop rotation--Enhances soil quality, disrupts weed, insect, and disease life and cycles and sequesters carbon and nitrogen, diversifies production (can have market benefits)
- Manure, compost, green manure use--Enhances soil quality, sequesters carbon, recycles nutrients, and contributes to productivity
- Cover cropping--Enhances soil quality, reduces erosion, sequesters carbon and provides nitrogen, prevents dust (protects air quality), improves soil nutrients, contributes to productivity

- ✳ Avoidance of synthetic fertilizers--Avoids contamination of surface and ground waters, enhances soil quality, sequesters carbon, mitigates salinization (in many cases)
- ✳ Avoidance of synthetic pesticides--Enhances biodiversity, improves water quality, enhances soil quality, prevents disruption of pollinators, reduces costs of chemical inputs
- ✳ Planting habitat corridors, borders, and/or insectaries--Enhances biodiversity, supports biological pest management, provides wildlife habitat
- ✳ Buffer areas--Improves water quality, enhances biodiversity, prevents wind erosion

These farming techniques benefit pollinators in many ways, but most importantly by bypassing fertilizers and pesticides. Floral resources are provided by the diversity of plants found in organic and sustainable farms, which tend to be smaller and grow a wider variety of crops than in large-scale commercial monocultures.

More Integrated Pest Management (IPM) could be incentivized in the newest version of the US Farm Bill as well, which would result in less pesticide use. IPM is “an approach to pest control that utilizes regular monitoring to determine if and when treatments are needed and employs physical, mechanical, cultural, biological, and educational tactics to keep pest numbers low enough to prevent unacceptable damage or annoyance” (BIRC.org). Systemic pesticides such as neonicotinoid seed treatments and Bt crops are opposed to IPM’s more targeted approach because neonicotinoid seed treatments indiscriminately kill

any and all insects, including beneficials, and natural biological control agents. Components of an IPM Program include a range of actions along a continuum: pest identification and their natural enemies; an ongoing monitoring and record keeping system for sampling of pest and natural enemy populations; determination of a pest threshold, above which action is taken, and below which pests are tolerated based on growing conditions, seasonal timing, and life stage of the pest/host; an integration of the least disruptive treatment programs relative to natural enemies and also least hazardous to humans; and an evaluation system to determine the outcome of treatment actions to determine the next step (BIRC.org). Indeed, IPM and systemic neonicotinoids are at opposite ends of the pest control spectrum in terms of their techniques and philosophy. IPM targets individual pests on an ad hoc basic when needed and then tries to use the most environmentally safe option to treat the pest, whereas neonicotinoids are used in a completely opposite way when applied indiscriminately as preventative and systemic seed treatments.

> "The world of systemic insecticides is a weird world, surpassing the imaginings of the brothers Grimm. It is a world where the enchanted forest of the fairy tales has become a poisonous forest. It is a world where a flea bites a dog and dies…where a bee may carry poisonous nectar back to its hive and presently produce poisonous honey" (Rachel Carson Silent Spring, 1962 via New Yorker).

This needn't necessarily be the case since neonicotinoids have a variety of application methods that can make them less systemic. For instance, if acetamiprid was used as a targeted foliar spray that quickly degraded, instead of as a preventative seed treatment, it could be a potential tool used in the IPM

arsenal, if conditions warranted. “Depending on the application method and timing, non-target organisms are not affected by neonicotinoids. Application into the soil by different methods allows the transport of the compound to the pest within the plant without harming beneficial organisms. On the other hand, selectivity in time allows, for example, foliar application against starting pest populations when beneficial arthropods are still absent” (Jeschke, 2011, p. 2900).

More neonicotinoid products can be expected to enter the global market as the initial patents expire and more inexpensive generic options are introduced. Insects are also growing resistant to some of the first generation neonicotinoids and increasing the need for novel new formulations. Many countries are currently evolving their pest control methods as their agricultural systems join the Green Revolution. This is especially true in China and other countries where growing population and increasing consumption patterns have led to increased pesticide use. China pesticide use is up 23% in 2012 (Agropages). In a familiar chain of events, the more toxic and persistent chemicals are being banned, and replaced by neonicotinoids and newer, greener pesticides. “With the ban of high-toxic pesticides such as methamidophos and fipronil, demand for the alternative nitenpyram is heating up in China. Meanwhile, there is constant demand in Southeast Asia, Europe, and South Africa” (Agropages, More Registration Approvals). “Imidacloprid, as the largest application amount of neonicotinoid insecticide in the world, is embracing a rapid development and becoming a hot spot in China. China records 13,620 tonnes of imidacloprid technical output in

2010, accounting for more than 50% of world's total, which is 20,000 tonnes" (Utrecht University).

This increase in demand comes as many of the national patents for first generation neonicotinoids imidacloprid, nitenpyram, and acetamiprid begin to expire, and are replaced by the newer generation neonicotinoids due to reports of emerging insect resistance and cross-resistance. After years of successful control, imidacloprid is now losing effectiveness on the dreaded Colorado Potato Beetle (Alyokhin et al., 2007). The more neonicotinoids are used, the more chances there are for insects to evolve resistance and active defense mechanisms. Cross-resistance to imidacloprid and thiamethoxam is also being noted due to overlapping application of different neonicotinoid insecticides (Alyokhin et al., 2007). The ultimate result will be new combinations of neonicotinoids with specialized formulations to combat the resistant insects. This is proving true in China, where Takeda Chemical's nitenpyram patent expired in 2008. Since then new nitenpyram formulations are being developed domestically by Chinese chemical firms, and registered by the Chinese Ministry of Agriculture to combat aphids, rice planthoppers, and greenhouse white fly on rice, tea, vegetables, and fruit trees (Agropages, More Registration). Chinese scientists from East China University of Science and Technology invented a new type of neonicotinoid that was registered in 2012 called "cycloxaprid" (Chemdatas.com). Cycloxaprid was then exclusively licensed to international chemical firm FMC, where it may one day be used in the US pending EPA registration. The cycle of

neonicotinoid use within the context of the Green Revolution will continue around the world as more nations seek to produce more crops more efficiently.

From their development, neonicotinoid insecticides have been revolutionary in their unmatched ability to control insects and protect food supplies. Since 1991, in a relatively short span, the neonicotinoid insecticides have proven far more safe and effective than anything previously used to control pests. As their use expanded, especially with seed treatments, neonicotinoids staged a rapid ascent to become the number one selling insecticide class. Even though bee studies are now beginning to tarnish the sterling reputation of the neonicotinoids, the positive qualities they possess still shine through, particularly in regard to human and animal safety. Regulators and risk managers must consider the relative benefits of the neonicotinoid class against the potential dangers they pose to bees while scientists seek to clarify complex links to bee declines. Additionally, it will take time to develop the next evolution of insecticides to replace the neonicotinoids. In the interim, regulation and safe use standards will need to be implemented to manage the risks accordingly. IPM, organic, and sustainable agriculture may offer alternatives to neonicotinoids. The perfect pesticide--one that is completely non-toxic and non-disruptive to non-target species, immune from pest resistance, and minimally persistent in ecosystems--is still in development. In the meantime, neonicotinoids are the best, albeit imperfect, option for crop protection in the Green Revolution.

APPENDIX OF TABLES AND FIGURES

Pesticide type	Year intro	LD_{50} (mg/kg)[b] Mammal	LD_{50} (mg/kg)[b] Bird	LC_{50} (ppm) Fish	LD_{50}[c] Honeybee	$t_{½}$ (days) Soil
Insecticides						
Paris green[d]	1867	22		Toxic	High	
DDT	1944	113 to > 1,000	Moderate	0.004–0.009	5	90–10,000
Lindane	1945	59–270	120–130	0.02–0.06	0.01	
Toxaphene[d]	1947	40–112	80–250	< 0.05	22–80	
Endosulfan	1955	70–110	205–1,000	0.002	Low	150–240
Carbaryl	1957	264–710	1,000–3,000	1.3–10	0.18	7–28
Chlorpyrifos	1965	135–2,000	32–490	0.002–0.54	0.36	7–56
Deltamethrin	1974	87 to > 10,000	> 2,250	0.00091–0.0014	0.023	8–28
Diflubenzuron	1975	> 4,640	> 5,000	> 65	> 100	3.2
Methoprene	1975	> 10,000		0.37	> 1,000	10
Abamectin	1985	10–221	85 to > 2,000	0.003–0.01	Toxic	Rapid
Imidacloprid	1991	450	31–152	211–237	High	0.17
Fipronil	1993	95–97	11 to > 2,000	0.085–0.43	High	
Tebufenozide	1994	> 5,000	> 2,150	3–5.7	> 234	7–66
Spinosad	1997	3,783 to > 5,000	> 2,000	3.5–30	0.0029	9–17
Flonicamid	2000	884–1,768	> 2,000	> 100	> 60	1.1
Tolfenpyrad	2002	107–386		0.0029		
Chlorantraniliprole	2006	> 5,000	> 2,250	> 14	> 104	< 60–365
Spirotetramat	2006	> 2,000	> 2,000	2.2–2.5	107	< 1
Pyrifluquinazon	2009	300–2,000	1,360	4.4		
Herbicides						
2,4-D	1942	138–764	472 to > 1,000	> 100	104	< 7
Atrazine	1957	> 1,332–3,992	940–4,273	4.3–76	> 97	16–117
Trifluralin	1961	5,545–6,293	> 2,000	0.088	> 100	25–201
Paraquat	1962	22–157	75–175	26–135	15	< 7
Alachlor	1969	930–1,350	1,536	2.1–5.3	> 94	8–17
Glyphosate	1974	3,530 to > 10,000	> 3,851	97 to > 1,000	100	27–146
Chlorsulfuron	1982	5,545–6,293	> 5,000	> 50 to > 980	> 100	28-42
Glufosinate	1981	200–2,000		710 to > 1,000	> 100	7–20
Mesotrione	2001	> 5,000	> 2,000	> 120	> 11	3–7
Fungicides						
Maneb	1950	> 5,000		1.8	Nontoxic	25
Captan	1952	9,000	2,000 to > 5,000	0.034–0.3	91	1
Benomyl	1970	> 5,000		0.27–4.2	> 50	0.8
Triadimefon	1976	250–1,000	> 2,000	4–10.		6–18
Metalaxyl	1979	633–788	923–1,466	> 100	269	29
Azoxystrobin	1996	> 5,000	> 2,000	0.47–1.6	> 25	70

Abbreviations: intro, introduced; LC_{50}, median lethal concentration; $t_{½}$, half-life.
[a]Data from Tomlin (2009) except as indicated. [b]Acute oral LD_{50} values are for the range of species described in the cited study. [c]LD_{50} data are presented as µg/bee by oral exposure except for benomyl, chlorsulfuron, and spinosad, for which data represent contact exposure. Toxicity levels are given as nontoxic, low, moderate, toxic, and high. [d]Data for Paris Green and toxaphene from Negherbon (1959).

Table 13. Some major pesticides used over time (Casida, 2012)

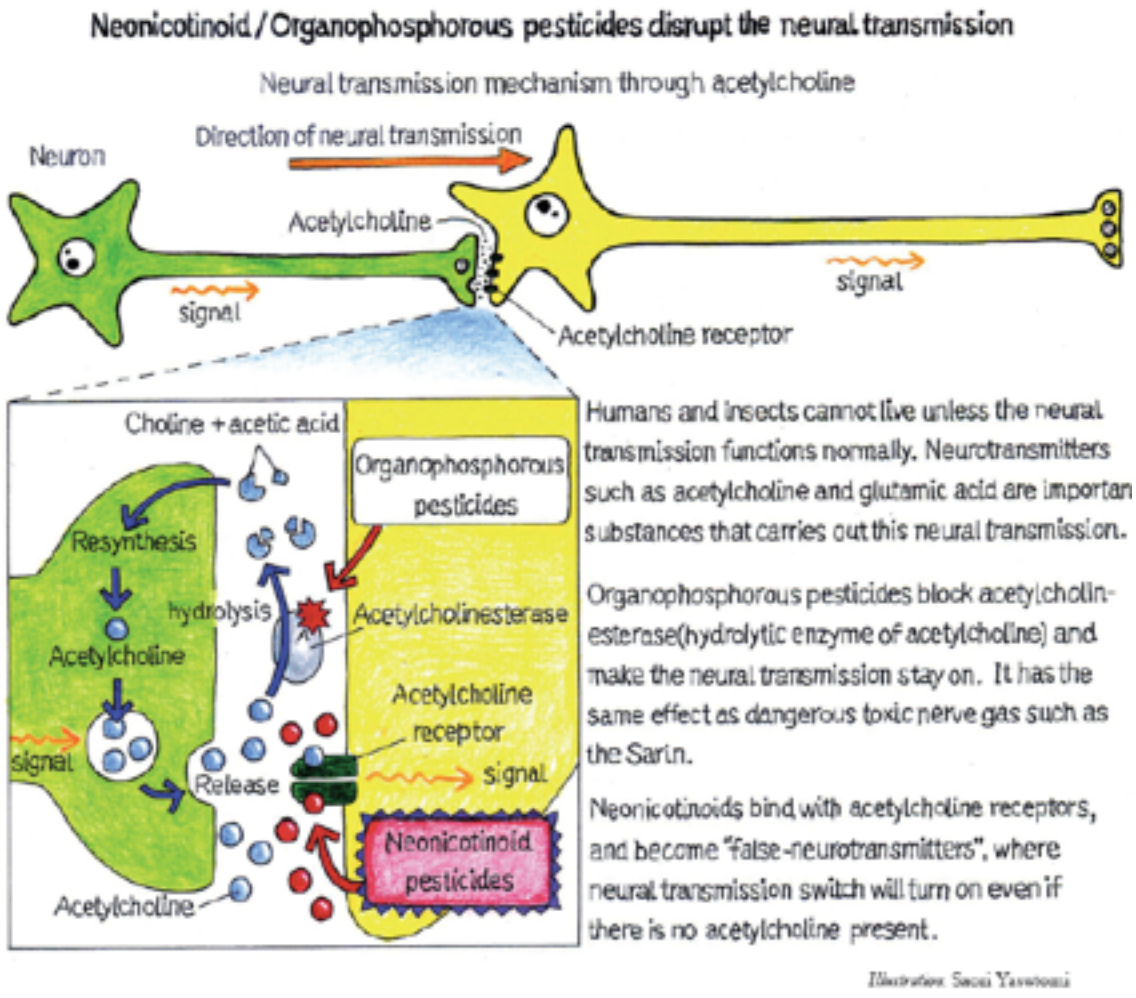


Figure 16. Mode of action for the most commonly used insecticide classes
(Japan Endocrine Disruption Preventive Action Online Resource)

Pretreatment effect of general insecticide synergists, DMI-fungicides, and a plant growth regulator on honey bee toxicity of neonicotinoid insecticides

Insecticide synergist[a]	n[b]	LD_{50} (μg/bee)[c]	95% CI[d]	Chi-square	Slope ± SE	SR[e]	95% CI[c]
Acetamiprid							
Alone	465	7.07	4.57–11.2	0.826	1.77 ± 0.105	1	
PBO	202	1.17	0.342–3.79	1.18	1.55 ± 0.181	6.04	4.29–8.51
DEF	124	2.39	0.278–12.4	5.85	2.96 ± 0.736	2.96	1.83–4.76
DEM	123	6.94	4.10–13.2	0.278	1.46 ± 0.140	1.02	0.783–1.33
Triflumizole	215	0.0290	0.0080–0.102	3.46	1.91 ± 0.240	244	171–347
Propiconazole	201	0.0675	0.0231–0.197	2.63	2.30 ± 0.242	105	76.7–143
Triadimefon	131	0.0844	0.0431–0.176	0.693	2.05 ± 0.198	83.8	64.2–110
Epoxiconazole	156	0.500	0.156–1.66	4.42	2.74 ± 0.404	14.1	10.0–20.0
Uniconazole-P	156	1.12	0.270–4.96	3.66	2.05 ± 0.349	6.31	4.22–9.45
Imidacloprid							
Alone	137	0.0179	0.0092–0.0315	0.303	1.70 ± 0.176	1	
PBO	152	0.0105	0.0061–0.0172	0.0889	1.66 ± 0.112	1.70	1.29–2.26
Triflumizole	125	0.0097	0.0052–0.0168	0.694	2.76 ± 0.284	1.85	1.67–3.09
Propiconazole	145	0.0118	0.0038–0.0303	1.01	2.12 ± 0.272	1.52	1.04–2.24
Thiacloprid							
Alone	158	14.6	9.53–25.4	0.480	2.73 ± 0.371	1	
PBO	193	0.0948	0.0406–0.211	0.424	1.64 ± 0.134	154	115–207
Triflumizole	160	0.0128	0.0031–0.0415	1.66	2.32 ± 0.363	1141	752–1740
Propiconazole	159	0.0261	0.0083–0.0690	1.05	2.27 ± 0.298	559	388–811

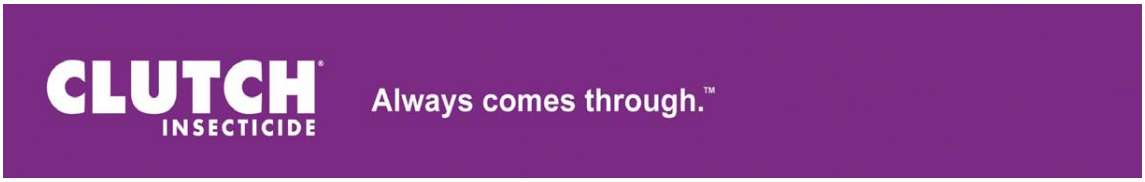
[a] In all, 10 μg of synergist was applied to the dorsal thorax of each worker honey bee 1 h prior to insecticide application.
[b] Number of insects tested.
[c] Results were corrected for control mortality. Dose is given in micrograms of active ingredient.
[d] CI, confidence interval.
[e] SR, synergism ratio (the LD_{50} of insecticide alone/LD_{50} of synergist and the insecticide).

Table 14. Neonicotinoid synergy with common DMI-fungicides (Iwasa et al., 2004)

Companies	products	Active ingredients	Markets
ArmorTech	Guillotine	clothianidin	U.S
Gowan	Scorpion®35SL	dinotefuran	U.S
Valent	Belay	clothianidin	U.S
	Arena50WDG	clothianidin	Canada
	Clutch50WDG	clothianidin	Canada
PBI/Godon	ZYLAM 20SG	dinotefuran	U.S
DuPont	Assail	imidacloprid	U.S
Valent	Safari 20SG	dinotefuran	U.S
Syngenta	Actara,	thiamethoxam	U.S
	Platinum75SG	thiamethoxam	U.S
	Cruiser	thiamethoxam	Japan France U.S
	CruiserMaxx	thiamethoxam difenoconazole metalaxyl-M	Canada
	Voliam Flexi	Chlorantraniliprole thiamethoxam	U.S
	Cruiser OSR	Imidacloprid Cyfluthrin clothianidin	U.K Germany
FMC	Brigadier®	Imidacloprid bifenthrin	Japan
Bayer CropScience	Titan	clothianidin	Canada
	Concept	Imidacloprid deltamethrin	Canada

Table 15. Major products and companies in the neonicotinoid market
(Agropages Neonicotinoid Insecticides Insight Online Resource)

****Note: This manufacturer refers to clothianidin as a third generation neonicotinoid (presuming nithiazine to be the first generation). Clothianidin and thiamethoxam are from the same generation of neonicotinoids, referred to as 2nd generation in Figure 9 and Table 2.**



Frequently Asked Questions

1. **How is Clutch® different from other neonicotinoid insecticides?**
   The active ingredient in *Clutch* is clothianidin, a 3rd generation neonicotinoid. Clothianidin has longer residual activity and less water solubility than other neonicotinoids, such as thiamethoxam. This decreases the potential and risk of leaching in the soil profile. It also means *Clutch* has superior rain-fastness. And clothianidin has faster movement within the leaf's tissue.

2. **How does *Clutch* move in the plant? How long does it take to start moving?**
   Clutch has local translaminar and systemic movement following a foliar spray. Results show translaminar movement in 30 minutes after application. *Clutch* also moves through the xylem of the plant when applied to soil. As expected, actively growing plants tend to move *Clutch* faster and more efficiently.

3. **Does *Clutch* get tied up in soil, or does it remain available for plant uptake?**
   Clutch does not get tied up with soil colloids like imidacloprid, which can be observed in those soils with moderate to high percentages of clay and organic matter. *Clutch* gets adsorbed by the colloids but it is still available for plant uptake. Thus, *Clutch* offers the best of both worlds, low leaching potential through the soil's profile and the most available to the crop. Higher rates are not needed in heavier soils.

4. **What MRLs are in place for *Clutch* in grapes as of January 2010?**
   a. US: 0.6 ppm – the US *Clutch* tolerance is based on foliar applications at the maximum labeled rate with a 0 day PHI
   b. Canada: 0.6 ppm (same as US)
   c. EU: table grape – 0.6 ppm; wine grape – 0.05 ppm (harmonized across the EU)
   d. Japan: 5 ppm (including wine grape)
   e. Mexico: 0.6 ppm (same as US)
   f. South Korea: 2 ppm
   g. .Codex MRL pending.

5. **Does *Clutch* break down in sunlight?**
   Yes, like many other insecticides, clothianidin is affected by sunlight.

6. **What is the rain fastness for *Clutch*?**
   Extremely good. Approximately 3 hours.

Figure 17. Neonicotinoid producer advertising (Clutch FAQ Online Resource)

CLUTCH® INSECTICIDE Always comes through.™

7. **Can *Clutch* be tank mixed or mixed with fertilizers, insecticides or herbicides?**

   Based on available data, there is no indication of antagonism with commonly used insecticides or with fungicides. However, since it is not possible to test all possible mixtures, the user should pretest to assure the physical compatibility and lack of phytotoxic effect of any proposed mixtures with *Clutch*. There have been indications of incompatibility with some fertilizer mixes and these are being further evaluated. We recommend conducting a jar test for compatibility. Caution in mixing is advised until additional information is available.

8. **What is the mixing order for a WDG formulation in a tank mix? (Does *Clutch* go like a WP or a liquid formulation?)**

   As with most pesticides, you should add ingredients for a tank mix in the following order: water, adjuvants (e.g., defoaming agents), dry products such as *Clutch* 50 WDG, liquids, then surfactants.

9. **Are there any adjuvant restrictions?**

   Clothianidin is compatible with adjuvants used for the neonicotinoid insecticide group.

10. **Can I leave *Clutch* in the tank over night?**

    No.

11. **How does pH affect *Clutch*?**

    Water pH could affect clothianidin's performance if it is less than 5.5 or higher than 8.5.

12. **How long does *Clutch* control key pests when applied as a foliar treatment?**

    Depending on the rate used by the grower, *Clutch* can provide from 10 to 14 days of residual control.

13. **How does *Clutch*'s mode of action compare with other neonicotinoids?**

    Clothianidin has the same mode of action of those products in the neonicotinoid group (IRAC MOA Group 4A).

14. **What are the risks of cross-resistance with other neonicotinoids such as imidacloprid, thiamethoxam and acetamiprid?**

    Cross-resistance development among insecticides that have the same mode of action and similar sites of action should always be considered. Efforts to minimize resistance development should be used.

15. **What other crops are already registered or in the process of being registered?**

    Clutch is EPA and CA DPR registered for use in grapes (soil and foliar), pears and apples, while Belay® Insecticide is labeled for us in potatoes. Longer term, between *Clutch* and *Belay*, which both contain clothianidin, labeled uses in California will expand to include such crops as vegetables, soybeans, fruits, nuts and cotton.

Figure 17. Neonicotinoid producer advertising (Clutch FAQ Online Resource)

CLUTCH® INSECTICIDE Always comes through.™

16. How does *Clutch* affect bees?

Clutch is acutely toxic to bees. It is labeled to minimize harm to the environment, including beneficial insects such as bees.

17. Do neonicotinoid insecticides cause colony collapse disorder?

The cause of CCD is unknown at this time, with speculation about a number of potential factors. There is no known causal evidence linking CCD to any crop protection product, including clothianidin, the active ingredient in *Clutch*. It appears that the more recent the registration of a neonicotinoid, the more stringent the bee language, although this is not a function of toxicity. Newer chemistries such as dinotefuran, clothianidin and thiamethoxam have more bee precautionary language on their labels than older compounds in the same class.

18. What is a 3rd generation neonicotinoid?

Third generation neonicotinoids are the latest innovation in this important class of insecticides. New neonicotinoids have unique physical and chemical properties that are different from older neonicotinoids. Clothianidin was first developed in 2001–2002.

Figure 17. Neonicotinoid producer advertising (Clutch FAQ Online Resource)

Usage and product names of Neonicotinoids

Product name (name of active ingredients)

Forestry
Prevention of pine wilt disease

Matsu Green solution (acetamiprid)
Starkle (dinotefuran)
Moriate SC (clothianidin)

Gardening
Flowers / lawn

Bestguard (nitenpyram)
Earth garden (imidacloprid)
Yielder SG (acetamiprid)
Kadan fertilizer with insecticides (acetamiprid)
Mospilan(acetamiprid)

Farming
Rice / Fruits / Vegetables

Dantotsu (clothianidin)
Bestguard (nitenpyram)
Admire (imidacloprid)
Mospilan (acetamiprid)
Albarin (dinotefuran)
Prince froable (fipronil)
Cruiser FS30 (thiamethoxam)
Starkle (dinotefuran)
Hustler powder (clothianidin)

Homes
Termite eradication / Building materials

Hachikusan (imidacloprid)
Agenda SC (fipronil)
Takelock (clothianidin)

Pets
flea control

Frontline (fipronil)
Advantage Plus (imidacloprid)

Residential
insecticides

Kobaega Hoihoi (dinotefuran)
Ari no su tettei shometsu chu (dinotefuran)
Bonfran (dinotefuran)
Black cap (fipronil)
Wiper one G (fipronil)

*Product name (name of active ingredients)
*Fipronil:A new type of insecticide (not a neonicotinoid type, but a phenylpyrazole type)
It is attracting attention in countries like France as a cause of honeybees losses

Figure 18. A sampling of neonicotinoid products for home and residential use in Japan (Japan Endocrine Disruption Preventive Action Online Resource)

Now ubiquitous on garden center shelves, neonicotinoids can be applied in much greater concentrations in gardens than on farms, and with fewer restrictions. These products do not carry any warning about hazards to bees or other pollinators. (Photograph: Matthew Shepherd/The Xerces Society.)

Figure 19. A sampling of neonicotinoid products for home and residential use in the U.S. (Xerces Society Online Resource)

Crop	Total acreage in 2010	Percentage treated in 2010 with one or more of three neonicotinoids for which use data patterns can be compiled (clothianidin, imidacloprid, thiamethoxam)	Total projected treated acreage
Corn	88 million (2011 – 92..3 million acres)	94%	82.72 million – 2010
Soy	77.4 million (2011 – 75.2 million acres)	32%	24.77 million – 2010
Wheat,	53.6 million (2011- 56.4 million acres)	42% (Cereals)	22.51 million – 2010
Cotton	11 million (2011 – 13.7 million)	42%	4.62 million – 2010
Sorghum	5.4 million (2011 – 5.3 million acres)	75%	4.05 million – 2010
Sugarbeets	1.17 million (2011 – 1.24 million acres)	65%	0.76 million – 2010
Sunflowers	1.95 million (2011 – 1.9 million acres)	Unknown	Unknown
Canola	1.4 million (2011 – 1.1 million)	100%	1.4 million – 2010
Rice	3.6 million (2.6 million acres)	51%	1.84 million – 2010
Alfalfa	20 million (19.3 million acres)	Unknown	Unknown
Peanuts	1.3 million (1.1.5 million acres)	Unknown	Unknown
Projected total			*at least* 142.67 million acres

Table 16. Neonicotinoid crop use patterns in the U.S.
(Pesticide Action Network State of the Science Online Resource)

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