Original Article

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A Short Review on Neonicotinoids; Use in Crop Protection and Issues on Honeybee and Hive Products

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Abstract

Neonicotinoids are a relatively new class of insecticides. Due to higher field efficacy, flexible application procedure and claim of low vertebrate toxicity neonicotinoid became a promising insecticide candidate, surpassed 2.6 billion USD in 2009 global scale and in the consecutive years had highest market share among all insecticides. But also, growing volume of evidence suggested that neonicotinoids affect honeybee adversely primarily via impairments on learning and memory, and ultimately foraging success leading to colony collapse. In order to protect honeybee and bee diversity, European Union, Korea banned the use of some neonicotinoids, such as clothianidin, thiamethoxam, imidacloprid. Undoubtedly the restriction of the use of these neonicotinoids on nectar and pollen-rich crops could be expected to reduce a potential threat to bees and at the same time, the policy allowed time for investigation in a field-realistic manner. In this present review, we focus on the concerns of neonicotinoids related to honeybee and hive contamination.

Key words: Neonics, Honeybee, Learning and memory, Foraging success, Policy, Toxicity

INTRODUCTION

Plant pests including plant-feeding invertebrates, pathogens and weeds are significant constraints on crop production. Pesticide has become an indispensable component of present day's agriculture in order to feed the rapidly growing global population. A sizable portion of pesticide industry has been occupied by insecticide. Insecticides are natural and synthetic chemicals used to control insect pests (Table 1). At the same time, synthetic insecticides are often received attention for appearing as an environmental pollutant, insect resistance development and especially non-target toxicities. It stands true for neonicotinoids, a class of relatively new insecticides introduced in the 1990s. The use of this class is of particular concern due to their potential non-target effect primarily on insect pollinators, honey bee in particular. At first, information came out based on observation of huge bee colony loss in 2006 (EPA, 2017a) and research on bees. Since the approval and marketing of neonicotinoids, two decades almost passed. During this period, many investigations have come up with evidence of the toxic effect of neonicotinoid on many different terrestrial and aquatic animals other than bees (Gibbons *et al*., 2015). Another concern is the long-term persistence of neonicotinoids in the environment. Despite having a voluminous dataset on the lethal and sub-lethal effect of neonicotinoids on bees and other terrestrial and aquatic organisms, information on the actual field-based usage of the insecticide are limited. As responsive policy few countries

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Table 1. Classification of insecticides relative to the mode of action (modified from IRAC, 2017)

Nerve and muscle target	
Acetylcholinesterase (AChE) inhibitors	Carbamates, Organophosphate
GABA-gated chloride channel blockers	Organochlorine
Sodium channel modulators	Pyrethroids, DDT
Nicotinic acetylcholine receptor (nAChR) modulators	Neonicotinoids, Spinosyns
Glutamate-gated Cl channel (GluCl) modualtors	Avermectins, Milbemycins
Octopamine receptor agonists	Amitraz
Growth and development target	
Juvenile hormone mimics	
Mite growth inhibitor	Clofentezine, Etoxazole
Inhibitors of chitin biosynthesis	Buprofezin
Molting disruptors,	Cyromazine
Respiration target	
Inhibitors of mitochondrial ATP synthase	Diafenthiuron, Organotin miticides
Mitochondrial electron transport inhibitors	METI acaricides
Midgut target	
Microbial disruptors of insect midgut membranes	*Bacillus thuringiensis*

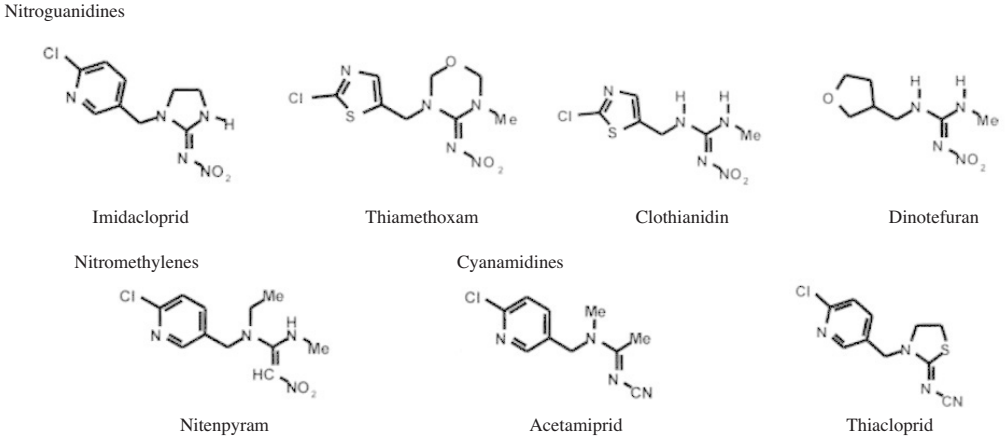

Fig. 1. Chemical structures of seven known active ingredients of neonicotinoids relative to 3 chemical categories of nitroguanidines, nitromethylenes, and cyamidines.

impose time-limited restriction on the use of three active ingredients of neonicotinoids namely clothianidin, thiamethoxam, and imidacloprid. However, the scientific debate continues. In this present review, we focus on the concerns of neonicotinoids related to honeybee and hive contamination and on-going regulatory debates for risk assessment and management relative to pollinator protection.

Use of neonicotinoids in crop protection

Insect control with chemical began about 2000 years ago and most natural products dominated as controlling agents. In the year 1940, first synthetic insecticide has been introduced as dichlorodiphenyl trichloroethane (DDT) and the journey of synthetic insecticide began. Neonicotinoids, synthetic derivatives of nicotine, are relatively new class of insecticides used worldwide to control a number of insect pests, particularly plant-chewing, -piercing and -sucking insects primarily belonging to hemipterans including aphids, leafhoopers, planthoopers, thrips and whiteflies (Elbert *et al*., 1991; Tomizawa and Casida, 2005). It is also effective managing some insect pests belonging to lepidopteran, dipteran and coleopteran insects (Elbert *et al*., 1998). According to IRAC (Insecticide Resistance Action Committee, 2017), there are seven active ingredients like

Table 2. Chronology of development of neonicotinoid pesticides with physico-chemical and biological properties

Year	Compound	Manufacturers[1]	Physico-chemical properties[2]			Application on			
			MW	WS	DC	Pests	Foliar	Soil	Seed
1991	Imidacloprid	Bayer	255.7	0.61	-	Thrips, mealynugs, leafminers, termites	++	+++	++(+)
1995	Nitenpyrum	Sumi Take	270.72	590	3.1	-	++	+	-
	Acetamiprid	Nippon Soda	222.67	2.95	0.7	Codling moth, diamondback moth	+++	+	-
1998	Thiamethoxam	Syngenta	291.71	4.1	-	Mealybugs, plantbugs, leafminers, termites	+++	+++	++
2000	Thiacloprid	Bayer	252.72	0.184	-	Codling moth, pollen beetle	+++	-	-
2001	Clothianidin	Sumi Take/ Bayer	249.7	0.34	11.1	Woolly aphid, oriental fruit moth, corn rootworm	++	++	+++
2002	Dinetofuran	Mitsui Chem.	202.21	39.83	12.6	Soft scales, thrips, mealybugs	+++	++	-

[1]Elbert *et al*. 2008; [2]Bonmatin *et al*., 2015.

*MW=Molecular weight (g/mol), WS=Water solubility (g/L), DC=Dissociation constant.

Table 3. Insects resistance to neonicotinoids and the resistance development mechanisms

Common name	Scientific name	Compound	Resistance mechanism	Ref.
Cotton whitefly	*Bemisia tabaci*	Imidacloprid	Detoxification by microsomal monooxygenase. Overexpression of cytochrome P450 (CYP6CM1)	1
Green peach aphid	*Myzus persicae*	Imidacloprid, Clothianidin	Overexpression of cytochrome P450 (CYP6CY3)	2
Cotton-melon aphid	*Aphis gossypii*	Imidacloprid, acetamiprid, clothianidin	Target-site-nutation (β1 nAChR subunit)	3
Brown plant hopper	*Nilaparvata lugens*	Imidacloprid	Target-site-mutation (Y151S) in two nAChR subunits, N1α1 and N1α3.	4
Housefly	*Musca domestica*	Imidacloprid, Thiamethoxam	Increased expression of P450 genes (CYP6A1, CYP6D1, CYP6D3, CYP6G4)	5
Colorado potato beetle	*Leptinotarsa decemlineata*	Imidacloprid	P450-mediated detoxification	6
Grennhouse whitefly	*Trialeurodes vaporariorum*	Imidacloprid	Enhanced expression of the P450 CYP6CM1	7

1: Nauen *et al*., 2002; Rauch and Nauen, 2003; Karunker *et al*., 2008; 2009, 2: Bass *et al*., 2013; Puninean *et al*., 2010, 3: Koo *et al*., 2014; Shi *et al*., 2012, 4: Liu *et al*., 2005, 5: Markussen and Kristensen, 2010; Hojland *et al*., 2014, 6: Zhao *et al*., 2000; Mota-Sanchez *et al*., 2006, 7: Karatolos *et al*., 2011.

imidacloprid, nitenpyram, acetamiprid, thiamethoxam, thiacloprid, clothianidin, dinotefuran. Out of these seven active ingredients, imidacloprid, thiamethoxam, clothianidin and dinotefuran belong to nitroguanidines; nitenpyram belongs to nitromethylenes; acetamiprid and thiacloprid belong to cyanamidines (Fig. 1). The physico-chemical properties, pest spectrum, and application have been represented in Table 2.

Foliar application of neonicotinoids is generally targeted against pests attacking crops such as cereals, maize, rice, potatoes, vegetables, sugar beet, cotton, tobacco and deciduous fruits (Elbert *et al*., 1998). It has been shown that 2.5~5 ppm a.i. (active ingredient) application in the soil controlled typical soil insect pests such as *Agriotes* sp., *Diabrotica balteata* or *Hylemyia antiqua* (Elbert *et al*., 1991). A new horizon of crop protection strategy i.e. seed treatment has been opened up with the development of neonicotinoids. Seed dressing, film coating, pelleting or multilayer coating allow for environmentally safe and perfect protection of crops against insect pests (Elbert *et al*., 2008). Neonicotinoids are widely used in seed coatings of a variety of crops such as cotton, corn, cereals, sugar beet, soybean, oilseed rape and are taken up systemically by growing plants and distributed to all tissues (Elbert *et al*., 2008; Rundlöf *et al*., 2015). Table 2 represents the pest spectrum and biological profile (foliar use, soil use, and seed treatment) of neonicotinoids.

Due to higher field efficacy, flexibility in application methods and most importantly in comparison to alternative insecticides like organophosphate lower vertebrate toxicity

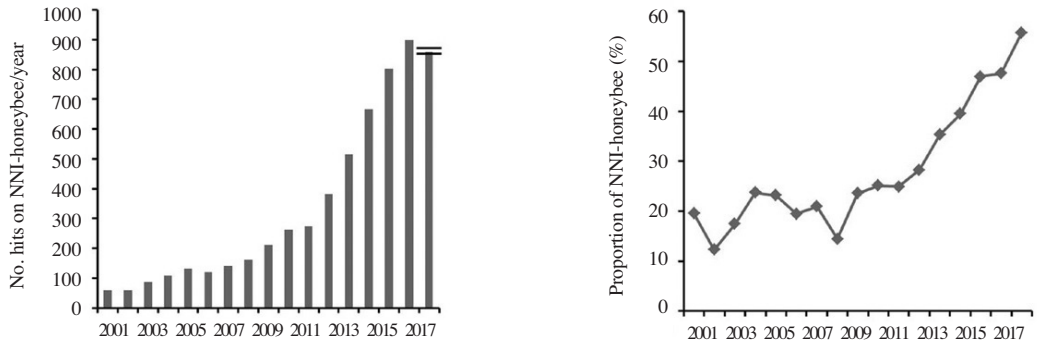

Fig. 2. Search results in Google Scholar on 'Neonicotinoids and honeybee' (left), and proportion (%) of 'Neonicotinoids and honeybee' among 'Insecticides and honeybee'.

Table 4. LD_{50} value (ng bee^{-1}) from acute toxicity testing of neonicotinoids on honeybee

Compound	*Apis mellifera*							*Apis cerana*
	oral[1]	oral 24h[2]	oral 48h[3]	oral 48h[4]	contact 24h[5]	contact 48h[3]	contact 48h[4]	oral 24h[2]
Imidacloprid	116	8.6	--	3.7	--	--	81	2.7
Nitenpyram	80.5	--	--	--	138	--	--	--
Acetamiprid	--	--	14500	--	7070	8090	--	--
Thiamethoxam	11.8	--	5	5	30	24	24	--
Thiacloprid	23000	--	17300	--	14600	38800	--	--
Clothianidin	18	2	3	3.79	22	44	27.5	0.5
Dinotefuran	--	--	23	--	--	--	--	--

[1]Codling *et al*., 2016; [2]Li *et al*., 2017; [3]Decourtye and Devillers, 2010; [4] EFSA 2015a,b,c; [5]Iwasa *et al*., 2004.

neonicotinoids became a promising insecticide candidate, surpassed 2.6 billion USD in 2009 global scale (Jeschke *et al*., 2011; Tomizawa and Casida, 2005 *cf*. LaLone *et al*., 2017). In the consecutive year, neonicotinoids have replaced organophosphate and became the largest category of insecticides with the market share of 25% of total insecticides globally. This rise continued for next few years. In 2014, the global neonicotinoids market value reached USD 3.9 billion, up by 7.1% compared with that of 2013.

However, there are some other concerns about resistance development and nontarget impact on beneficial invertebrates. Several insect pests have developed resistance to neonicotinoids insecticides (Table 3) with known resistance mechanisms. Apart from the list, neonicotinoid resistance has also been reported in several other insect pests such as white-backed planthopper *Sogatella furcifer*a (Horvath), small brown planthopper *Laodelphax striatellus* (Fallèn), Asian citrus psyllid *Diaphorina citri* (Kuwayama), codling moth *Cydia pomonella* L., and western flower thrips *Frankliniella occidentalis* (Pergande) (Bass *et al*., 2015). Increasing list of neonicotinoid resistant insects (Bass *et al*., 2015), evidence suggesting the adverse primarily sub-lethal effects of neonicotinoids on terrestrial and aquatic vertebrate wildlife (Gibbons *et al*., 2015) are found to be threats to continue neonicotinoid applications.

Exposure of neonicotinoids to honeybee

In 2006 large number of worker honeybees disappeared from honeybee colony in the US. Sudden loss of colony's worker bees led to losses of 30 to 90% of beehives in the US during 2006-2007. This phenomenon was called 'colony collapse disorder' (CCD) which has been characterized by the disappearance of majority of the worker bees, leaving behind a queen, plenty of food and a few nurse bees to care for remaining broods and immature bees and queen. Worldwide 35% of the crop production depends on pollinators (Klein *et al*., 2007) and reduction in

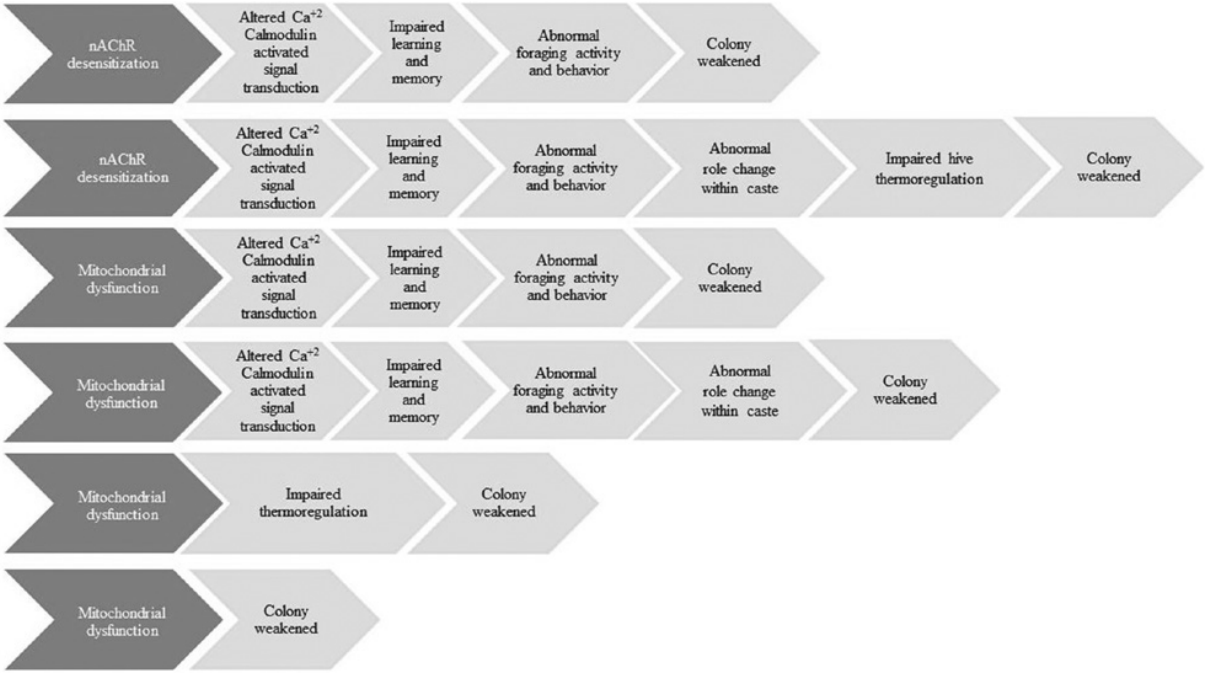

Fig. 3. Effect of neonicotinoids on honeybee - adverse outcome pathways (after Lalone *et al*., 2017).

honeybee colony would negatively effect on human nutrition (Chaplin-Kramer *et al*., 2014; Smith *et al*., 2015). Thus there was growing concern and initiatives to investigate the possible causes of the CCD and it included pesticide, habitat degradation, malnutrition, pathogens and *Varroa* infestations as possible factors.

Since the introduction of neonicotinoids in the global market and wide use in a variety of crops, several approaches also have been taken in order to investigate their lethal and sub-lethal (including foraging behavior, memory, locomotion, navigation or orientation) effects on honeybees (Auteri *et al*., 2017). The attention of the scientific communities has been observed by the increasing trend of publications (Fig. 2). Google scholar platform has been used to search the results. Year wise hit number was considered against 'neonicotinoids and honeybee' and 'insecticides and honeybee' and proportion of 'neonicotinoid and insect' in 'insecticides and honeybee' was calculated for the respective year. Growing volume of evidence suggested that neonicotinoids affect honeybee adversely primarily via impairments on learning and memory, and ultimately foraging ability (LaLone *et al*., 2017). Table 4 demonstrates the acute toxic level of neonicotinoids on honeybee. It has found that clothianidin and thiamethoxam possessed comparatively higher toxicity to honeybee. Metabolites of neonicotinoids also contribute to the toxicity. However, N-demethyl acetamiprid, 6-chloro-pyridilmethyl alcohol and 6-chloro-nicotinic acid (metabolites of acetamiprid) possesses less toxicity with a higher value of LD_{50} (>50000 ng bee^{-1}) (Iwasa *et al*., 2004). Several studies have been carried out on different metabolites of imidacloprid viz. olefin (LD_{50} 36 ng bee^{-1}), 5-OH-imidacloprid (LD_{50} 159 and 153 ng bee^{-1}), Di-OH-imidacloprid (LD_{50} > 49 ng bee^{-1}), urea-metabolite (LD_{50} > 100000 ng bee^{-1}) and 6-chloronicotinic acid (LD_{50} 122000 ng bee^{-1}) (Nauen *et al*., 2001; Decourtye *et al*., 2003). Genetic differences also exist in the response to neonicotinoids toxicity in honeybee (Laurino *et al*., 2013). A significant difference in LD_{50} value was found in case of imidacloprid and thiamethoxam for *A. mellifera ligustica*, *A. mellifera mellifera* and *A. mellifera carnica* and in case of clothianidin for *A. mellifera ligustica* and *A. mellifera mellifera* (Laurino *et al*., 2013). This is true for indirect contact toxicity i.e. LC_{50} also. However, existing reports on

semi-field condition shows variable results. Test on *Bombus terrestris* foraging on plants grown from imidacloprid treated sunflower seed with 0.7mg $seed^{-1}$ with chronic exposure did not demonstrate any foraging and colony vitality (Tasei *et al*., 2001).

In contrast, chronic exposure of 11 weeks with 200mg l^{-1} to 10μg l^{-1} imidacloprid contaminated sugar syrup with or without foraging resulted in LC_{50} 59μg l^{-1} (ppb) and 20μgl^{-1} (ppb) respectively (Mommaerts and Smagghe, 2011). Effects on learning and memory under filed based condition are yet to be demonstrated and the small volume of existing data under variable conditions is not enough to draw conclusive remarks.

Most of the risk assessments of NNI on honeybee or pollinators were conducted in the laboratory or semi-field environments, which make the policy implementation difficult because of the lack of evidence in a realistic situation. Recently a country-specific field-based study has come up with evidence of negative effects of neonicotinoids on honeybee, bumblebee and solitary wild bees linked to neonicotinoid residues across the landscape (Woodcock *et al*., 2017). Winter-sown oilseed rape was grown commercially with either seed coating containing neonicotinoids (clothianidin or thiamethoxam) or no seed treatment (control) across three countries namely Hungary, Germany, and the UK. The investigation revealed variations in response among countries. Clothianidin resulted in significant reduction of overwintering colony size in Hungary and UK, in contrast, no effect was found in Germany. Further, reproduction of *B. terrestris* and *Osmia bicornis* was negatively correlated with neonicotinoid residues. In another field-based study in Canada demonstrated honeybee colonies located in neonicotinoid treated cornfield had higher *Varroa* loads than those in untreated corn fields (Alburaki *et al*., 2017).

Mechanisms on lethal and sub-lethal effect on honeybee

Neonicotinoids are neurotoxins affecting the nervous system of the organisms. Fig. 3 represents the plausible adverse outcome pathways (LaLone *et al*., 2017). Nicotinic acetylcholine receptors (nAChR) belong to the cys-loop superfamily of ligand-gated ion channel, responsible for rapid neurotransmission and are conserved across vertebrates and invertebrates (Karlin, 2002; Jones and Sattelle, 2010). Because of diverse functional architecture, the toxicological responses differ. Neonicotinoids are nAChR agonists i.e. a substance that initiates a physiological response when combined with a receptor. Upon prolonged and repeated exposure to neonicotinoids, desensitization occurs at the receptors resulting initial opening of the ion channel, ion exchange across cell membrane followed by rapid channel closure, effectively inhibiting neurotransmission (Quick and Lester, 2002; *cf*. LaLone *et al*., 2017). This is true for the case of honeybee, alterations in gene transcripts such as increasing abundance of nAChRα1 subunit in brain, vitellogenin, and genes related to immune and memory formation in response to?neonicotinoids (Christen *et al*., 2017). In another study (Li *et al*., 2017) comparing between *Apis mellifera* and *A. cerana*, it was found that *A. cerana* was more sensitive to imidacloprid and clothianidin. The same pattern was found for imidacloprid (Lee *et al*., 2016). However, they possess distinct mechanisms to mount an innate immune response against neonicotinoid exposure. In contrary to upregulation of carboxylesterase, prophenol oxidase and acetylcholinesterase activities in *A. cerana*, they were found significantly downregulated in *A. mellifera* after 48 hours imidacloprid treatment whereas during clothianidin exposure AChE was downregulated and glutathione S-transferase activity was upregulated in both the species. Different response was observed in different developmental stages of honeybee, increasing activities of glutathione S-transferase and carboxylesterase para were found in pupal stage in response to thiamethoxam exposure (Tavares *et al*., 2017). Strong downregulation of gene coding for major jelly proteins (MRJPs) was observed in response to imidacloprid exposure which weakens bee colony (Wu *et al*., 2017).

Another key mechanism is mitochondrial dysfunction. Mitochondria play a critical role in cellular respiration resulting in the production of adenosine triphosphate

(ATP), biological energy currency. Further, mitochondria are also associated with several processes such as Ca^{+2} storage and release for cell signaling, heat production, mediates cell growth and cell death. Thus mitochondrial dysfunction manifests perturbations in these processes. However, uncertainty exists as to the presence of the nAChR in invertebrate mitochondria (LaLone *et al*., 2017). Studies with honeybee and bumblebee (*Bombus terrestris*) demonstrated adverse impacts on mitochondria while exposed to nAChR agonists (Moffat *et al*., 2015; Nicodemo *et al*., 2014). Moffat *et al*. (2015) described mitochondrial depolarization (i.e. loss of membrane potential) in bumblebee neurons upon 48 hours 1nM imidacloprid exposure. Change in foraging behavior of bumblebee in response to clothianidin has been confirmed by one recent study (Arce *et al*., 2017). Not only managed bee population but the study suggests that sub-lethal effects of neonicotinoids could also scale up causing loss of wild bees diversity (Woodcock *et al*., 2016).

Contamination of hive products

The laboratory experiments demonstrated well about the mechanism of how neonicotinoids affect honeybees. With the increasing volume of evidence, the doubt no longer exists about the negative effect of neonicotinoids on honeybee. However, one question still remains unanswered. The amounts detected in nectar and pollen was found often less than 10 ppb (Chauzat *et al*., 2006; 2009) whereas at least 40 ppb dose is necessary for abnormal honeybee foraging behavior, 0.5 ppm for missing bee and 3 ppm for 100% failure to return to a source of sugar offered to them (Yang *et al*., 2008). Guttation was found the overlooked source that contained a higher amount of neonicotinoids. Guttation, a physiological phenomenon occurring in many vascular plants especially grasses, is a formation of drops of xylem sap on the tips or along the edges of leaves. As neonicotinoids are systemic insecticides, it dissolves with water entering roots, mixing in the xylem sap and be exuded through hydathodes of leaf margins. In a study by Girolami *et al*. (2009) showed that leaf guttation drops of corn plants germinated from neonicotinoid-coated seeds contained insecticides constantly higher than 10mg/l (=10 ppm) with maxima up to 100mg/l (=100 ppm) for thiamethoxam and clothianidin and up to 200mg/l (=200 ppm) for imidacloprid. However, the analyses of environmental neonicotinoid pesticide residual level in plant, bees and bee products become an essential tool for risk assessment of the insecticides. Although environmental neonicotinoid residues were found to be lower than acute or chronic toxic level, it does not outskirt the possibility of being a source of bee poisoning (Blacquière *et al*., 2012; Codling *et al*., 2016). Because of their systemic property, neonicotinoids translocate from seed treatment to different plant parts and could be the cause of bee intoxication. Thus, there is increasing interest to examine the concentration of neonicotinoids in bee-collected materials such as pollen, nectar and bee products like honey and bee wax and bee itself to measure the exposure of the neonicotinoids (Mullin *et al*., 2010; Blacquière *et al*., 2012; Barganska *et al*., 2013; Kasiotis *et al*., 2014; Gbylik-Sikorska *et al*., 2015). However, the results revealed wide variations. In one study investigating the neonicotinoid residues in honey from beehive with proximity to canola, alfalfa, dandelions, and willow found that clothianidin and thiamethoxam were most frequently detected neonicotinoids found in 68 and 75% of honey samples with the mean concentration of 8.2 and 17.2 ng g^{-1} (ppb) respectively (Codling *et al*., 2016). 239 honey samples collected from 24 apiaries in different parts of France analyses found that 17.6 and 21.8% samples were positive with 6-chloronicotinic acid (a metabolite of imidacloprid) and imidacloprid with maximum 10.2 and 1.8μg kg^{-1} (ppb) concentration respectively (Chauzat *et al*., 2011). One monitoring study in Belgium published in 2007 showed only 4.6% of honey samples were found positive for imidacloprid contamination (Pirad *et al*., 2007). Another study in 2009 revealed, out of 48 honey samples collected different areas of Belgium in the proximity of maize field treated with 0.05 to 2.48% imidacloprid, 8.4% samples were found positive with mean concentration 0.275μg kg^{-1} (Nguyen *et al*., 2009). However, 91 honey samples from 73 apiaries in North

West Spain analyses did not find any sample positive with neonicotinoid residues (García-Chao *et al*., 2010). In the most recent study of 198 honey samples from all over the world demonstrated that overall, 75% of samples contained quantifiable neonicotinoid residues (Mitchell *et al*., 2017). However, the proportion varied considerably, being highest in North American honey (86%) followed by Asian (80%), European (79%) and the least for South American (57%) with an average of 1.8 ng g^{-1} (ppb) neonicotinoid in contaminated honey and reached to a maximum 56 ng g^{-1} (ppb) which lies in the range bioactive range causing deficits in learning behavior (Mitchell *et al*., 2017). 33 and 40.5% pollen samples out of 187 samples in Chauzat *et al*. (2011) study were detected positive with 6-chloronicotinic acid and imidacloprid with highest concentration of 9.3 and 5.7μg kg^{-1} (ppb) respectively. In contrast, Higes *et al*. (2010) and Bernal *et al*. (2010) did not find any neonicotinoid residues in pollen samples collected from different vegetation in Spain. To measure the exposure of the neonicotinoid, the best way is to determine the concentration in honeybees. In contrast to many findings with negative results (Pirad *et al*., 2007; Nguyen *et al*., 2009; Mullin *et al*., 2010). Chauzat *et al*. (2011) study found 18.7 and 11.2% honey bee samples with positive with the mean concentration of 1 and 1.2μg kg^{-1} (ppb) for 6-chloronicotinic acid and imidacloprid respectively. However, in majority, the concentration of neonicotinoid residues is lower than MRL (maximum residue limits) set by EC (European Commission) (EU MRLs: 50 ng/g for acetamiprid, imidacloprid, and thiacloprid and 10 ng/g for clothianidin and thiamethoxam *cf*. Tanner and Czerwenka, 2011).

Regulatory decision

The loss of honeybee colony has become a concern for the present and future food security and environmental sustainability although neonicotinoids share a sizable global economy. This provides the scope of scientific debate. Mounting evidence not funded by pesticide industry indicates even low level of exposure affects the ability of honeybee to communicate, can suppress their immune system, becomes more susceptible to viruses and *Varroa* mites (Vidau *et al*., 2011; Sánchez-Bayo *et al*., 2016; Brandt *et al*., 2016; Gregorc *et al*., 2016). In spring 2008, serious colony losses were reported in Italy and the Italian authority suspended the use of clothianidin, thiamethoxam, imidacloprid along with fipronil treated maize seed on the temporary basis (Auteri *et al*., 2017). Considerable amount investigations examining the impact of neonicotinoids on honeybee colony survival and development produced diverging data. Based on scientific reports (Whitehorn *et al*., 2012 on imidacloprid; Henry *et al*., 2012 on thiamethoxam; Schneider *et al*., 2012 on clothianidin) and data obtained from APENET during 2009 to 2011 in June 2012 European Food Safety Authority (EFSA) urged for further experimental data in order to draw a definite conclusion. In May 2013 European Commission declared restriction on the use of clothianidin, thiamethoxam, and imidacloprid in seed treatment. Subsequent to the 'ban regulation', in July 2013 EFSA also mandated to perform the risk assessment for foliar application and all uses other than seed treatment in these 3 neonicotinoids. In response to the EU decision, the Rural Development Administration (RDA) in Korea imposed a limited-time ban on the further inclusion in the list of application of those 3 chemicals in February 2014 (RDA, 2014). Following the open call in 2015, EFSA carried out an updated risk assessment in 2015 and 2016. In March 2017 EC presented its draft regulation to ban neonicotinoids to the Member States and later the deadline for this evaluation has been proposed 30 November 2017 (Pesticide Action Network, Europe 2017; EURACTIVE.com). EFSA is currently working on the new mandate to review the risk assessment on neonicotinoids and the recent investigations with the field-based data are of immense importance to draw a conclusion which fosters the sustainable use of the chemical. Similarly U.S. Environmental Protection Agency (EPA) 2017 assessment also finds that imidacloprid possesses a risk to aquatic organisms. However, EPA keeps clothianidin, thiamethoxam and dinotefuran out the list of neonicotinoid possesses significant risk. The agency is scheduled to make

the final decision in 2018 (EPA, 2017b). Canada's Pest Management Regulatory Agency (PMRA) has targeted completion of the pollinator risk assessment for the neonicotinoids by December 2017 and consultation has been scheduled in early 2018 (Health Canada, 2017).

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