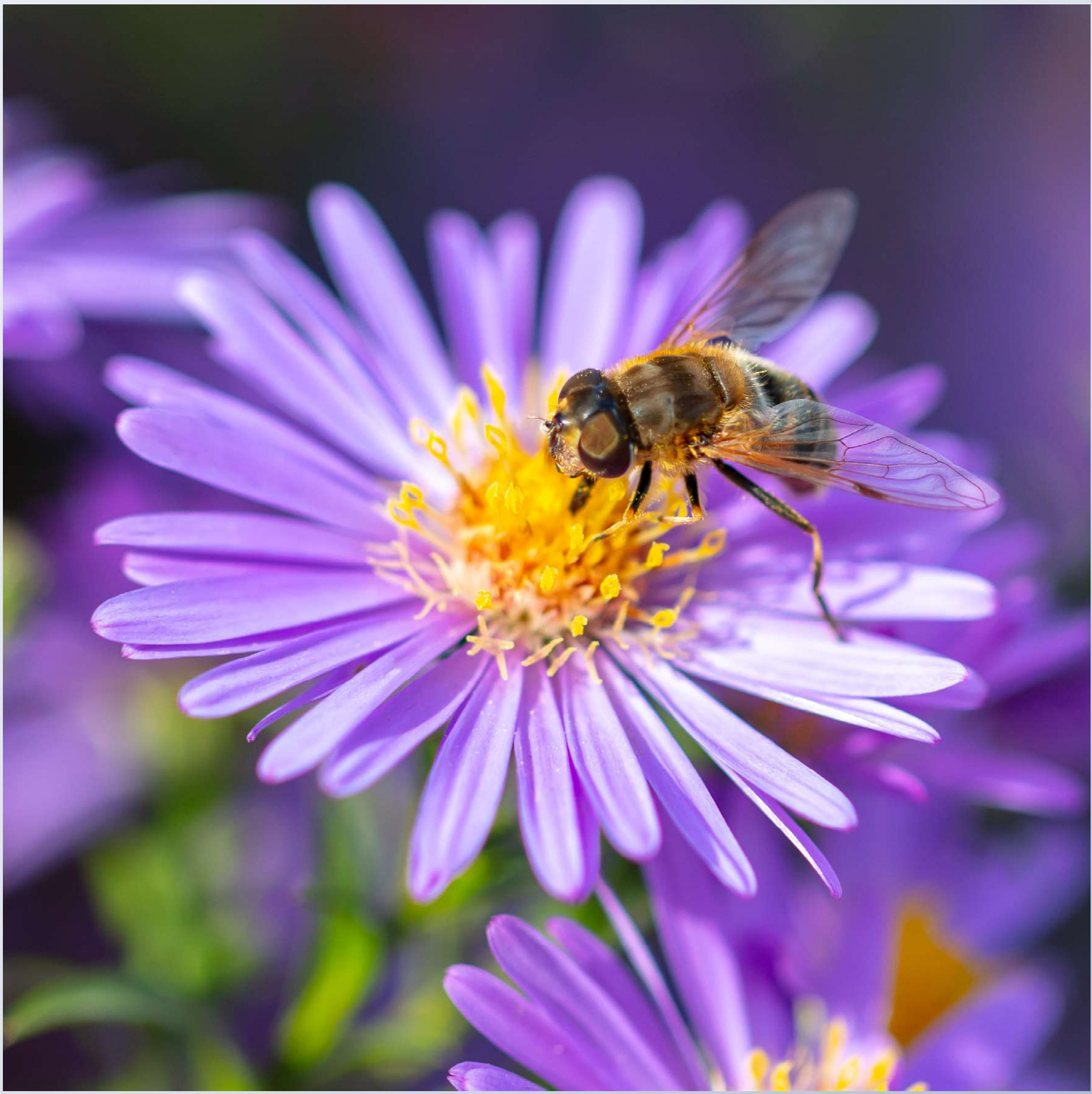
Risk Assessment of Neonicotinoids in the Asia-Pacific Region



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Who is AASSA?

The Association of Academies and Societies of Sciences in Asia is a non-profit international organization with science, technology, and innovation (STI) interests. It consists of scientific and technological academies and societies in Asia and Oceania. It was launched in 2012 through the merger of two organizations, i.e., AASA (Association of Academies of Sciences in Asia, founded in 2000) and FASAS (Federation of Asian Societies and Academies of Sciences, founded in 1984). Its current members are 32 national academies and societies of sciences from 30 countries and one regional academy of engineering and technology. The principal objective of AASSA is to act as an organization in Asia and Oceania which plays a major role in the development of the region through science and technology. AASSA serves as a forum to discuss and provide advice on issues related to science and technology, research and development, and the application of sciences and technology for socio-economic development.

Vision

To promote initiatives, networking in Asian and Oceanian academies and societies of science to address global challenges through science and technology.

Mission

To strengthen the capacity of Asian and Oceanian academies and societies in their efforts to reduce hunger and poverty; to improve health & education; to combat against climate change through sustainable development, ensuring inclusivity and equitability. And to provide evidence-based science policy advice.

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Foreword

Pollination by insects is an integral step in agriculture. It is estimated that approximately 75% of the crops traded worldwide depend to some degree on pollinators, both natural and cultured.

During 2006–07 in certain areas of the USA, it was noted that there had been a sudden, unexplained collapse of honeybee colonies which resulted in a loss of around 40% of the honeybee population. The same phenomenon was also noted in other regions of the world. Since honeybees are one of the most important pollinators, this phenomenon immediately rang alarm bells not only to honeybee keepers but to agriculture in general. It is also a grave concern for world food security.

Among many probable causes that could affect honeybee colony collapse, such as climate change and global warming, urbanization and habitat loss, emergence of parasites, ecosystem disruption, etc., one that drew most attention was a new class of pesticides, the neonicotinoids, which had entered the market in 1991 and subsequently gained wide use.

In 2013, having considered the benefits and risks of using neonicotinoid pesticides, the European Commission restricted certain uses of them. However, the reaction to this restriction was not uniformly favourable, and the European Commission requested EASAC (the European Academies' Science Advisory Council) to study this problem. EASAC studied neonicotinoids from the wider perspective of the interaction between agriculture and ecosystem services. It published its findings in a report, 'Ecosystem Services, Agriculture and Neonicotinoids', in 2015.

In 2019, NASAC (the Network of African Science Academies), in collaboration with the IAP (InterAcademy Partnership), also published its study on neonicotinoids in Africa, 'Neonicotinoid Insecticides: Use and Effects in African Agriculture'. In 2021, the IAP also requested AASSA (the Association of Academies and Societies of Sciences in Asia) to similarly study the Asia-Pacific region to supplement the European and African studies.

AASSA designated KAST (the Korean Academy of Science and Technology) as the host academy of this project. AASSA also assembled a working group of 25 experts and a report writing team of 15 from 25 member countries in Asia-Pacific region led by Professor Chuleui Jung, Andong National University, South Korea. They held six hybrid meetings (by Zoom and in-person) and one in-person symposium. The final draft was then sent to distinguished external reviewers; their suggestions, comments and corrections were incorporated into this report.

In closing, I express my heartfelt gratitude to IAP for its financial support, to the working group, writing team and reviewers for their valuable time and effort, and to AASSA and KAST staff for their administrative support in making this project a success.

I also sincerely hope that this report will show scientists a direction for their additional future researches and provide policy-makers with a useful tool for formulating scientifically sound, evidence-based guidelines and regulations on the use of neonicotinoids.

Professor Yoo Hang Kim
Immediate Past President, AASSA

Executive summary

Neonicotinoids, a group of synthetic insecticides, were designed to control several insect pests, particularly plant-piercing and -sucking species such as aphids, leafhoppers, planthoppers, and whiteflies (hemipterans) and thrips (thysanopterans). Neonicotinoids are also effective in managing some lepidopteran, dipteran, and coleopteran insect pests. The first neonicotinoid, imidacloprid, came onto the market in 1991; thereafter, up to 2002, seven further neonicotinoids (imidacloprid, nitenpyram, acetamiprid, thiamethoxam, thiacloprid, clothianidin, and dinotefuran) were marketed. Another two neonicotinoids, cycloxaprid, and paichongding, have since been developed.

Neonicotinoids are applied in agriculture in three different ways: by foliar application, seed treatment, and soil treatment. However, the use of this class of insecticide is of particular concern because of its potentially non-target effects, primarily on insect pollinators and natural enemies. Initially, information came out on the basis of observations of huge bee-colony losses in 2006 in the USA, which drew the attention of honeybee researchers. Since the first approval and marketing of neonicotinoids, three decades have passed. During this period, many investigations have shown evidence of the toxic effects of neonicotinoids not only on bees but also on many different terrestrial and aquatic animals. Another concern is the long-term persistence of neonicotinoids in the environment. As a responsive policy, several countries have imposed time-limited restrictions on the use of three active ingredients of neonicotinoids, clothianidin, thiamethoxam, and imidacloprid, especially in the European Union.

Following the reports published on neonicotinoid use and their impacts on agriculture, associated ecosystems, and the environment in Europe and Africa, a group of researchers was assembled to review the available scientific evidence and examine the situation and risks imposed in the Asia-Pacific region. As Asia is the largest and the most populous continent in the world, and the region is heterogeneous with wide variations in climate (tropical to temperate), high diversities of plant and insect species, as well as different agricultural practices ranging from highly mechanical and automated cultivation to smallholder manual farming practices. In most of Asia, the tropical environment increases the risk of disease, weeds, and pest infestation. Pesticides and fertilisers are important components in driving seasonal cycles of planting, harvesting, and production in the region to help counteract the effects of tropical climates and increasingly irregular weather patterns because of climate change. Over the past 30 years, crop yield in Asia has steadily increased and the total amount of pesticide use has been relatively stable. However, the proportion of neonicotinoids in insecticide use has sharply increased, as seen in South Korea and Japan, although great uncertainty remains because of limited or scarce datasets. The impact of neonicotinoids by seed coating might be of relatively less concern in Asia than in North America and Europe because the use of neonicotinoids in Asian agriculture mostly involves foliar application.

This report provides a comprehensive risk assessment of neonicotinoid insecticides in the Asia-Pacific region. It has been divided into six chapters. The first chapter describes the current use of agrochemicals with special emphasis on neonicotinoids and agricultural production in Asia-Pacific countries. The second chapter discusses the scientific knowledge about the molecular mechanisms of action of neonicotinoids on insects. The third chapter deals with the risk assessment of neonicotinoids on the environment. The fourth and fifth chapters discuss the risk assessment of neonicotinoids on pollinator and natural enemy populations in the Asia-Pacific region. The sixth chapter of the report discusses regulation and risk mitigation programmes in the region.

In the third chapter, on the risk assessment of neonicotinoids on the environment, we show that research on the environmental impacts of neonicotinoids in the Asia-Pacific region has been slow in following that in other parts of the world. Monitoring studies of the residues in soil, water, and sediments are, on the contrary, numerous and comprehensive, and help our understanding of the sources and extent of the contamination caused by this group of soluble and mobile pesticides. In general, the findings are consistent with the widespread contamination of surface waters. Studies on the toxicity and toxicokinetics of neonicotinoids in several aquatic taxa have highlighted that chronic exposures to low neonicotinoid levels are the main driver of these insecticides' impacts on organisms. The ecological effects on aquatic and terrestrial ecosystems are linked. Although contamination of water bodies by neonicotinoids was not seriously considered for decades, data show alarming signatures on the risks to aquatic biodiversity with trophic cascades, exemplified by the case of Lake Shinji in Japan and its fishery collapse. Current contamination levels are expected to increase owing to the continuous use of neonicotinoids in all kinds of crops and urban settings, and the persistence of parent compounds and their toxic metabolites. Consequently, the environmental risks of neonicotinoids, particularly those risks to the aquatic environment, will only get worse unless restrictions are applied.

In the context of honeybee colonies, two different trends have been observed in Asian countries. Countries such as China, Korea, India, Iran, Israel, Myanmar, Pakistan, and Vietnam have shown increasing trends in the honeybee hive numbers during the past three decades. In contrast, Australia, Japan, Mongolia, Taiwan, and Uzbekistan have shown either decreasing trends or no change. Chapter 4, on the risk assessment of neonicotinoids on Asian pollinator populations, reveals the diverse situation of Asian honeybees in relation to population dynamics and possible neonicotinoid exposure, the toxicity of neonicotinoids to honeybees and other pollinators such as bumblebees and solitary bees, and the geographical and environmental conditions and varying patterns of insecticide use. On the basis of the meta-analysis of available data, the risk quotient is high for honeybees compared with bumblebees. Similar to the toxicity results, the risk quotient also reveals that imidacloprid, thiamethoxam and clothianidin are more toxic than acetamiprid and thiacloprid.

Chapter 5, on the risk assessment of neonicotinoids on natural enemies, gives a detailed report in the Asia-Pacific context. The meta-analysis demonstrates the significant negative effect of neonicotinoid pesticides on the behaviour of predators and beneficial parasitoids. Among them, the highest negative impact is observed in predators, with an 84% reduction in the number of prey consumed. The application of insecticides may cause sublethal effects on biological and behavioural parameters including developmental time, fecundity, longevity, sex ratio, feeding activity, predation rate, orientation, and the mobility of natural enemies. The negative effects of sublethal doses of neonicotinoids on the longevity, survival, and fecundity of hoverflies and ladybirds is also documented. The findings suggest that continuous exposure to pesticides in agroecosystems is driving the decline of biodiversity and functional interactions responsible for ecosystem services.

Chapter 6 shows the regulations of the uses of neonicotinoid insecticides in the Asia-Pacific region mainly consist of registration of pesticide products, post-registration review, and requirements for risk mitigation. Registration of the uses of neonicotinoids in the region follows local pesticide regulatory systems, which are different from country to country. This results in very diverse data requirements and decision-making standards throughout the region. Continuous efforts are needed to further harmonise the regulatory systems and promote science-based risk assessment that takes into account not only the toxic effects of the chemicals but also local agricultural conditions and practices.

In the Asian countries with the highest number of registered neonicotinoid uses, such as Japan, China, and Australia, concerns about environmental issues are invoking risk assessments of those uses. On the basis of those assessments, some neonicotinoids might be discontinued or restricted. At the same time, because of the importance of neonicotinoids to agriculture in these countries, any regulatory decisions need to carefully balance the benefits and risks.

In most Asian countries, the authorities require precautionary statements on labels where potential risks are identified, and risk mitigation measures are needed. The mitigation measures are required to be acceptable to local farmers and implementable in the countries. Levelling up the review system as well as implementing risk mitigation programmes with education for farmers seems important in some countries.

Overall, a large volume of scientific evidence indicates that neonicotinoids have a serious negative effect on pollinators, natural enemies and the environment including aquatic ecosystems. Regular monitoring, restricted application of neonicotinoids and enforced review systems are essential components for monitoring neonicotinoid use to avoid potential hazards to non-target species and ecosystems.

1 Introduction

Pesticides are indispensable in agricultural production. They are used to target and control weeds, diseases, and pest insects. The increase in the world's population in the 20th century could only have been possible with a parallel increase in food production. About one-third of agricultural products would not be grown without agricultural pest control. Agricultural pesticide use can be divided into three eras: the first using natural compounds, until 1870; then an era of inorganic compounds, until 1945; and finally an era of synthetic organic compounds, which have been used since then. In the first era, smoking with plant and animal materials or pyrethrum were used. In the second era, sulfur compounds and Bordeaux mixture provided significant efficiency. In the third era, with advances in organic chemistry stimulated by the development of DDT (dichlorodiphenyltrichloroethane), BHC (benzene hexachloride), and 2,4-D (2,4-dichlorophenoxyacetic acid), a series of innovations were made in pesticide development and consequently their use expanded with such chemicals as organochlorines, carbamates, organophosphates, pyrethroids, insect growth regulators, and NNIs.

Neonicotinoids (NNIs) are a class of synthetic, systemic insecticides that are widely used on agricultural crops globally. They mostly target plant-piercing and -sucking insect pests such as aphids, leafhoppers, planthoppers, and whiteflies (hemipterans) and thrips (thysanopterans). Since the first NNI, imidacloprid, was introduced into the market in 1991, seven more NNIs (imidacloprid, nitenpyram, acetamiprid, thiamethoxam, thiacloprid, clothianidin, and dinotefuran) have been developed. Because of their characteristics favoured by users, NNIs have rapidly replaced previously developed insecticides such as carbamates or organophosphates not only in agriculture but also in human residential environments. The attractive characteristics of NNIs are (1) predicted lower mammalian toxicity because of their selectivity for insect nicotinic acetylcholine receptors over mammalian ones; (2) their higher persistence; (3) they are active against a broad spectrum of crop pests; (4) their systemic properties (e.g. transferring to all parts of treated plants from root to flower, and fruit food produced by those plants); (5) their versatility in application (e.g. foliar sprays, prophylactic seed coating, and soil drenching treatment); (6) high water solubility; and (7) assumed lower impacts on fish and other wildlife (Craddock *et al*. 2019).

Since the introduction of imidacloprid in 1991, neonicotinoids have come to represent approximately 30% of the global insecticide market with registration in more than 120 countries (Jeschke *et al*. 2011). The rapid increase in their use has been accompanied by non-target effects such as threatening beneficial insects as well as other environmental concerns. Since the beginning of the 21st century, NNIs have been implicated in the colony collapse disorder of honey bees (Woodcock *et al*. 2017). Three NNIs, thiamethoxam, clothianidin, and imidacloprid, were banned from use on flowering crops by the European Union in 2013 for 2 years. Even with NNIs' relatively lower acute toxicities, chronic exposure to sublethal doses of them hampers honey bees' learning and memory behaviour, resulting in failure to return to their hives. Further emerging evidence has shown endocrine disruption and toxicity to humans and wildlife throughout trophic levels in food webs (EFSA PPR Panel 2013).

Following the reports published on NNI use and their impacts on agriculture, associated ecosystems, and the environment in Europe (EASAC 2015) and Africa (NASAC 2019), a special risk assessment of NNIs in the Asia-Pacific region was requested. A group of researchers was assembled to review the available scientific evidence and examine the situation and risks in the Asia-Pacific region. The Asia-Pacific region is different from other continents: Asia is the largest and the most populous continent in the world; the region is heterogeneous with wide variations in climate (tropical to temperate); it has high diversities of plant and insect species; it also has different agricultural practices, ranging from highly mechanical and automated cultivation to smallholder manual farming practices, so we expected the risks from NNI use would differentially affect the environment in the region. In most of Asia, the tropical environment increases the risk of disease, weeds, and pest infestation. Pesticides and fertilisers are important components in driving seasonal cycles of planting, harvesting, and production in the region to help counteract the effects of tropical climates and, increasingly, irregular weather patterns caused by climate change. Also, wetted paddy fields occupy most of the farmland in many parts of Asia, which is in contrast to large dry plantations of corn, oilseed crops, or wheat in America and Europe. Over the past 30 years, crop yield in Asia has steadily increased and the total amount of pesticide use has been relatively stable. However, the proportion of NNIs in insecticide use has sharply increased, as seen in South Korea and Japan, although great uncertainty remains because of limited or scarce datasets.

Thus experts from diverse disciplines such as agronomics, agricultural chemistry, pest management science, pollination and beekeeping, biological control of crop pests, pesticide registration and management, and environmental monitoring and analysis were invited to participate in this risk assessment. Also, delegates from most Asian countries were invited for periodic meetings, online or offline, to communicate results and concerns from their local, unique environments.

This report first reviews agriculture and pesticide use with emphasis on NNIs in Asia-Pacific countries. Then, we discuss the scientific knowledge about the mode of action of NNIs on insects, specifically honeybees and molecular mechanisms. This is followed by a risk assessment of acute, chronic, and sublethal toxicities of NNIs on pollinators and the natural enemies of crop pests. The risk assessment was expanded to the wider environment, especially soil and water systems. Since NNIs have high solubility and mobility in water, and water organisms have high sensitivities to them, a significant contribution to understanding the risks of NNIs in these environments is detailed. Then, we review the registration and regulation of insecticides in the Asian countries. Finally, policy recommendations for the risk management of NNIs are made to mitigate the challenges identified in the report and to protect ecosystem services and biodiversity in the region.

2 Status of agrochemical use in Asia-Pacific countries

Chapter summary

Pesticides are an important component in driving crop productivity because they are designed to control agricultural pests. Owing to its enormous agricultural base, the Asia-Pacific region is the largest user of pesticides, and their importance has increased with the growing demand for food crops. At the same time, concern about the unintentional impact on the environment of pesticides such as neonicotinoid insecticides (NNIs) has increased. The use of insecticides in the region has been relatively stable over the past 30 years. NNIs have been applied to all kinds of crops in the region, but the proportions of registered items based on applicable crop types are quite different by country. This, combined with the heterogeneous environment of Asia, means it is necessary to perform a risk assessment of NNIs that considers each country's agricultural environments such as crops, pests, farming practices, and habitat characteristics in order to maintain biodiversity and crop productivity in the region. Neonicotinoid impact by seed coating treatment might be relatively low in Asia compared with North America and Europe. However, application for outdoor use, particularly during the flowering period, is cautioned because of the high possibility of exposure to forager honeybees and other pollinator insects, regardless of the application method. In major rice-producing countries, it is important to be aware of the environmental impact of NNIs in water systems from rice paddy fields, because of these insecticides' high water solubility.

2.1 Introduction

The Asia-Pacific region is heterogeneous with wide variations in climate (Figure 2.1), a high diversity of plant and insect species, as well as different agricultural practices ranging from highly mechanical and automated cultivation to smallholder manual farming. In most of the region, the tropical environment increases the risk of disease, weeds, and pest infestation. Pesticides and fertilisers are important components in driving seasonal cycles of planting, harvesting and production to help counteract the effects of tropical climates and increasingly irregular weather patterns because of climate change.

The market for crop protection chemicals in the Asia-Pacific region is projected to register a compound annual growth rate of 3.8% from 2020 to 2025 (Mordor Intelligence 2021a). Crop protection chemicals are a type of agrochemical that is used to keep insects, diseases, weeds, and other pests from destroying crops. Synthetic crop protection chemicals are the most common type, with biopesticides accounting for a small percentage of the market (Mordor Intelligence 2021a). Pesticides have been used to boost crop yields as the demand for food crops has increased with the region's growing population. Furthermore, in nations such as India and other Southeast Asian countries, where arable land per person is dwindling at an alarming rate, pesticides can play a key role in raising average crop yields per hectare. Both government and private initiatives, such as the proper application of pesticides in terms of quantity and application methods, are raising awareness of pesticide overuse among the region's farmers (Mordor Intelligence 2021a).

Agricultural chemicals have been used more frequently in the region as demand for agricultural products has grown and agriculture has become more commercialised. Insects cause the most agricultural damage, followed by pathogens and weeds. Insecticides make up a significant portion of total pesticide use in the region. Crop protection businesses around the world are establishing production facilities and introducing products to meet the demands of farmers in this region (Mordor Intelligence 2021a). Because of its enormous agricultural base, the Asia-Pacific region is the largest user of crop protection chemicals (Mordor Intelligence 2021b). In 2020, China, Japan, India, and Australia accounted for more than 80.0% of the region's market for crop protection chemicals (Mordor Intelligence 2021b). Synthetic pesticides have been widely used to reduce crop losses caused by pests and diseases. Higher demand for food grains, restricted arable land availability, rising exports, increasing farming in horticulture and floriculture, and raising public awareness about pesticides are all driving forces for the crop protection chemicals industry. Companies place a high priority on research and development, which is the backbone of the launch of new and enhanced pesticide products, and the rising demand for crop protection chemicals throughout the region (Mordor Intelligence 2021b).

With the growing importance of pesticides in crop production, concerns about their unintentional impact on non-target organisms have been raised worldwide. In particular, neonicotinoid insecticides (NNIs) have been speculated as a culprit causing negative impacts on bee colonies and other beneficial insects such as pollinators that help the fertilisation of flowering plants. The Asia-Pacific region has a high diversity of pollinator species including honeybees such as *Apis mellifera* and *Apis cerena* (Osterman *et al.* 2021). Their contribution to crop yields varies from region to region but overall is estimated to be greater than 10% in most Asian countries (Figure 2.2 and Box 2.1). The recently raised

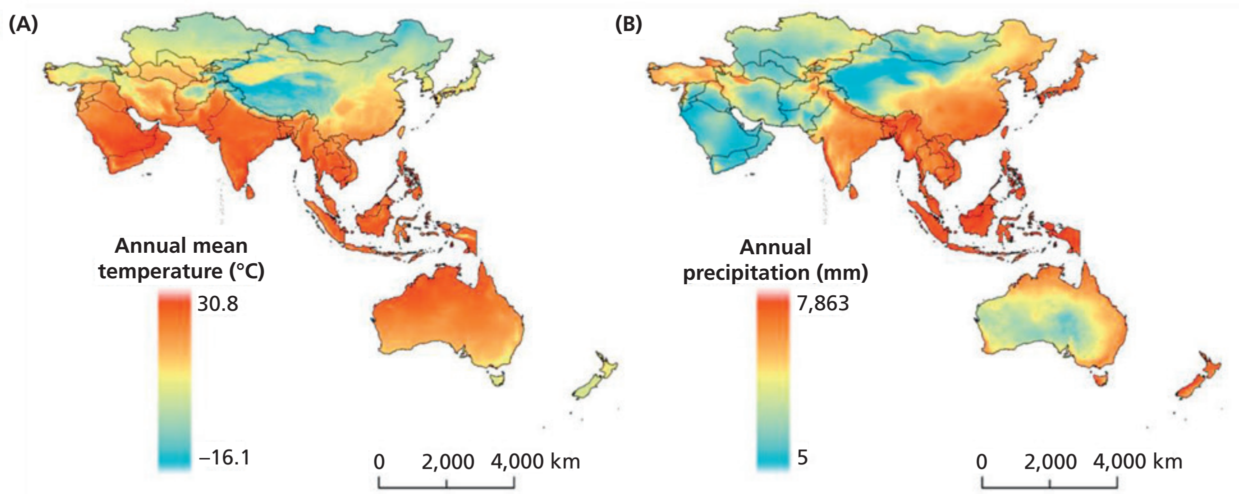


Figure 2.1 Climatic variability of (A) temperature and (B) precipitation in the Asia-Pacific region (https://www.worldclim.org/).

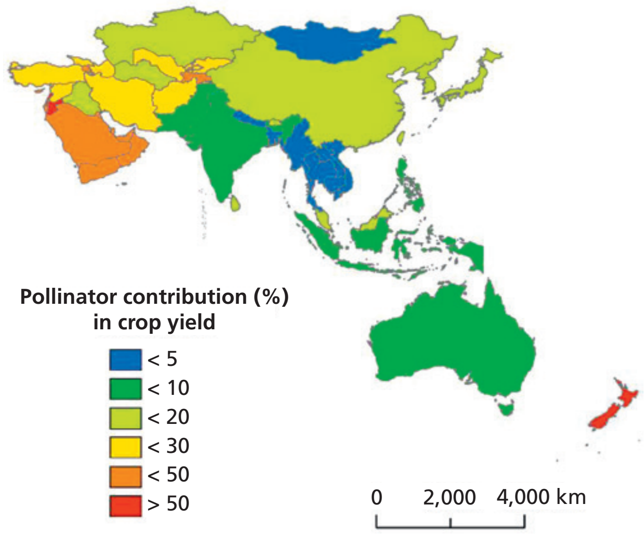


Figure 2.2 Estimated contribution of pollinators to crop yield in the Asia-Pacific region.

Box 2.1 Pollinator-dependent crop yield in the Asia-Pacific region

We analysed the status of pollinator contribution to crop yield in the Asia-Pacific region. The data for crop production (tonnes) and area harvested (hectares in the latest available year (2020) were obtained from the database of the Food and Agriculture Organization of the United Nations (FAOSTAT) website (https://www.fao.org/). The key crops paired with pollinator dependency (%) are listed on the basis of Jung (2008), Smith *et al.* (2015), and Ghosh and Jung (2016) (Table 2.1). Total crop yield (Y_{Total}) and pollinator-dependent crop yield (Y_{PD}) were calculated using following equations countrywise:

$$Y_{\text{Total}} = \frac{\sum_{i=1}^{n} P_i}{\sum_{i=i}^{n} A_i}$$

$$Y_{PD} = \frac{\sum_{i=1}^{n} P_i \times \text{PD}_i}{\sum_{i=i}^{n} A_i}$$

where P_i, A_i, and PD_i are the production, crop harvested area and the pollinator dependency of the *i*th crop, respectively. The mid-point of the pollinator dependency range on each crop was applied to equations (Table 2.1). The overall contribution of pollinators to crop yield in each Asian country was calculated as $Y_{\text{PD}}/Y_{\text{Total}}$ (Figure 2.2).

Continues on next page

Box 2.1 *(continued)*

Table 2.1 Crops and pollination dependency (PD) for evaluation of pollinators' contributions to agricultural productivity in the Asia-Pacific region (adapted from Smith *et al.* (2015) and Ghosh and Jung (2016))

Crop	PD (range)[1]	Crop	PD (range)
Cereal, nes[2]	0 (0–0)	Rape seed	0.25 (0.1–0.4)
Maize green	0 (0–0)	Sesame seed	0.25 (0.1–0.4)
Maize	0 (0–0)	Soybean	0.25 (0.1–0.4)
Millet	0 (0–0)	Strawberries	0.25 (0.1–0.4)
Pulses, nes	0 (0–0)	Almonds, with shell	0.65 (0.4–0.9)
Rice, paddy	0 (0–0)	Apples	0.65 (0.4–0.9)
Sorghum	0 (0–0)	Apricots	0.65 (0.4–0.9)
Tea	0 (0–0)	Avocado	0.65 (0.4–0.9)
Vegetables, fresh nes	0 (0–0)	Berries, nes	0.65 (0.4–0.9)
Walnuts, with shell	0 (0–0)	Blueberries	0.65 (0.4–0.9)
Wheat	0 (0–0)	Buckwheat	0.65 (0.4–0.9)
Beans, green	0.05 (0–0.1)	Cashew nuts, with shell	0.65 (0.4–0.9)
Citrus fruit, total	0.05 (0–0.1)	Cherries	0.65 (0.4–0.9)
Cow peas, dry	0.05 (0–0.1)	Cherries, sour	0.65 (0.4–0.9)
Groundnut, with shell	0.05 (0–0.1)	Cranberries	0.65 (0.4–0.9)
Papaya	0.05 (0–0.1)	Cucumber and gherkins	0.65 (0.4–0.9)
Persimmon	0.05 (0–0.1)	Mango, mangosteens, and guavas	0.65 (0.4–0.9)
Pigeon peas	0.05 (0–0.1)	Peaches and nectarines	0.65 (0.4–0.9)
Broad beans and horse bean	0.25 (0.1–0.4)	Pears	0.65 (0.4–0.9)
Coconut (including copra)	0.25 (0.1–0.4)	Plums and sloes	0.65 (0.4–0.9)
Coffee, green	0.25 (0.1–0.4)	Quinces	0.65 (0.4–0.9)
Currants	0.25 (0.1–0.4)	Raspberries	0.65 (0.4–0.9)
Eggplant	0.25 (0.1–0.4)	Cocoa beans	0.95 (0.9–1.0)
Figs	0.25 (0.1–0.4)	Kiwi fruit	0.95 (0.9–1.0)
Gooseberries	0.25 (0.1–0.4)	Melons, other (including cantaloupes)	0.95 (0.9–1.0)
Oilseed, nes	0.25 (0.1–0.4)	Pumpkins, squash, and guards	0.95 (0.9–1.0)
Okra	0.25 (0.1–0.4)	Watermelons	0.95 (0.9–1.0)

[1] PD is assumed to be the mid-point of the ranges in parentheses.
[2] nes, not elsewhere specified.

China, Korea, Japan, New Zealand, and some Middle Eastern countries such as Oman, Israel, Jordan, Turkey, Bahrain, and Kuwait showed high crop yields in the Asian region (Figure 2.3A). Oceania, Indochina, and Central Asian countries were at a relatively moderate level. Pollinator-dependent crop yields were relatively high in New Zealand and some Middle Eastern countries in the Asia-Pacific region (Figure 2.3B).

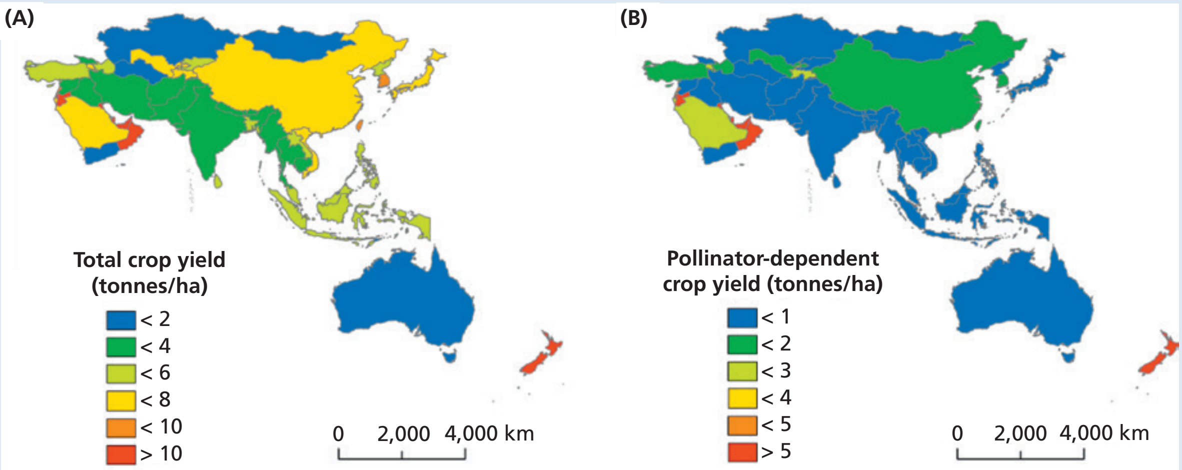


Figure 2.3 (A) Total crop yield and (B) pollinator-dependent crop yield in the Asia-Pacific region.

negative impact of NNIs on pollinators might thus impair crop productivity in the Asia-Pacific region (Stanley *et al.* 2015). Therefore, it is especially important to analyse the use of pesticides to estimate their potential impact on pollinators and crop yields in the region.

The cultivated crops in the Asia-Pacific region are quite different from those on other continents, and they can induce different exposure routes of pesticides on non-target organisms. For example, rice is a staple diet in many Asian countries, and is self-sufficient for most of its consumption. Because of the distinctive cropping system of rice that requires a lot of water, applied pesticides may have an exposure route to ecosystems through the water system in paddy fields. This route deserves to be examined in major producers of rice crops such as Indochina countries, Southeast Asia, and East Asia (Figure 2.4). Crop production patterns and pesticide application methods can provide key information to estimate pesticide exposure routes and their risk to the environment.

2.2 Crop productivity and insecticide use in the Asia-Pacific region

According to FAOSTAT, crop production in the Asia-Pacific region has been progressively increasing over the years. China and India have been following the increasing trend (Figure 2.5A). On the other hand, a downward trend has been observed in Japan, Korea, and Taiwan (Figure 2.5B).

The increase in crop production in China and the decline in Japan, Korea, and Taiwan might be due to the change in area where crops have been cultivated: increasing in China and India and decreasing in Japan, Korea, and Taiwan over the years (Figure 2.6).

Despite the change in crop production and area harvested, there has been no loss in crop productivity from the yield point of view. An increase in yield on a per hectare basis has been observed in Australia, China, India, Japan, South Korea, and Taiwan. Over the years, Australia and China have consistently been the leading countries in terms of yield per unit area (Figure 2.7).

On the basis of FAO data, insecticide use for agricultural purposes in the Asia-Pacific region has been relatively stable while the production quantity of primary crops has increased over the past 30 years (Figure 2.8). Understandably, the increased production has been mainly driven by China and India (Figure 2.9). On the other hand, there seems to be a downward trend for crop production in Japan, Korea, and Taiwan. A similar trend of decline in insecticide use has also been observed (Figures 2.10 and 2.11).

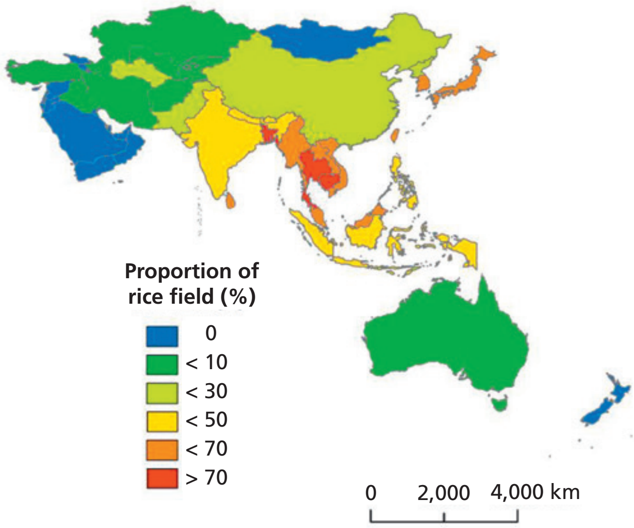


Figure 2.4 Proportion of rice field (%) to total area of crop harvested in the Asia-Pacific region (FAOSTAT).

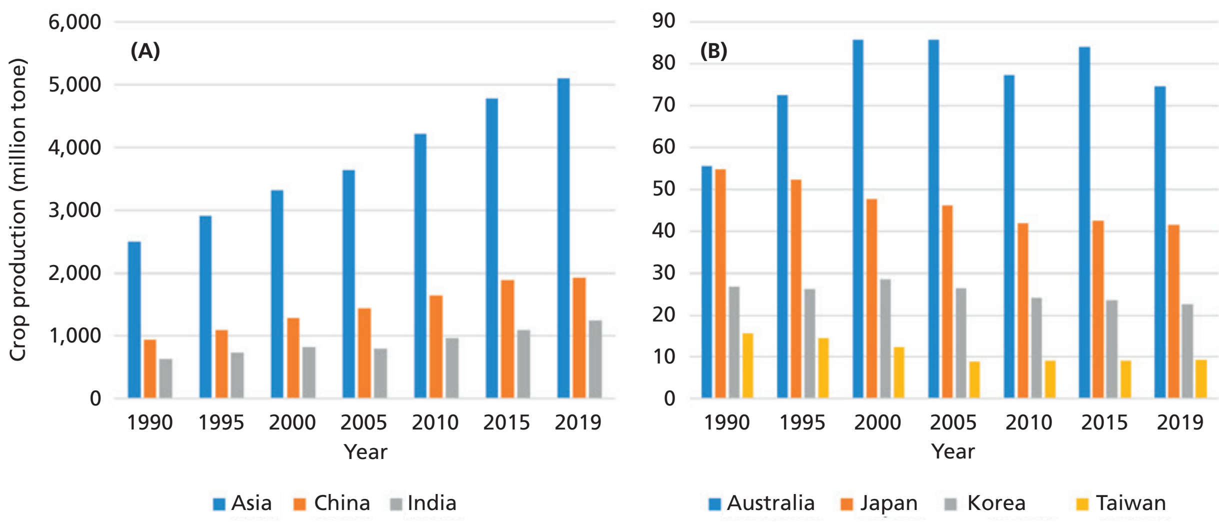


Figure 2.5 Temporal changes in crop production (million tonnes) in the Asia-Pacific region.

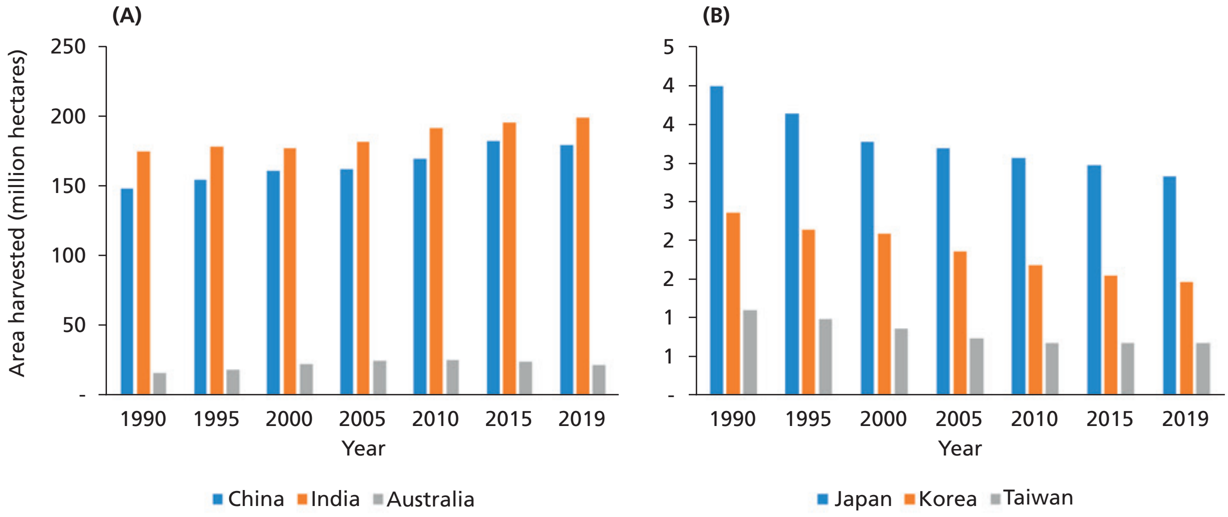


Figure 2.6 Temporal changes in area harvested in the Asia-Pacific region.

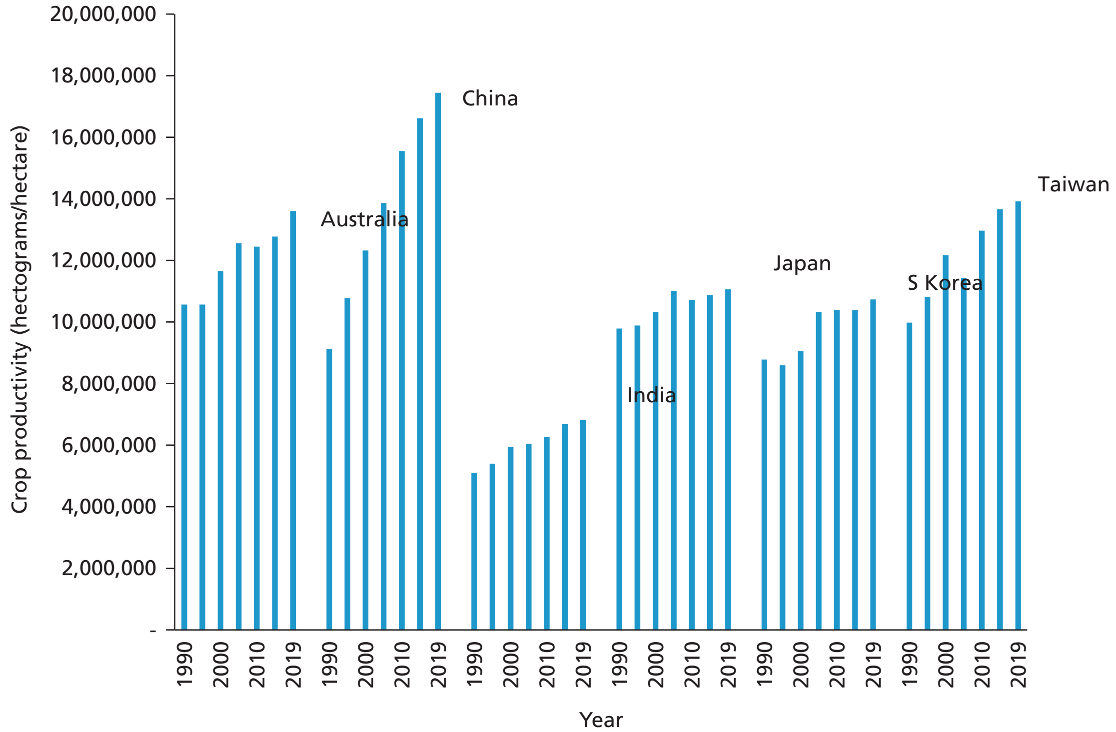


Figure 2.7 Trends in crop productivity (hectograms per hectare) in the Asia-Pacific region.

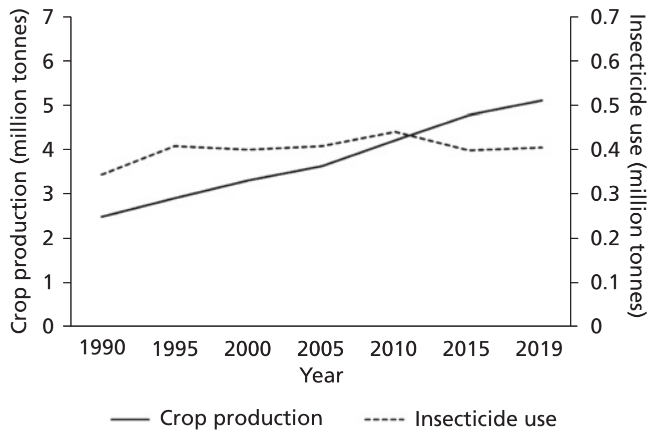


Figure 2.8 Crop production and insecticide use in the Asia-Pacific region.

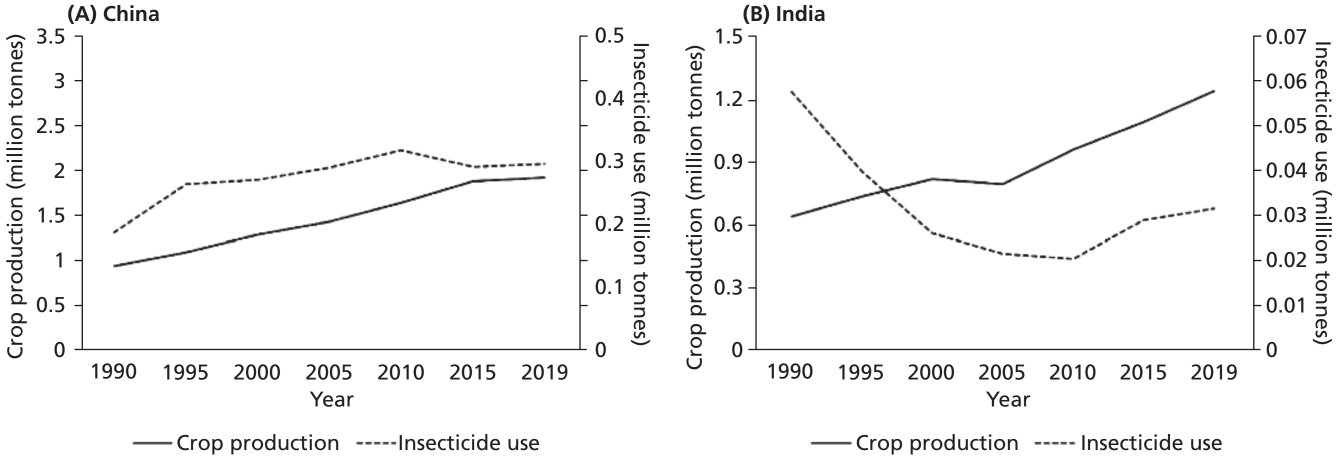


Figure 2.9 Crop production and insecticide use in (A) China and (B) India.

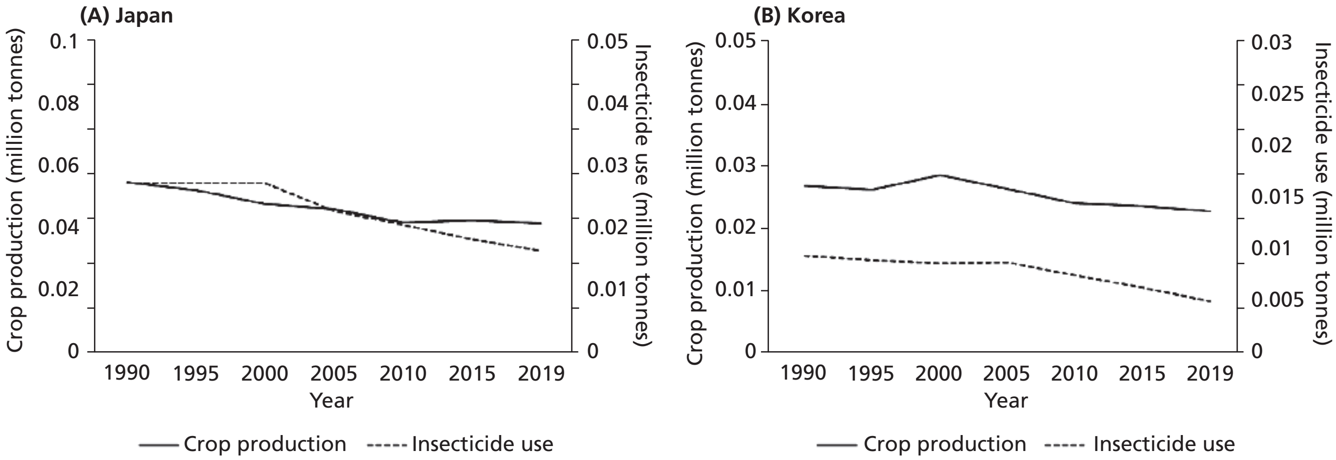


Figure 2.10 Crop production and insecticide use in (A) Japan and (B) Korea.

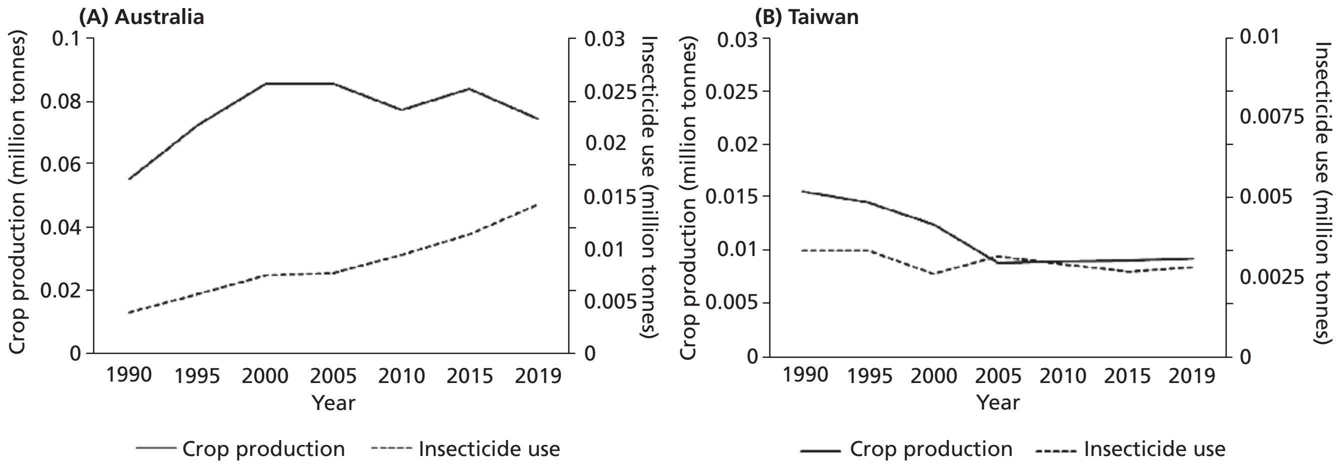


Figure 2.11 Crop production and insecticide use in (A) Australia and (B) Taiwan.

Specific data on each NNI as an insecticide group are not publicly available. However, there is some information from Japan and Korea.

In Japan, seven types of NNI have been registered since the first, imidacloprid, in 1992 (Taira 2014). The amount of NNIs distributed in Japan has continually increased since the first registration but has flattened since 2008 (Figure 2.12).

Korea is a major market with high use of NNI pesticides. Six types of NNI have been registered for agricultural

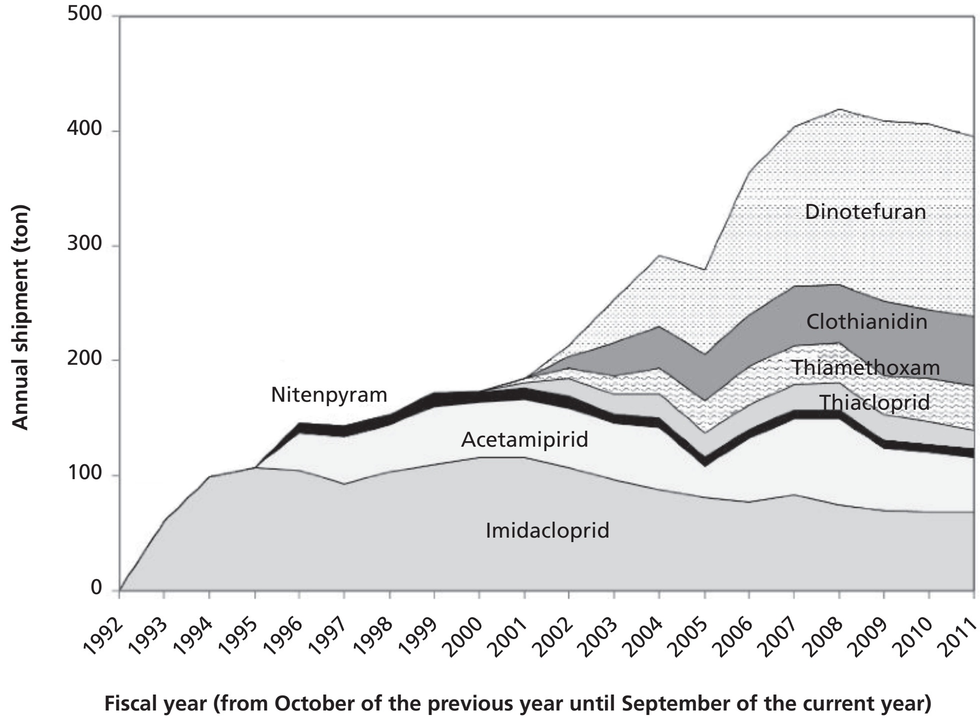


Figure 2.12 The shipment of NNIs in Japan (Taira 2014).

purposes since the first, imidacloprid, in 1993 (Korea Crop Protection Association). The imported amount steadily increased until the middle of the 2010s, and has been stable since. Currently, dinotefuran is the most widely used NNI in terms of the amount of active ingredient (Figure 2.13).

2.3 Neonicotinoids: registered uses in the Asia-Pacific region

NNIs are important for growers and are widely used in different regions of the world. There are more than 16,000 uses registered globally. Among the regions, Asia-Pacific has the highest number of registered uses, more than 5000, or 30% of total registered uses in the world (Figure 2.14) (Homologa).

In the Asia-Pacific region, there are 5,115 uses of Neonicotinoids registered in total. Japan, Taiwan, South Korea, Australia, and China are the top five countries in terms of the number of registered NNI uses (Figures 2.14 and 2.15).

Imidacloprid, acetamiprid, thiamethoxam, clothianidin, and dinotefuran are the top five NNIs registered in the Asia-Pacific region. They make up 91% of the number of registered uses in the region (Figure 2.16).

Neonicotinoids are applied to all kinds of crops in the region. Among them, vegetables, ornamentals, and fruits are the top three crop groups in terms of the number of registered NNI uses (Figure 2.17).

2.3.1 Japan

In Japan, acetamiprid, dinotefuran, clothianidin, imidacloprid, and thiamethoxam are the top five NNIs registered, making up 91% of the total number of registered uses (Figure 2.18A).

Ornamentals (e.g. flowers), vegetables, fruits, forestry, and cereals are the top five crop groups in terms of the numbers of registered NNI uses. Unlike all the other countries, close to 50% of registered uses are in ornamentals (Figure 2.18B).

2.3.2 Taiwan

In Taiwan, imidacloprid, acetamiprid, dinotefuran thiamethoxam, and clothianidin are the top five NNIs registered, making up 98% of the total number of registered uses (Figure 2.19A).

Vegetables, fruits, and ornamental are the top three crops in terms of the number of registered NNI uses, amounting to more than 90%. More than 60% of registered uses are focused on vegetables (Figure 2.19B).

2.3.3 South Korea

In South Korea, acetamiprid, imidacloprid, dinotefuran, thiamethoxam, and clothianidin are the top five NNIs registered. Making up more than 85% of the total number of registered uses, they are quite equally spread (Figure 2.20A).

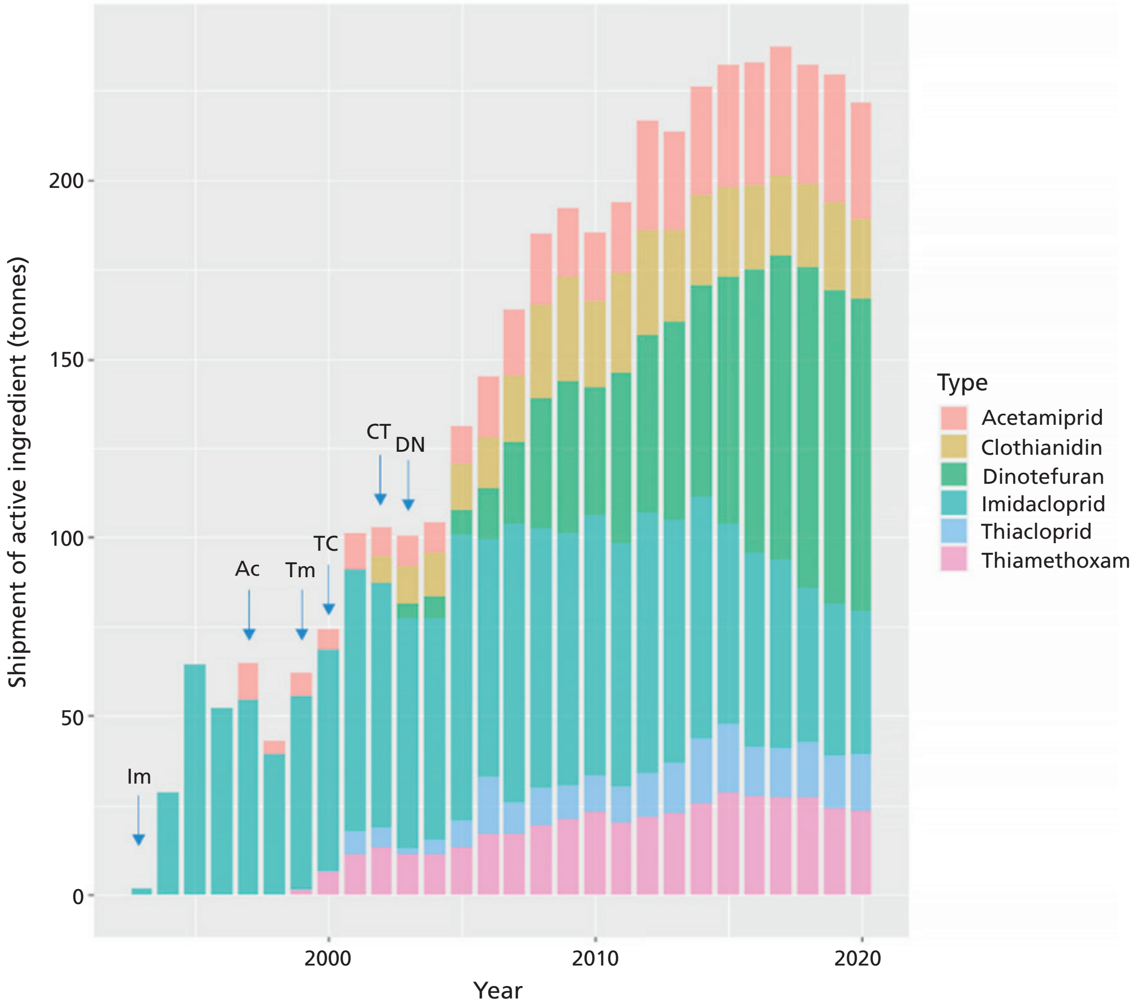


Figure 2.13 Trends in NNI use estimated from the shipment of active ingredients per year in Korea from 1993 (Korea Crop Protection Association).

Vegetables, fruits, and ornamental are the top three crop groups in terms of the number of registered NNI uses, amounting to more than 80%. More than half of the 80% registered uses are in vegetables (Figure 2.20B).

2.3.4 Australia

In Australia, imidacloprid, thiamethoxam, and clothianidin are the top three NNIs registered, making up more than 80% of the total number of registered uses. Imidacloprid occupies close to half of the total registered uses (Figure 2.21A).

Like South Korea, vegetables, fruits, and ornamental are the top three crop groups in terms of the number of registered NNI uses in Australia, amounting to more than 70%. More than 40% of registered uses are in vegetables (Figure 2.21B).

2.3.5 China

In China, imidacloprid, thiamethoxam, and acetamiprid are the top three NNIs registered, making up close to 80% of the total number of registered uses. Imidacloprid occupies 30% of the total registered uses. Some less common NNIs are also registered in China (Figure 2.22A).

Unlike other countries, more than 60% of the registered uses are in vegetables, with 10% in fruits (Figure 2.22B).

2.3.6 India

In India, imidacloprid, acetamiprid, and thiamethoxam are the top three NNIs registered, making up close to 90% of the total number of registered uses. Imidacloprid occupies close to 50% of the total registered uses (Figure 2.23A).

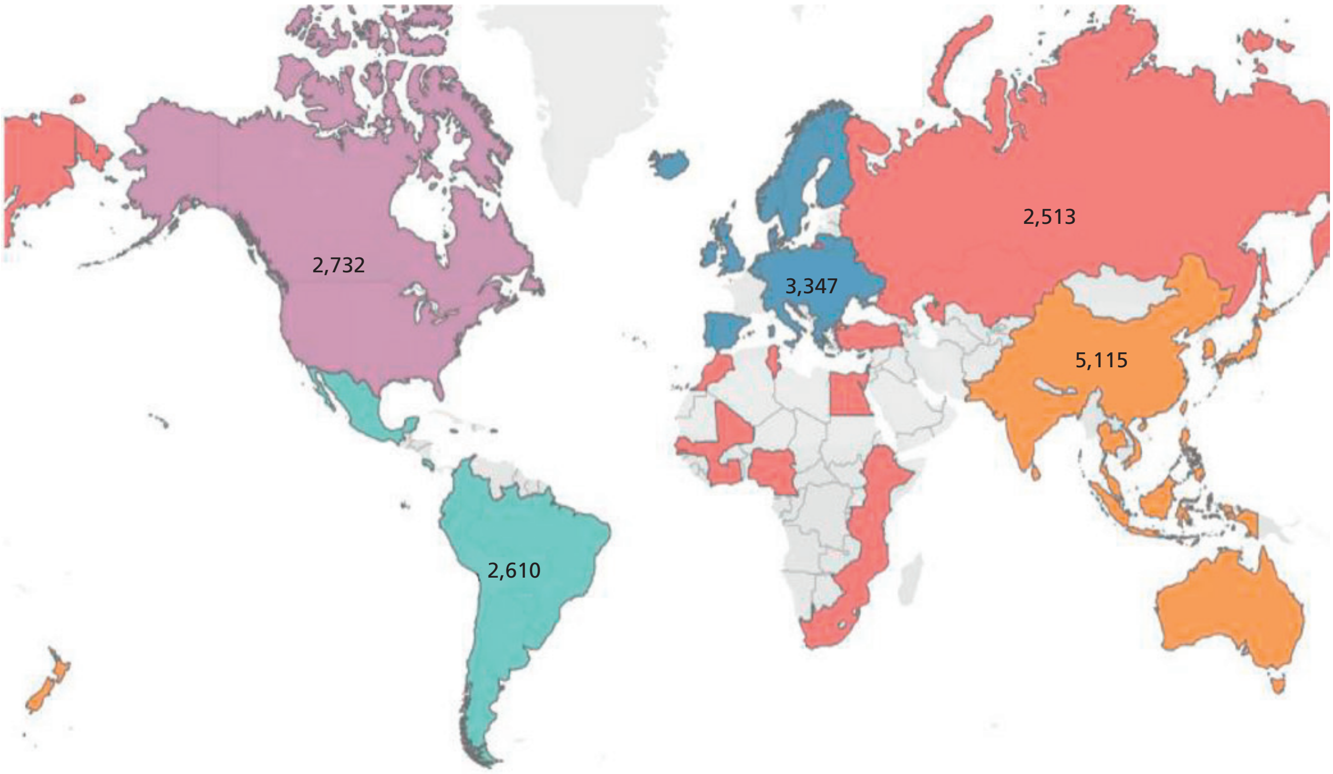
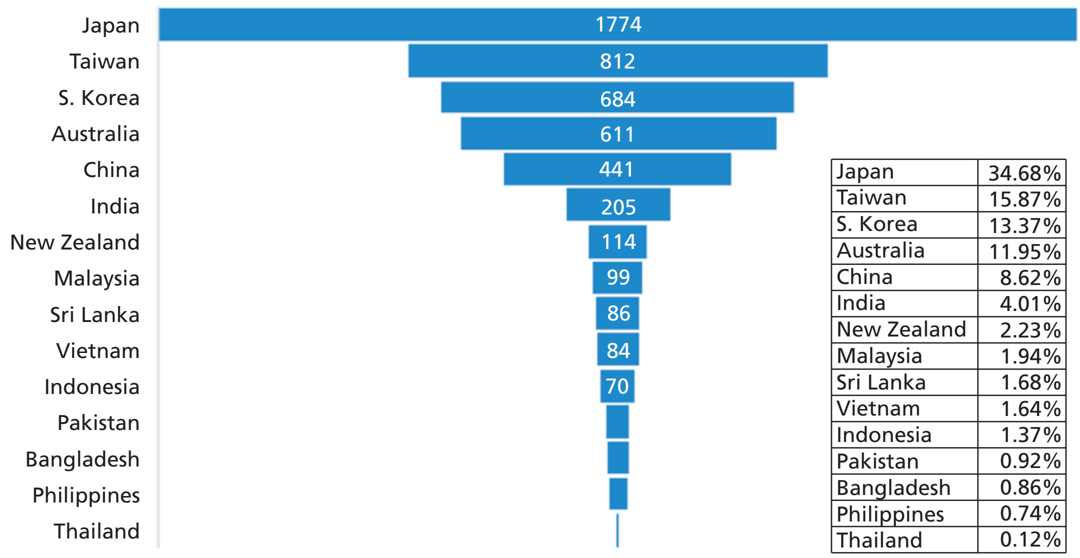


Figure 2.14 Number of NNI registered uses in the world.



Japan	34.68%
Taiwan	15.87%
S. Korea	13.37%
Australia	11.95%
China	8.62%
India	4.01%
New Zealand	2.23%
Malaysia	1.94%
Sri Lanka	1.68%
Vietnam	1.64%
Indonesia	1.37%
Pakistan	0.92%
Bangladesh	0.86%
Philippines	0.74%
Thailand	0.12%

Figure 2.15 Numbers of NNI registered uses in the Asia-Pacific region.

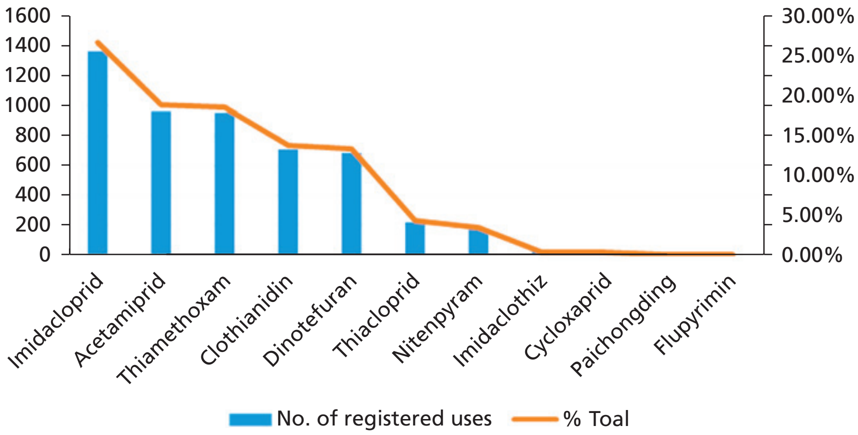


Figure 2.16 Number of NNI registered uses in the Asia-Pacific region.

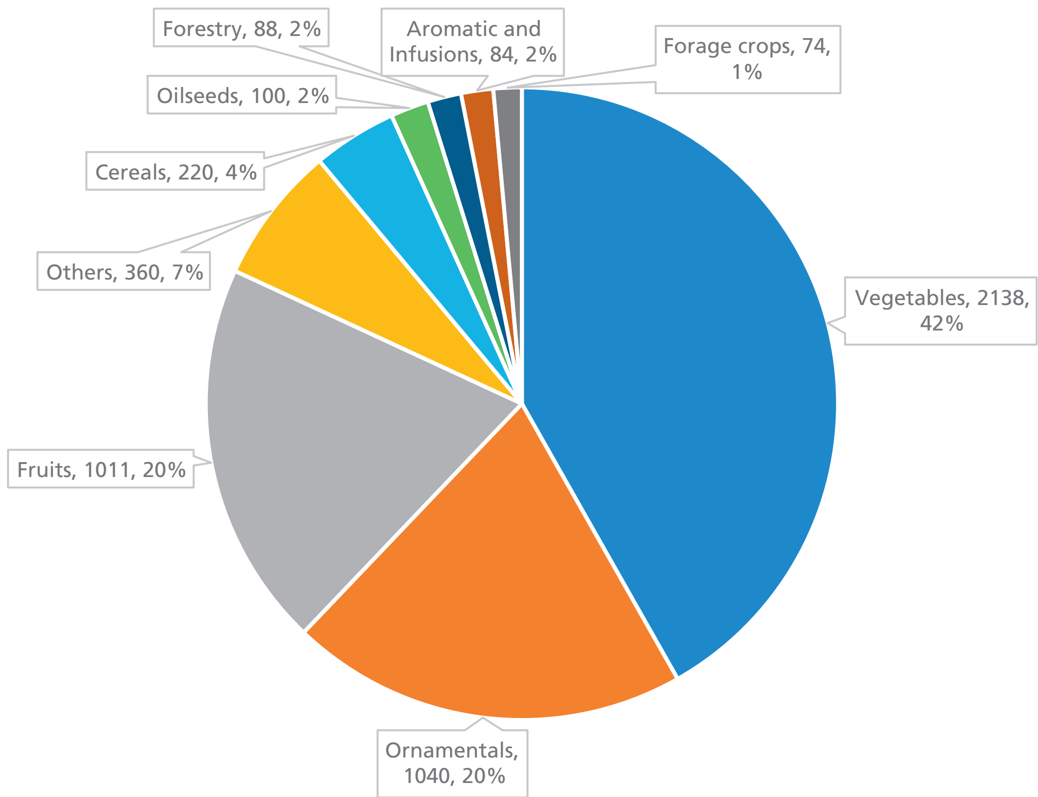


Figure 2.17 Number of NNI registered crop uses in the Asia-Pacific region.

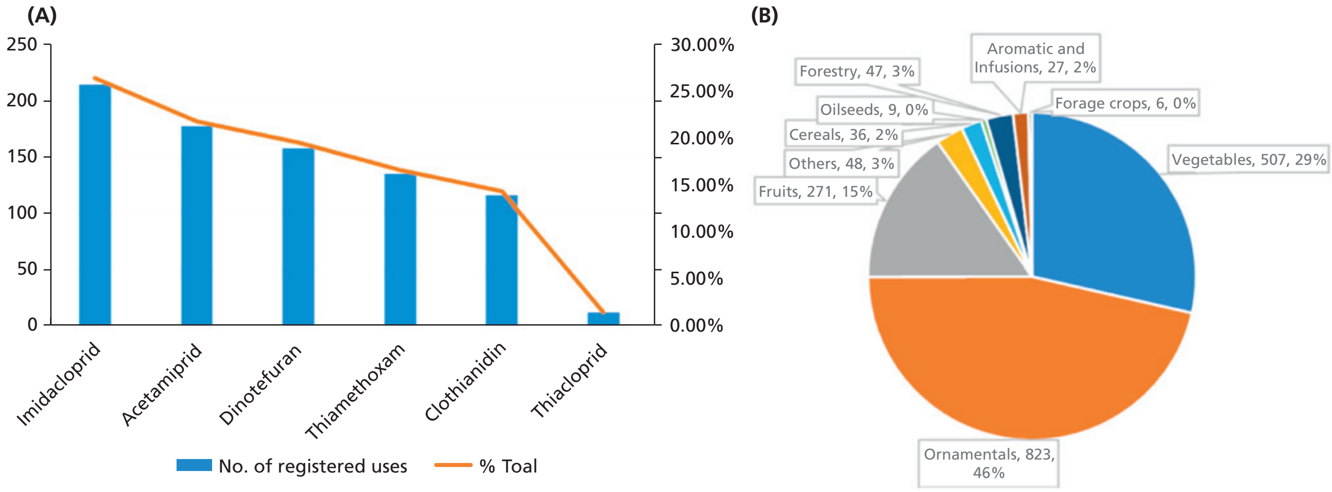


Figure 2.18 NNIs (A) registered and (B) by crop use in Japan.

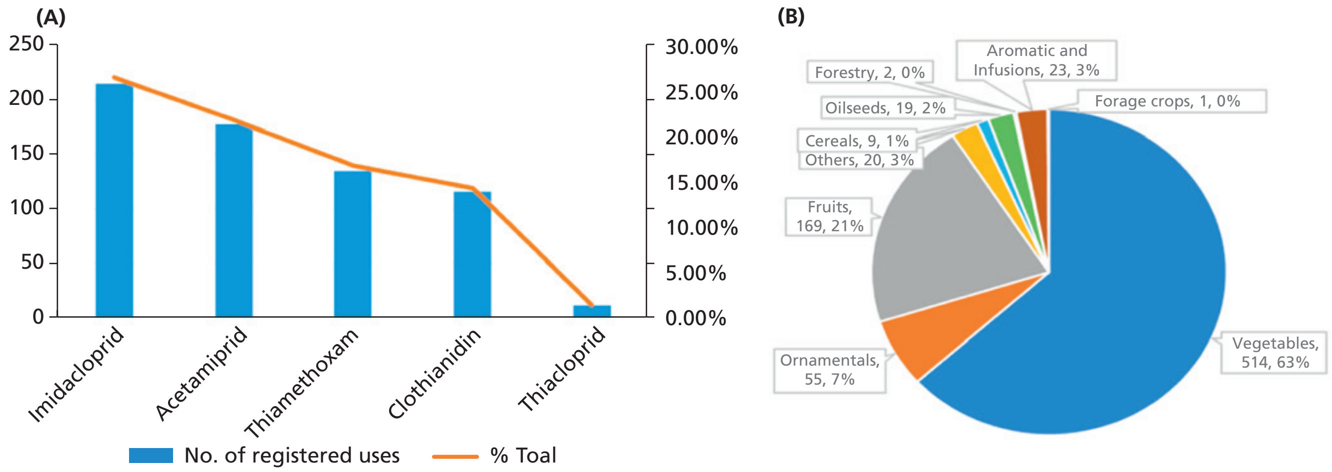


Figure 2.19 NNIs (A) registered and (B) by crop use in Taiwan.

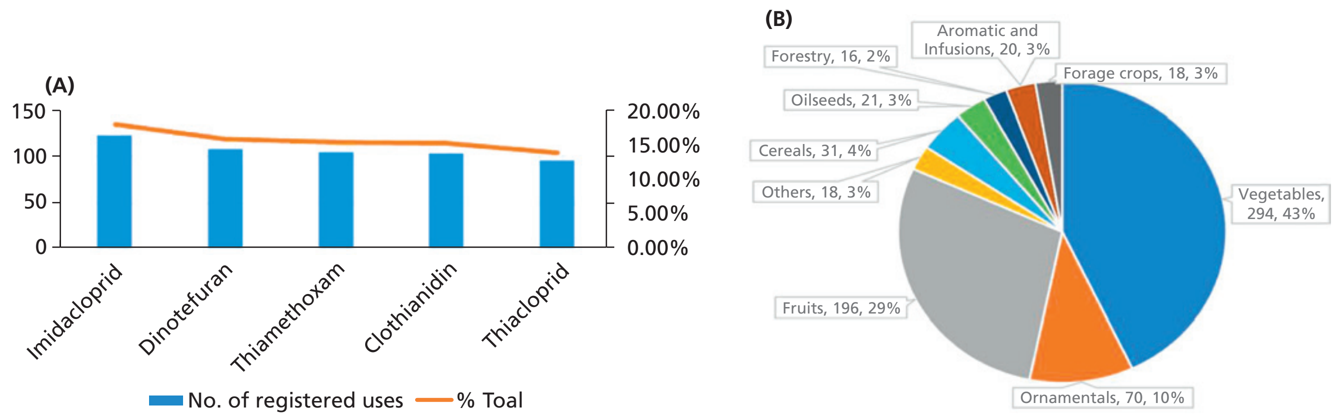


Figure 2.20 NNIs (A) registered and (B) by crop use in Korea.

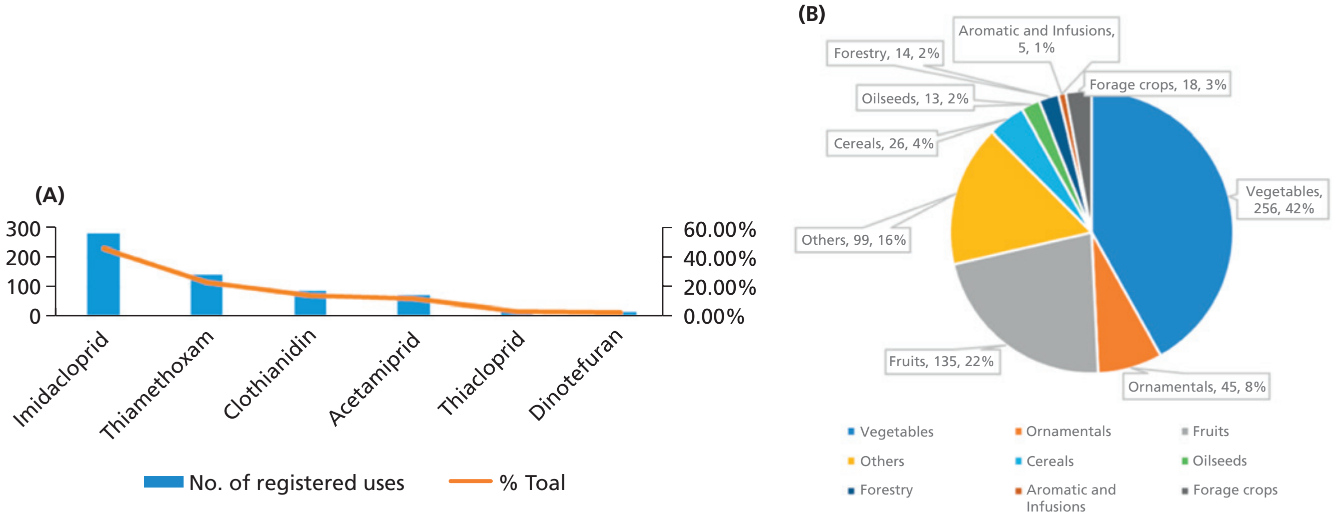


Figure 2.21 NNIs (A) registered and (B) by crop use in Australia.

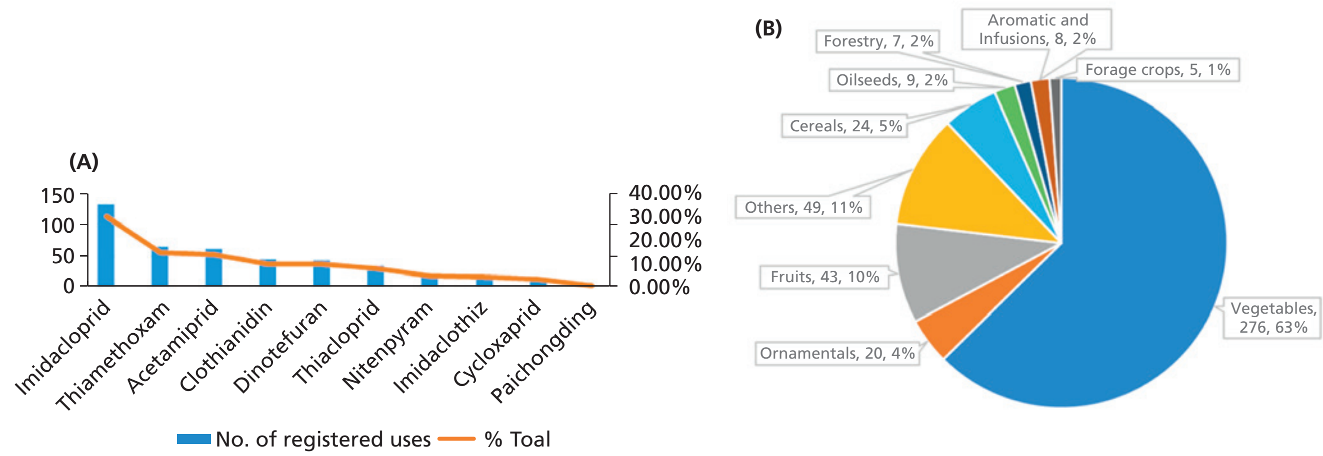


Figure 2.22 NNIs (A) registered and (B) by crop use in China.

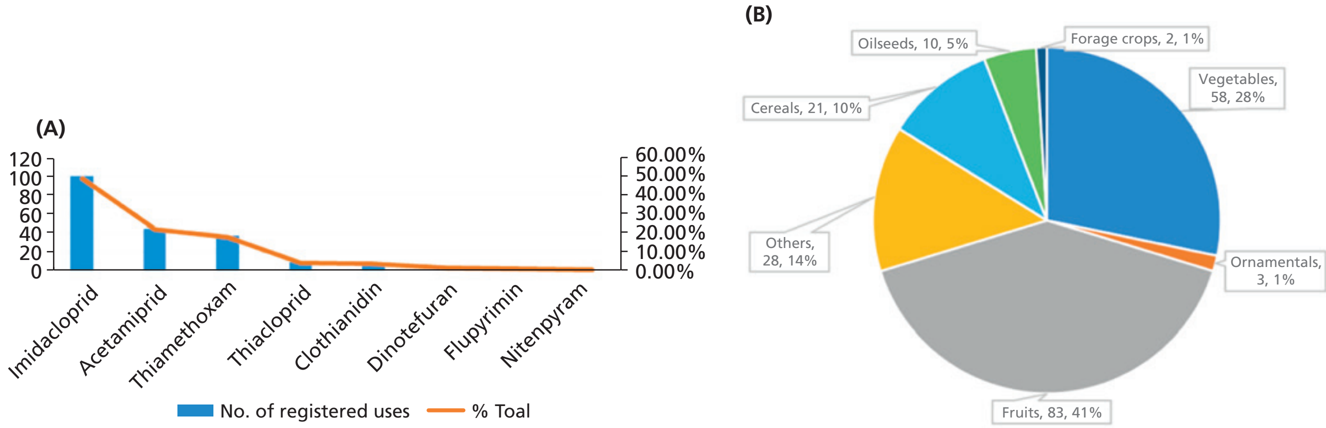


Figure 2.23 NNIs (A) registered and (B) by crop use in India.

Vegetables, fruits, and ornamentals are the top three crops in terms of the number of registered NNI uses, amounting to almost 70%. Unlike other countries where the highest registered uses are in vegetables, more than 40% of the registered uses in India are in fruits (Figure 2.23B).

2.4 Method of application of neonicotinoids in crop use

On the basis of currently registered applications for different crop uses in the Asia-Pacific region, spray/aerial treatment is the predominant method, accounting for 74%, while seed treatment accounts for only 7% (Homologa) (Figure 2.24).

Further examination of the top three countries in the Asia-Pacific region in terms of the number of registered crop uses, namely Japan, Taiwan, and South Korea, provides additional understanding of how NNIs are being applied in crops.

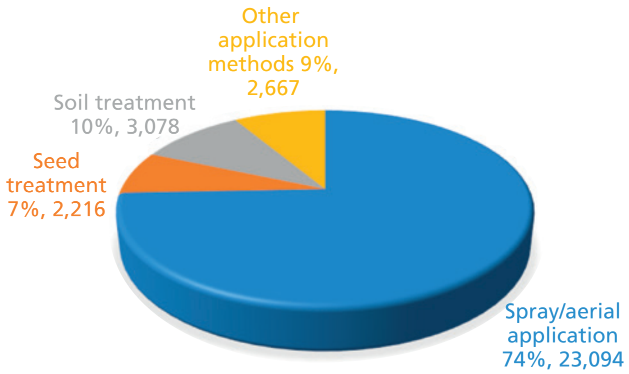


Figure 2.24 NNI registered applications in the Asia-Pacific region.

On the basis of sales data collected by the Japan Crop Protection Association, about 90% of NNIs have been consistently distributed for the past 15 years for application as a foliar spray for different crop uses. Application of NNIs as seed treatment was less than 5% (Figure 2.25).

In Taiwan, as noted in the National Pesticide Registration Record by the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, the principal method of application for NNI products is foliar . There are no registered applications of NNI products through seed treatment in Taiwan. Among all the registered applications of NNI products in Taiwan, some products can also be used for seedling boxes.

Similarly in South Korea, 80% of the registered crop uses are by foliar application (Korea Crop Protection Association) (Figure 2.26). Seed treatment by NNIs is negligible.

2.5 Conclusion

The Asia-Pacific region is heterogeneous for climate, agricultural environments, and cultivated crops. Meanwhile, it has a high diversity of pollinators that provide ecosystem services including crop pollination. Over the past 30 years, crop yield in the region has steadily increased, while the use of insecticides that

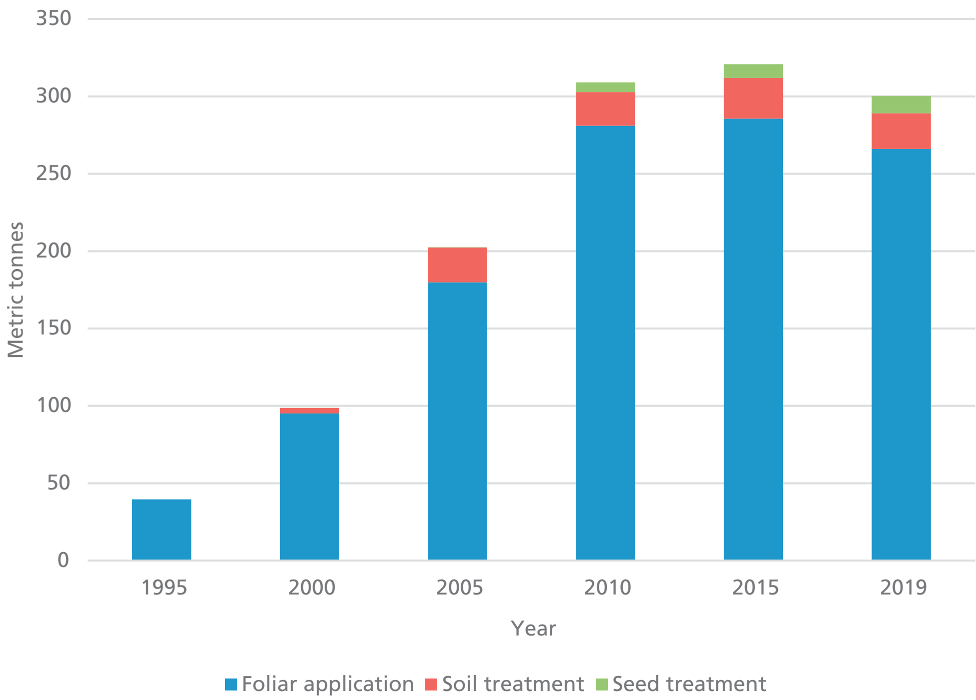


Figure 2.25 Methods of NNI application for crop use in Japan.

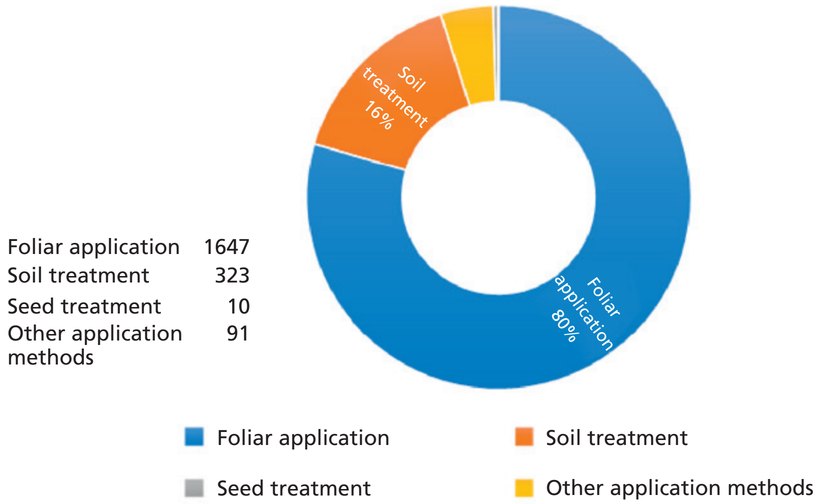


Figure 2.26 Registered crop use application of NNIs in South Korea.

potentially affect beneficial organisms has been relatively stable. This trend in use in Asia may lie in the phase-out of highly toxic and broad-spectrum pesticides, and the development of new products in terms of pesticide resistance management. However, the proportion of NNIs in insecticide use is expected to increase, as seen in South Korea and Japan, although there is high uncertainty owing to the lack of data.

NNIs have been applied to all kinds of crops in the Asia-Pacific region, but the proportions of registered items based on applicable crop types are quite different by country. This, combined with the heterogeneous environment of Asia, means it is necessary to perform a risk assessment of NNIs that considers each country's agricultural environments such as crops, pests, farming practices, and habitat characteristics in order to maintain biodiversity and crop productivity in the region. Neonicotinoid impact by seed coating treatment might be relatively low in Asia compared with North America and Europe. However, application for outdoor use, particularly during the flowering period, is cautioned because of the high possibility of exposure forager honeybees and other pollinator insects, regardless of the application method. In major rice-producing countries, it is important to be aware of the environmental impact of NNIs in water systems from rice paddy fields, because of these insecticides' high water solubility.

3 Molecular mechanisms of negative impacts of neonicotinoids on honeybees

Chapter summary

Extensive use of neonicotinoid insecticides (NNIs) in agriculture, particularly for seed dressing, has been proposed as a possible cause of the colony collapse disorder of honeybees. Colony collapse disorder has been officially reported in the USA, Canada, and European countries, but not in Asian countries. In an attempt to evaluate the risk of NNIs as a potential cause of colony collapse disorder in Asian countries, we have reviewed the literature about the negative impacts of NNIs on honeybees and summarised it on the basis of two possible exposure scenarios (acute versus chronic exposure). NNIs can affect honeybees by impairing diverse aspects of physiology, including learning and memory, metabolism, immunity, foraging activity, flight performance, development, etc. Numerous studies suggest that NNIs possess chronic hazards to honeybees that can be linked to colony collapse disorder under both exposure scenarios. Assuming little difference in the NNI hazard level between different geographical regions, however, the potential risk of NNIs to honeybees in a particular region is probably determined by the chronic exposure route present in the agroecosystem. With this in mind, the absence of such chronic exposure routes in Asian countries may currently reduce the risk of NNIs substantially.

3.1 Introduction

Neonicotinoid insecticides (NNIs) are a class of pesticide categorised as the competitive modulators of nicotinic acetylcholine receptors (nAChRs) (Insecticide Resistance Action Committee, MOA 4A). NNIs compete with the excitatory neurotransmitter acetylcholine (ACh) for binding to nAChR in the cholinergic nervous systems of vertebrates and arthropods, causing over-excitation depending on dose. Whereas low doses of NNIs can stimulate synaptic transmission, over-excitation of the cholinergic nervous system by high doses of NNIs causes synaptic block, resulting in acute paralysis and eventual death (Yamamoto 1999). In addition, because nAChR is distributed not only in neuronal but also in non-neuronal tissues of insects, particularly of honeybees (Pamminger *et al.* 2017), NNIs can potentially disrupt various cellular signalling cascades mediated by ACh in non-neuronal tissues.

Since NNIs have low mammalian toxicity and are highly selective to insects (Wood and Goulson 2017), they have rapidly replaced the predecessor groups of insecticides such as organophosphates, carbamates, and pyrethroids in the market. NNIs are relatively more water-soluble and highly systemic than other groups of contact insecticides, enabling the rapid adsorption and translocation of treated NNIs inside the plant (Sur and Stork 2003). Owing to their systemic properties, NNIs can be treated by various means including not only the typical foliar spray but also seed dressing (coating), soil drenching, and trunk injection, which are particularly effective against sucking pests (Elbert *et al.* 2008) On the basis of these advantages, NNIs have been the most broadly used class of insecticides worldwide, with seed dressing being the primary application method. Following seed treatment, substantial amounts of NNIs are also taken up by wildflowers growing near arable land and translocated into the pollen and nectar at concentrations that are sometimes even higher than those found in crops (Botías *et al.* 2015).

Following extensive use of NNIs in pest control, numerous studies have reported the negative impacts of NNIs on honeybees and other pollinators since the mid-2000s (reviewed by Lu *et al.* 2020). In addition, possible connections between the negative impacts of NNIs and colony collapse disorder have been proposed (reviewed by LaLone *et al.* 2017). The wide implementation of NNI seed dressing in agriculture has established a unique chronic exposure route to pollinators, in particular to honeybees (*Apis mellifera*) and bumblebees. Translocated NNIs from dressed seeds can eventually reach and thus contaminate the nectar and pollen when treated crops grow to the flowering stage. Once the contaminated nectars and pollens are collected and brought to the colony by forager bees, the entire colony including developing brood is exposed to the NNI-contaminated diet. Although the detected concentrations of NNIs in the nectar and pollen in the field are extremely low, with typical ranges of 2–20 parts per billion (p.p.b.) in the case of imidacloprid (Schmuck *et al.* 2001; Tosi *et al.* 2018; Wen *et al.* 2021), chronic exposure over entire developmental stages probably results in adverse impacts on the honeybee colony, as demonstrated in many studies simulating natural exposure scenarios (Dively *et al.* 2015; Chambers *et al.* 2019; Colin *et al.* 2019; Kim *et al.* 2022).

Many studies on the adverse impacts of NNIs on honeybees and other pollinators have been criticised because their experimental design did not reflect actual exposure. In most studies reporting the negative impacts of NNIs, higher doses of NNIs rather than field-realistic ones were used without considering the typical chronic exposure route formed through NNI-contaminated nectar and pollen, thereby not simulating natural exposure routes in agroecosystems.

Nevertheless, exposure scenarios to sublethal doses of NNIs higher than field-realistic ones are still possible if one assumes possible routes through foliar residues and contaminated water, although these exposure routes are more probably acute and temporary. In this review, therefore, we categorise the possible adverse effects, with a particular focus on their molecular mechanisms, on the basis of the two possible exposure scenarios: (1) acute exposure to sublethal doses of NNIs and (2) chronic exposure to field-realistic doses of NNIs through contaminated diet. When substantially higher sublethal doses than the field-realistic ones were used in a toxicity assessment, the observed negative effects were defined as the acute sublethal hazard level, which can result from accidental exposure to the foliar residues of NNI or NNI-contaminated water in the natural setting. In contrast, when the negative impacts were evaluated using extremely low NNI doses over longer periods of exposure, we assumed the observed hazard level as the outcome of diet-mediated chronic exposure to NNIs.

3.2 Components of the cholinergic system and their roles in honeybees

The term 'cholinergic system' indicates any system in which ACh is used as a signalling molecule. Components of cholinergic systems include ACh, ACh-synthesising choline acetyltransferase (ChAT), ACh-hydrolysing acetylcholinesterase (AChE), and nAChR and muscarinic ACh receptor (mAChR) which use ACh as a ligand. It is known that the cholinergic system of insects is confined to the central nervous system, whereas that of mammals is located in both the peripheral and central nervous systems (Yamamoto 1999). As the insecticidal activity of NNIs is primarily conferred by blocking synaptic transmission in cholinergic neurons in the central nervous system, the presence and function of a cholinergic system in a non-neuronal system has been neglected. In recent years, however, evidence supporting the presence of a cholinergic system in non-neuronal tissues of honeybees has indicated possible adverse effects of NNIs on physiological metabolism in these tissues as well (Kim *et al.* 2012; Wessler *et al.* 2016; Pamminger *et al.* 2017).

3.2.1 Acetylcholine-hydrolysing acetylcholinesterase

Acetylcholine-hydrolysing acetylcholinesterase (AChE), which is primarily found in the postsynaptic membrane, hydrolyses ACh into acetic acid and choline to terminate synaptic transmission. The change of AChE activity in the brain modulates learning and memory, and the labour division of honeybees (Gauthier *et al.* 1992; Shapira *et al.* 2001). There are two paralogous AChEs, AmAChE1 and AmAChE2, in honeybees. AmAChE2 is a typical AChE involved in synaptic transmission in that it shows high catalytic activity, neuronal distribution (brain and ganglia), membrane-binding property, and is constitutively expressed. In contrast, AmAChE1 has a significantly lower catalytic activity (i.e., 2,500-fold lower than AmAChE2), exists as a soluble form, and is expressed in both neuronal and non-neuronal tissues (Kim *et al.* 2012). The expression of AmAChE1 is inducible by brood rearing suppression, heat shock, and crowding, all of which could be a serious threat to honeybee colonies (Kim *et al.* 2017; 2019).

3.2.2 Nicotinic acetylcholine receptor

Nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel made up of homo- or hetero-pentameric subunits. Binding of a chemical messenger, such as ACh and nicotine, opens the nAChR channel pore leading to influx of cations such as sodium and calcium ions, which causes depolarisation of the plasma membrane and generates the action potential in postsynaptic neurons (Hogg *et al.* 2003). There are nine α-subunits and two β-subunits in honeybee nAChR (Jones *et al.* 2006). The transcription levels of each subunit differ depending on the organ, with the expression levels of α7-, α2-, and β1-subunits being high in the brain whereas those of α9- and β2-subunits are high in the fat body for instance (Pamminger *et al.* 2017). nAChRs are found in the optic lobes, antennal lobes, mushroom bodies, vertical lobes and medial lobes in the brain, and suboesophageal ganglion (Gauthier and Grünewald 2012). Cultured Kenyon cells from the mushroom bodies, the main area for olfactory learning and memory of honeybees (Hammer and Menzel 1995), were induced to generate inward currents by ACh and nicotine but not by oxotremorine, the muscarinic agonist, indicating the presence only of functional nAChRs in Kenyon cells (Goldberg *et al.* 1999). The injection of nicotinic antagonists, mecamylamine, α-bungarotoxin, or methyllycaconitine, into the brain impaired the performance of learning and memory (Lozano *et al.* 1996; 2001; Dacher *et al.* 2005; Gauthier *et al.* 2006). Different subunits of nAChRs seem to be in charge of different timescale memory acquisition: medium-term memory was inhibited by mecamylamine, contrary to long-term memory inhibition by α-bungarotoxin, each of which act on different subunits of nAChR (Kempsill and Pratt 2000).

3.2.3 Muscarinic acetylcholine receptor

Muscarinic acetylcholine receptor (mAChR) is a G-protein-coupled receptor, to which ligand binding triggers several intracellular signalling cascades through the activation of G protein (Haga 2013). There are currently two annotated mAChRs in honeybees, GAR-2 and DM1, but their properties and functions are not clearly understood. The activation of mAChR is involved in neuropile growth of worker bees following foraging activity (Ismail *et al.* 2006; Dobrin *et al.* 2011), nestmate recognition (Ismail *et al.* 2008), and memory retrieval in worker bees (Lozano and Gauthier 1998). NNIs do not directly work as agonists of mAChR (Buckingham *et al.* 1997). However, diverse functional roles of nAChR and mAChR are interconnected (Green *et al.* 2005; Ellis *et al.* 2006; Grilli *et al.* 2009), and excitation and inhibition of the receptors can be regulated by each other (Marchi and Grilli 2010), suggesting the possibility of mAChR dysfunction following NNI exposure.

3.2.4 Acetylcholine-synthesising choline acetyltransferase

Acetylcholine-synthesising choline acetyltransferase (ChAT) is a transferase enzyme synthesising ACh. In honeybees, it is abundantly expressed in the brain, moderately expressed in haemocytes, and little is expressed in fat body and the midgut (Pamminger *et al.* 2017). It was also reported that ChAT is present in hypopharyngeal glands, the exocrine glands producing major royal jelly proteins, with millimolar concentrations of non-neuronal ACh secreted into royal jelly, which is related to the development of brood (Wessler *et al.* 2016).

3.3 Mode of action, detoxification mechanism, and selective toxicity of neonicotinoids

NNIs work as agonists on nAChRs (Yamamoto 1999). Contrary to nAChR activation and subsequent excitation of neurons by low concentrations of NNIs, the continuous excitation of neurons through exposure to high concentrations of NNIs leads to paralysis and death of organisms. The binding affinities of NNIs to nAChRs differ depending on the types of NNI and the subunit compositions of nAChRs (Simon-Delso *et al.* 2015). It is known that imidacloprid and thiacloprid are partial agonists, whereas acetamiprid, dinotefuran, nitenpyram, and clothianidin are more effective agonists to nAChRs (Tan *et al.* 2007). α-Bungarotoxin is a commonly used nicotinic antagonist for the binding affinity assay of nicotinic agonists as it competitively binds to and irreversibly inhibits nAChRs (Morley *et al.* 1979). In the nAChRs of insect species, there can be high- and low-affinity binding sites for α-bungarotoxin (e.g. both in *Locusta migratoria* nAChR, only high-affinity binding sites in *Myzus persicae* nAChR, and only low-affinity binding sites in *Drosophila melanogaster* and *Acyrthosiphon pisum* nAChRs) (Taillebois *et al.* 2018), but only one specific binding site has been found in honeybee nAChRs so far (Eldefrawi 1995; Nauen *et al.* 2001).

In honeybees, NNIs are rapidly detoxified into metabolites. The half-life of imidacloprid ranges between 4.5 and 5 hours (Suchail *et al.* 2004). Both phase I and II metabolisms are involved in NNI detoxification of honeybees. The detoxification of xenobiotics by cytochrome P450s (CYP) in honeybees is largely dependent on the CYP3 clan (CYP6 and CYP9 families) (Berenbaum and Johnson 2015). Several CYPs belonging to the CYP6 and CYP9 families were induced in honeybee larvae following imidacloprid exposure (Derecka *et al.* 2013). Exposure to nicotine, the natural compound having a similar structure to NNIs, significantly upregulated the transcriptions of two glutathione *S*-transferases which belong to phase II detoxification (Rand *et al.* 2015). Thiamethoxam exposure altered the activities of carboxylesterase, glutathione *S*-transferases, catalase, and alkaline phosphatase in honeybees, suggesting their possible role in NNI detoxifications (Badiou-Bénéteau *et al.* 2012).

The global use of NNIs for pest control results from the high selective toxicity of NNIs to insects. The difference in binding affinities of NNIs between mammalian and insect nAChRs is one of the factors making NNIs selectively high toxic to insects (Tomizawa and Casida 2003) along with differences in activation/detoxification metabolism and possibly permeability through the blood–brain barrier (Yamamoto *et al.* 1995; Tomizawa and Casida 1999; Tomizawa and Casida 2005). These NNIs, however, exhibit differential toxicities to honeybees. NNIs belonging to the nitroguanidine group, such as imidacloprid, clothianidin, thiamethoxam, and dinotefuran, were 94- to 820-fold more toxic to honeybees than acetamiprid and thiacloprid, in the cyanoamidine group, in tests using LD_{50} (the dose lethal to 50% of animals tested) (Iwasa *et al.* 2004). This differential toxicity was significantly reduced by pretreatment with piperonyl butoxide, the general CYP inhibitor, suggesting that detoxification through CYPs confers the selective toxicity. Manjon *et al.* (2018) revealed that the CYP9Q subfamilies (CYP9Q1, CYP9Q2, and CYP9Q3), which primarily metabolise NNIs, showed differential detoxification capabilities towards NNIs depending on structure: for instance, imidacloprid is poorly degraded but thiacloprid is efficiently degraded by CYP9Q3. This explains the differential toxicity by their different detoxification mechanisms, but not by their differential binding affinities to nAChRs.

3.4 Exposure routes of neonicotinoids to honeybees

The exposure routes of NNIs can largely vary depending on the application method (Fischer and Moriarty 2011). Owing to the systemic property of NNIs, seed dressing establishes the primary chronic exposure route to honeybees through the guttation fluid or honeydew on leaves, nectar, and pollen. Since queens, drones, and brood can be subsequently exposed through processed pollen and processed nectar, this systemic exposure route can affect the entire colony (Fairbrother *et al.* 2014), which affects both worker bees and larvae. Other application methods, such as foliar spray, soil drenching, and trunk injection, also form exposure routes to nectar and pollen although the exposure is more likely intermittent. In contrast, NNIs treated with

Table 3.1 Potential exposure routes and their exposure targets of honeybees (reproduced and modified from Fischer and Moriarty (2011))

Source of neonicotinoid contamination	Exposure type	Exposure route	Exposure target (honeybee developmental stage)	
			Worker	Brood
Seed dressing (primary)	Chronic	Nectar	+++[a]	+
		Pollen	+ to +++	++
Foliar spray or dust drift from treated seeds	Acute to subchronic	Drinking water[b]	+ to +++	+
Foliar spray	Acute to subchronic	Foliar residue	+++	–
	Acute	Direct contact to spray	+++	–

[a] + to +++: low to high probability; –: little probability.
[b] Includes guttation fluid, honeydew, and other water sources containing residues such as damp soil or puddles.

foliar spray can be deposited on flowering crops and wild plants, thus forming a direct exposure route to forager bees. Foliar-sprayed or seed-dressed NNIs can contaminate water systems, in which the contaminated water can be exposed to honeybee adults and larvae. Exposure of adult bees to direct spray is also possible but the effect is rather lethal, causing acute poisoning. The potential exposure routes and their exposure targets are summarised in Table 3.1.

3.5 Adverse effects of neonicotinoids on honeybees and underlying molecular mechanisms

A wide variety of experimental designs in terms of the treatment method, duration, and dose of NNIs have been used for assessing the potential hazard and risk of NNIs to honeybees. Depending on the exposure duration and dose of NNIs, the exposure of honeybees to NNIs can be divided into two scenarios regardless of experimental setting (i.e. laboratory, semi-field or field): acute exposure to sublethal doses of NNIs and chronic exposure to field-realistic doses of NNIs. In this review, we define 'acute exposure to sublethal doses' as the single time exposure through diet spiking or topical application to sublethal doses that typically range in levels of parts per million in oral administration or nanograms per bee in topical application without causing any apparent acute lethality. In contrast, 'chronic exposure to field-realistic doses' is defined as the constant exposure (single day to weeks) through NNI-treated diet with field-realistic doses that typically range within parts per billion levels.

3.5.1 Acute exposure of honeybees to sublethal doses of neonicotinoids

Learning and memory

As stated earlier in this chapter, the activation of nAChR is closely related to learning and memory (Gauthier and Grünewald 2012); thus, exposure to NNIs significantly affects the learning and memory abilities of honeybees. Exposure to 12 ng of imidacloprid per bee impaired medium-term olfactory memory and the proboscis extension reflex (Decourtye *et al.* 2004). A much lower dose, 0.21 ng of imidacloprid per bee, decreased the waggle dance circuit and increased the sucrose response threshold of honeybees (Eiri and Nieh 2012). In addition, imidacloprid at a dose of 7.5 ng per bee damaged the navigation memory of honeybees, which was also observed following treatments with 2.5 ng of clothianidin or 1.25 µg of thiacloprid per bee (Fischer *et al.* 2014). Thiacloprid at a dose of 200 ng per bee impaired learning and memory (Tison *et al.* 2017), and acetamiprid also increased antennal sensitivity at 1 µg per bee and damaged olfactory learning at 0.1 µg per bee (El Hassani *et al.* 2008).

Immune system

The adverse effects of NNIs on the immune system of honeybees have been reported. Topical application of 2–21 ng of clothianidin per bee reduced honeybee immune response, promoting viral load and the proliferation of *Varroa* mites (Di Prisco *et al.* 2013; Annoscia *et al.* 2020). Oral exposures for 24 hours to 200 µg/L thiacloprid, 1 µg/L imidacloprid, or 50 µg/L clothianidin reduced the haemocyte density, encapsulation response, and antimicrobial activity of honeybees (Brandt *et al.* 2016). In mammals, nAChR regulates immune pathways through cholinergic nerves or direct activation of immune-related cells (Cui and Li 2010; Kawashima *et al.* 2012; Jun *et al.* 2018). Considering the presence of nAChRs in haemocytes (Pamminger *et al.* 2017), the immune-related cells of honeybees might also be directly affected by NNI exposure. One of the mechanisms underlying immune suppression by NNIs is the negative modulation of NF-κB immune signalling, which deteriorates immune defence and leads to virus proliferation (Di Prisco *et al.* 2013).

Energy metabolism

NNIs can negatively affect mitochondrial respiration by altering mitochondrial Ca^{2+} homeostasis (Xu *et al.* 2022). The altered energy metabolism following acute exposure to NNIs seems to result from the malfunction of mitochondria. In mammals, nAChRs were reported to be present in the mitochondrial outer membrane, suggesting that Ca^{2+} homeostasis could be affected by NNI exposure (LaLone *et al.* 2017). Ca^{2+} accumulation in mitochondria activates ATP production by modulating the activities of enzymes related to the tricarboxylic acid cycle but excessive accumulation could lead to cell apoptosis (Rizzuto *et al.* 2012). Although the presence of nAChRs in insect mitochondria has not been reported, there have been several cases of mitochondrial dysfunction caused by NNI exposure in honeybees. Reduced mitochondrial activity was observed from isolated honeybee mitochondria after treatment with 50, 75, and 100 µM imidacloprid (Nicodemo *et al.* 2014). Acute oral administration of 0.2 ng of thiamethoxam per bee significantly increased thorax temperature following a heat shock (Tosi *et al.* 2016). Exposure to a low concentration of imidacloprid (5 µg/L) also increased cluster temperature (Meikle *et al.* 2018).

Foraging activity

Even a single exposure to field-realistic doses of NNIs can negatively affect the foraging activity of forager bees. Honeybees exposed to more than 50 µg/L of imidacloprid showed delay in homing from foraging and had a problem in locating feeding sites when exposed to more than 1,200 µg/L of imidacloprid (Yang *et al.* 2008). When honeybees were fed 1.34 ng of thiamethoxam, their survival rate decreased and their failure rate at foraging increased (Henry *et al.* 2012). Similarly, imidacloprid exposure altered foraging activity at more than 17 p.p.b. and decreased avoidance from predators of honeybees at more than 34 p.p.b. (Tan *et al.* 2014). It was revealed by radio-frequency identification tracking that foraging activity of forager bees decreases by exposure to more than 0.5 ng of clothianidin per bee and 1.5 ng of imidacloprid per bee (Schneider *et al.* 2012). One of the reasons for the decreased foraging activity following NNI exposure is a direct impairment of flight ability. A single oral administration of thiamethoxam at a dose of 1.34 ng per bee significantly altered flight performance of forager bees (Tosi *et al.* 2017) and treatments of 2.18 ng of clothianidin per bee and 7.5 ng of dinotefuran per bee reduced the homing flight rate of foragers (Matsumoto 2013), which seemed to result from disturbed energy metabolism. Decreased learning and memory abilities caused by NNIs also affect failure of foraging activity (Dukas and Visscher 1994).

Development

The role of ACh in the development of honeybees and the adverse effects of NNIs on development was reviewed in detail by Grünewald and Siefert (2019). Briefly, disturbance of neuronal and non-neuronal nAChRs alters the endocrine system such as the titre of juvenile hormone and vitellogenin, which affects the metabolism and development of honeybees. Impaired development caused by NNIs mainly happens through chronic rather than acute exposures. Vitellogenin plays a crucial role in regulating the development of adult honeybees along with juvenile hormone (Amdam *et al.* 2012). Expression of vitellogenin significantly decreased following topical application of 0.02 p.p.m. imidacloprid (Chaimanee *et al.* 2016) whereas upregulations of vitellogenin were also observed after treatments with acetamiprid, clothianidin, imidacloprid, and thiamethoxam (Christen *et al.* 2016).

3.5.2 Chronic exposure of honeybees to field-realistic doses of neonicotinoids

Learning and memory

NNI-induced impairment of cognitive abilities, including learning, memory, olfaction, gustation, navigation, and orientation, can alter foraging performance, resulting in harmful consequences to colony survival. Meta-analysis of pesticide impacts on honeybee learning and memory revealed that chronic exposure produces more negative impacts on the memory ability than acute exposure, indicating the more apparent hazard of chronic exposure on honeybee memory (Siviter *et al.* 2018). Honeybees exposed to syrup containing 24 p.p.b. imidacloprid showed decreases in both the foraging activity on the food source and activity at the hive entrance as well as impaired olfactory learning in both semi-field and laboratory conditions (Decourtye *et al.* 2004). Chronic exposure to a field-realistic concentration of clothianidin (4 p.p.b.) impaired olfactory learning in the laboratory (Piiroinen and Goulson 2016). Reduction of cognition was observed from worker bees exposed to thiamethoxam only during the brood stage, implying that NNIs could have a long-term effect on honeybee learning and memory, possibly by affecting neuronal development (Peng and Yang 2016; Papach *et al.* 2017).

Metabolism

When subchronically treated with 2 p.p.b. imidacloprid in syrup for a period of 15 days, the exposed honeybees exhibited multifaceted physiological alterations, as indicated by the upregulation of genes associated with lipid–carbohydrate–mitochondrial metabolism, elevation of P450 genes, downregulation of genes in glycolytic and sugar-metabolising pathways, and downregulation of the environmentally responsive *Hsp90* gene (Derecka *et al.* 2013). This finding suggests that subchronic exposure to the field-realistic dose of imidacloprid can impair energy metabolism in adult pollinators. Along with energy metabolism, the carbohydrate metabolism and lipid content of forager bees were affected by chronic exposure to 5 and 20 p.p.b. imidacloprid in field conditions (Kim *et al.* 2022). Protein, lipid, glucose, and glycogen contents were changed following chronic oral administrations of low (5 p.p.b.) or high (50 p.p.b.) doses of clothianidin or imidacloprid (Cook 2019). Non-flight metabolic rate of forager bees increased following oral administration of 5 p.p.b. imidacloprid for 48 hours (Gooley and Gooley 2020). One possible reason for the metabolic changes following NNI exposures, especially for lipid and carbohydrate metabolism, was suggested as the development of insulin resistance. Endoplasmic reticulum stress induced by the membrane-depolarising property of NNIs or altered calcium signalling can induce insulin resistance (Ozcan and Tabas 2016; Wang *et al.* 2018; Park *et al.* 2022). Several findings (e.g. alteration of insulin signalling-related gene transcription levels, enriched insulin signalling pathway from transcriptome analysis, and phenotypic changes in physiology) have been proposed as the putative evidence for the development of insulin resistance in honeybees (Cook 2019; Gao *et al.* 2020; Kim *et al.* 2022).

Interactions with microbial pathogens

Nurse bees orally exposed to syrup containing 5.1 p.p.m. thiacloprid showed increased mortality if they were infected with *Nosema ceranae* compared with uninfected ones (Vidau *et al.* 2011). This result supports the hypothesis that the combination of *N. ceranae* prevalence with pesticide exposure may contribute to honeybee colony reduction. In another study where nurse bees chronically exposed to 5 and 20 p.p.b. imidacloprid were challenged with *Nosema* spp., the *Nosema* infection rate increased significantly compared with control bees, demonstrating an indirect effect of imdacloprid on pathogen growth in honeybees (Pettis *et al.* 2012). Genes related to immune response, detoxification, and oxidation–reduction were confirmed by quantitative polymerase chain reaction to be affected while workers were treated with sublethal imidacloprid (Chaimanee *et al.* 2016; Alburaki *et al.* 2017; De Smet *et al.* 2017; Gregorc *et al.* 2018) On the basis of this finding, it can be suggested that interactions between imidacloprid and pathogens serves as a major contributor to the increased mortality of honeybee colonies, and eventually to colony collapse disorder. In a similar study to reveal the potential interactions between microbial pathogens and an NNI, field-realistic doses of thiacloprid were treated individually to larval (0.1 mg/kg) and adult (5 mg/L) honeybees along with infection by two common microbial pathogens, *Nosema ceranae* and black queen cell virus (Doublet *et al.* 2015). An additive interaction between black queen cell virus and thiacloprid was found as judged by decreased host larval survival, suggesting the elevated viral loads were due to thiacloprid. In adult bees, a synergistic interaction was also observed between *N. ceranae* and thiacloprid. A similar synergistic interaction was also reported between chronically exposed sublethal doses of imidacloprid and the microbial pathogens *N. ceranae* and deformed wing virus in Africanised honeybees (Balbuena *et al.* 2022). In this study, imidacloprid affected the expression of some genes associated with immunity, resulting in an altered physiological state, and caused significant changes in gut microbiota when administered to bees infected with *N. ceranae*.

Colony performance

Honeybee colonies chronically exposed to field-realistic concentrations of thiamethoxam (5 p.p.b.) and clothianidin (2 p.p.b.) over two brood cycles exhibited decreased performance in the short-term colony such as declining numbers of adult bees and brood as well as reductions in honey production and pollen collections (Sandrock *et al.* 2014). The NNI-exposed colonies recovered in the medium term and overwintered successfully but exhibited significantly slowed growth during the following spring, probably because of queen failure, further supporting previously documented long-term impacts of NNIs on queen supersedure. Taken together, this study also demonstrates the short- and long-term negative impacts of NNIs on colony performance and queen fate, thus contributing to colony failure in a complex manner.

Flight performance

Prolonged exposure (24 hours) to field-relevant doses of four NNIs (imidacloprid, thiamethoxam, clothianidin, dinotefuran) and the plant toxin nicotine was reported to affect basic motor function and postural control in forager bees (Williamson *et al.* 2014). This finding illustrates that exposure for 24 hours to sublethal doses of NNIs probably influences behaviour of honeybees that can eventually affect normal function in a field setting. Forager bees showed a concentration-dependent decrease of flight performance following treatments of 5, 20, and 100 p.p.b. imidacloprid, resulting in a significant difference between control and 100 p.p.b.-treated groups (Kim *et al.* 2022). The reduction of flight performance is likely to be a consequence of the combination of altered energy metabolism, mitochondrial dysfunction (Lu *et al.* 2020), and immature flight muscles due to the precocious onset of foraging (Colin *et al.* 2019).

Foraging activity

When chronically exposed to thiacloprid, widely conceived as less toxic to honeybees, in the field for several weeks at a sublethal concentration, bees exhibited impaired foraging behaviour, homing success, navigation performance, and social communication (Tison *et al.* 2016). In a study using radio-frequency identification tags to track honeybees' lifetime flight behaviour, bees exposed to 5 p.p.b. imidacloprid in sugar syrup during their larval stage showed precocious onset of foraging, fewer orientation flights, and reduced their lifetime foraging flights (Colin *et al.* 2019). Transcriptomic approaches suggested that phototransduction, behaviour, and somatic muscle development were also affected (Wu *et al.* 2017). These results suggest the potential of NNIs in disturbing the normal age-based labour division in a colony, thereby impairing foraging efficiency. Interestingly, colonies chronically exposed to 100 p.p.b. imidacloprid showed higher honey storage than control colonies, which might result from the avoidance to imidacloprid-containing honey or reduced energy metabolism as shown in bees treated with 100 p.p.b. imidacloprid (Dively *et al.* 2015; Kim *et al.* 2022).

Development

Chronic exposure to clothianidin and thiacloprid can affect larval development both through the direct impacts on larvae and through the indirect effects mediated by the increased feeding time of nurse bees (Siefert *et al.* 2020). Honeybee queens from colonies treated with 4 p.p.b. thiamethoxam and 1 p.p.b. clothianidin for 36 days showed an increased number of ovarioles but a compromised physiology such as a reduced quality and quantity of spermathecal-stored sperm, probably related to reduced queen success (Williams *et al.* 2015). Newly emerged bees and nurse bees showed a concentration-dependent decrease in body weight after treatments of 5, 20, and 100 p.p.b. imidacloprid (Kim *et al.* 2022). The underlying molecular mechanisms for the adverse effects of NNIs on honeybee development have recently been reviewed (Chen *et al.* 2021). Exposure to imidacloprid during the larval stage caused transcriptomic profile changes in larvae, pupae, and adults, and the function of affected genes varied by the ages of workers (Tesovnik *et al.* 2019; Chen *et al.* 2021a). Worker bees exposed to 1, 10, and 50 p.p.b. imidacloprid for 4 consecutive days during the larval stage can develop normally; yet, foraging-related transcription factors were found to be upregulated in 14-day-old workers, at the same age as nurse bees. Furthermore, the transcriptome of imidacloprid-treated workers was highly similar to that of 20-day-old controls, suggesting a precocious forager will be induced if workers are exposed to sublethal levels of imidacloprid during the larval stage (Chen *et al.* 2021a).

Genetic diversity

Queens reared in colonies exposed to field-realistic doses of NNIs (4 p.p.b. thiamethoxam and 1 p.p.b. clothianidin) showed a reduced mating frequency compared with control queens, suggesting that NNIs can reduce the genetic diversity of colonies by increasing intracolonial relatedness (Forfert *et al.* 2017). Since the reduction in genetic diversity among worker bees can negatively influence colony vitality, NNIs may have a cryptic effect on colony health by impairing the mating frequency of queens.

3.6 Concentration-dependent biphasic effects of neonicotinoids on honeybees

There have been several reports that some of the adverse effects on honeybees show biphasic patterns depending on the exposure concentration (Table 3.2). The survival rates of honeybees were greater in comparatively high doses than in low doses when treated with imidacloprid, clothianidin, and thiamethoxam (Suchail *et al.* 2000; Baines *et al.* 2017; Wong *et al.* 2018). Protein content increased and glucose content decreased following treatment with 5 p.p.b. clothianidin, but no change was observed after treatment with 50 p.p.b. clothianidin (Cook 2019). Food consumption also decreased after treatment with 5 p.p.b. imidacloprid but was not affected by 50 p.p.b. imidacloprid. Following chronic oral administrations of 5, 20, or 100 p.p.b. imidacloprid, body weight and lipid content decreased at low imidacloprid concentrations (5 and 20 p.p.b.), whereas they increased at a high imidacloprid concentration (100 p.p.b.) (Kim *et al.* 2022). Transcriptome analysis showed that energy metabolism was upregulated by treatment with a low concentration of imidacloprid but downregulated by treatment with a high concentration of it. There have been several other instances where the stimulation of energy metabolism only happened with NNI exposures at low concentrations. The non-flight metabolic rate and temperature of clustered honeybees increased in a group treated with 5 p.p.b. imidacloprid but they were constant following treatment with higher concentrations (20 and 100 p.p.b.) of it (Meikle *et al.* 2018; Gooley and Gooley 2020). In addition, the thorax temperature of honeybees increased following treatment with 0.2 ng of thiamethoxam per bee, whereas it decreased with treatment at 1 and 2 ng per bee (Tosi *et al.* 2016). Given that the activity of isolated honeybee mitochondria decreases in a concentration-dependent manner in imidacloprid treatments with concentration ranges of 0–100 μM (Nicodemo *et al.* 2014), the biphasic pattern shown in energy metabolism probably does not result from direct effects of NNIs on mitochondria.

The biphasic patterns observed in honeybees following NNI treatment may be attributed to the presence of different nAChR subunits along with the existence of two distinct binding sites (low versus high affinity) on each nAChR. The Kenyon cells in the honeybee brain, which contain the α2-, α8-, and β1-subunits of nAChR, show slow desensitisation by imidacloprid, whereas the antennal lobes, if they contain an additional α7-subunit, conversely undergo fast desensitisation by imidacloprid (Barbara *et al.* 2008; Dupuis *et al.* 2011). The compositions of nAChR subunits also differ between

Table 3.2 Concentration-dependent biphasic adverse effects of NNIs on honeybees

Index	Insecticide	Measurement method	Exposure route and duration	Observed pattern	Reference
Body weight	Imidacloprid	Direct measurement	Chronic oral administration	Decrease at 5 and 20 p.p.b. Increase at 100 p.p.b.	Kim *et al.* (2022)
Lipid content	Imidacloprid	Spectrophotometric method and transcriptome		Decrease at 5 and 20 p.p.b. Increase at 100 p.p.b.	
Energy metabolism	Imidacloprid	Transcriptome		Increase at 5 and 20 p.p.b. Decrease at 100 p.p.b.	
Insulin signalling pathway- and carbohydrate metabolism-related genes	Imidacloprid	qPCR		Significant changes at 5 and 20 p.p.b. No changes at 100 p.p.b.	
Longevity	Imidacloprid	Direct measurement	Chronic oral administration	Increase at 15 and 45 p.p.b. by phytochemicals Decrease at 105 and 135 p.p.b. by phytochemicals	Wong *et al.* (2018)
Mortality	Clothianidin Thiamethoxam	Observation	Acute oral administration	U-shaped mortality curve in dose-dependent manner	Baines *et al.* (2017)
Mortality	Imidacloprid	Observation	Topical treatment	U-shaped mortality curve at low concentration	Suchail *et al.* (2000)
Protein content	Clothianidin	Spectrophotometric method	Chronic oral administration for two weeks	Increase at 5 p.p.b. No change at 50 p.p.b.	Cook (2019)
Glucose content	Clothianidin			Decrease at 5 p.p.b. No change at 50 p.p.b.	
Food consumption	Imidacloprid	Direct measurement		Decrease at 5 p.p.b. No change at 50 p.p.b.	
Non-flight metabolic rate	Imidacloprid	CO_2 production rate	Oral administration for 48 hours	Increase at 5 p.p.b. No change at 20 p.p.b.	Gooley and Gooley (2020)
Thorax temperature	Thiamethoxam	Thermal camera	Acute oral administration	Increase at 0.2 ng/bee after cold shock Decrease at 1 and 2 ng/bee after cold shock	Tosi *et al.* (2016)
Cluster temperature	Imidacloprid	Temperature logger	Chronic oral administration	Increase at 5 p.p.b. No change at 100 p.p.b.	Meikle *et al.* (2018)

organs (Pamminger *et al.* 2017). As NNIs show different binding affinities to different subunit compositions of nAChRs (Ihara *et al.* 2003), the differential expression of nAChR subunits within and between organs might lead to biphasic responses to NNIs. Although the low-affinity binding site of nAChRs to imidacloprid has not been identified in honeybees so far, the presence of both low- and high-affinity binding sites has been reported in *M. persicae*, *Nilaparvata lugens*, *Aphis craccivora*, and *L. migratoria*, suggesting the possibility of the presence of a low-affinity binding site that leads to biphasic responses to NNIs (reviewed by Simon-Delso *et al.* 2015).

3.7 Factors that affect toxicities of neonicotinoids to honeybees

3.7.1 Age

Physiologies of honeybee workers change as they age. The oxidative stress responses, which reduce oxidative stress during detoxification of xenobiotics (El-Demerdash *et al.* 2018), decrease following age with decreasing expression of antioxidant genes such as vitellogenin, superoxide dismutase, and catalase (Amdam *et al.* 2012; Sagona *et al.* 2021). The damage from oxidative stress following flight activity is accumulated during ageing (Margotta *et al.* 2018). The cholinergic system of honeybees also changes. The ACh titre in the brain increases with age, and subtypes of nAChR change following ageing which leads to different responses against imidacloprid and its metabolites (Guez *et al.* 2003). With these changes, older bees showed higher sensitivities to imidacloprid than younger bees (Zhu *et al.* 2020).

3.7.2 Genetic background

The toxicities of NNIs can vary depending on differences in the genetic background between subspecies or within subspecies of honeybees. *Apis mellifera caucasica* showed higher sensitivity to contact exposure of imidacloprid than *A. m. mellifera* (Suchail *et al.* 2000). The toxicities of clothianidin, imidacloprid, and thiamethoxam varied among *A. m. mellifera*, *A. m. ligustica*, and *A. m. carnica*, and individual colonies with different genetic backgrounds within each subspecies also showed different sensitivities to the NNIs (Laurino *et al.* 2013). The toxicity comparison of imidacloprid and thiamethoxam among *A. m. carnica*, *A. m. ligustica*, and Russian honeybees showed different toxicities across subspecies, particularly with imidacloprid being 33-fold more toxic to *A. m. ligustica* than *A. m. carnica* (Rinkevich *et al.* 2015). The sensitivity differences in accordance with different genetic backgrounds seem to result from different body sizes and detoxification/immune responses as shown in the comparison of NNI toxicities between *A. mellifera* and *Apis cerana* (Li *et al.* 2017).

3.7.3 Temperature

It was previously reported that honeybees are more sensitive to imidacloprid and thiamethoxam at low temperature (24 °C) than at high temperature (35 °C) (Saleem *et al.* 2020). However, a larger-scale study with a wider temperature range and more diverse kinds of NNI needs to be done to generalise the idea that NNIs are more toxic to honeybees at lower temperature. Acetamiprid showed increasing toxicity to *Drosophila suzukii* in a temperature-dependent manner (Saeed *et al.* 2018), which was also reported in *Diaphorina citri* (Boina *et al.* 2009). However, *D. citri* showed biphasic mortalities to imidacloprid and thiamethoxam treatments, being low at 17 °C, high at 27 °C, and low again at 37 °C (Boina *et al.* 2009), suggesting that two temperature points are not enough to observe the influence of temperature on imidacloprid and thiamethoxam toxicities to honeybees.

3.8 Evaluation of neonicotinoid hazard versus risk in Asia

The potential threat from NNI seed coatings applied to flowering crops has been the subject of considerable debate. For a precise assessment of NNI risk under real-world agricultural conditions, large-scale field experiments considering different NNI formulations, land uses, and regional climates are needed. In a large-scale experiment crossing three European countries (Hungary, Germany, and the UK), the interannual viability of honeybee colonies following the winter period was affected by NNI seed treatment in a country-specific manner when using oilseed rape as a model commercial crop (Woodcock *et al.* 2017). The country-specific responses of honeybees were suggested to be due to differences in the use of oilseed rape as a foraging source for bees and the disease incidences in hives. Although the overall residues were extremely low (< 1.5 p.p.b.), chronic exposure to low levels of NNIs may reduce hive fitness which is affected by several interacting environmental factors that can be country- or region-specific.

No other comparative study focusing on the differential adverse effects of NNIs has been reported between different geographical regions, particularly between Europe/America where colony collapse disorder has been frequently documented and Asia where no official such disorder has been described. When the possible exposure potential of honeybees to NNIs was assessed on a global scale by analysing 198 honey samples across the world, at least one of five tested compounds (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam) was detected in 75% of all samples, two or more in 45% of samples, and four or five in 10% of samples (Mitchell *et al.* 2017). Interestingly, there was a subtle difference in the types of NNI detected in honey between continents (Mitchell *et al.* 2017). The three banned NNIs (thiamethoxam, imidaclroprid, and chlothianidin) from Europe were the top three NNIs detected in North America, whereas in South America they were imidaclorpid, acetamiprid, and thiamethoxam. In Europe, less toxic NNIs such as thiacloprid and acetamiprid were mainly detected, reflecting the banned status of the three toxic NNIs. Unlike America and Europe, Asia showed some different detection patterns, with acetamiprid occupying approximately 70% followed by imidacloprid, suggesting the qualitative difference in honeybee exposure to NNIs is probably through honey in Asia. The relatively lower levels of toxic NNIs in the honey collected from Asia also suggests that the use pattern of NNIs in Asia is different from other continents and the likelihood of NNI-induced negative impacts is lower in this region.

3.9 Conclusion

As reviewed and summarised in the main text, it is clear that NNIs pose a significant hazard to honeybees under both exposure scenarios by disturbing both neuronal and non-neuronal physiologies. Since there has been no apparent study demonstrating differences in the physiological and toxicological responses of honeybees to NNIs between different geographical regions, it is rational to assume that the potential risk of NNIs to honeybees in a particular region is not determined by differential hazard levels but mainly by the presence or absence of actual exposure routes in the agroecosystem, which is shaped by the cultivation crops, agricultural practices, and NNI application system. In Asian regions, including China, India, Australia, Japan, Korea, and Taiwan, most NNIs are largely foliar-sprayed for vegetables, ornamental crops, and fruit plants (see chapter 1), but are seldom used for commercial crops such as maize, soybean, oilseed rape, cotton, sunflower, etc., which are usually treated with NNIs through seed dressing in other regions such as America and Europe. Seed dressing of bee-pollinating crops with NNIs enables the systemic translocation of treated NNIs to the pollen and nectar and thus establishes the chronic exposure route to bee colonies through foraging activity. Therefore, the lack of such a chronic exposure route of NNIs in Asian countries probably reduces the risk of NNIs significantly at present. Nevertheless, any future changes in agroecosystems in different regions of Asia could affect the NNI exposure scenario, so the risk assessment and regulation of NNIs should be conducted on a regular basis by primarily examining any alteration to agricultural systems that can establish new routes of NNI exposure. Systematic analysis of NNI residues translocated to the nectar and pollen following foliar spraying at different developmental stages of crop plants would be also beneficial for more precise evaluation of possible exposure routes in bee-pollinating crop plants typically treated with NNIs by this method.

4 Environmental impacts of neonicotinoids in the Asia-Pacific region

Chapter summary

This chapter focuses on the research about the environmental characteristics and impacts of neonicotinoid insecticides (NNIs) in the Asia-Pacific region, excluding their impacts on pollinators, which are reviewed in chapter 4. The research output on environmental issues in this region has increased remarkably in the past decade, prompted by their widespread use in all kinds of crops and the awareness that these soluble and very toxic insecticides are highly mobile in the environment.

Monitoring their contamination in aquatic ecosystems and soils of agricultural regions constitutes about a third of this research, and studies on the fate, transport, persistence, and degradation another third. Together, they show that the physico-chemical properties of these chemicals explain their mobility in the environment, which occurs mainly through water run-off and leaching. While microbial degradation helps in removing part of the residue loads, many of the metabolites thus produced are still toxic and impact equally on organisms. Of concern is the pervasive contamination of drinking waters with multi-residues of these insecticides, which may affect human health as well.

Studies on the toxicity and toxicokinetics of NNIs to several aquatic taxa have highlighted that chronic exposures to low NNI levels are the main driver of their impacts on organisms. The ecological effects on aquatic and terrestrial ecosystems have been studied in detail using experimental mesocosms in various countries and complemented with some field studies. These highlight the wide range of effects of NNIs on aquatic insects and crustaceans. Specific impacts include the disappearance of *Sympetrum* dragonflies from rice fields in Japan and the general decline of macroinvertebrates in rivers. The latter declines have resulted in the collapse of the fishing industry in Lake Shinji, Japan, as the food resource of smelt and eels was reduced dramatically. Given that protective environmental thresholds are often exceeded, and since residue loads are on the increase in most regions, the impacts of NNIs on aquatic ecosystems will only exacerbate in the future unless their use is restricted.

4.1 Introduction

Concerns about the environmental impacts of neonicotinoid insecticides (NNIs) have attracted the attention of researchers worldwide, many years after these pesticides were first introduced to agricultural markets in the mid-1990s. The research output on environmental issues in the Asia-Pacific region has increased remarkably in the past decade, with China and Japan leading the way (Figure 4.1).

This chapter focuses on the available research about the environmental characteristics and impacts of NNIs in this region. A literature search among 14 countries using SCOPUS yielded 324 publications from 2015 until 2022, of which 112 (34%) related to environmental issues alone. Excluded from this list were papers that dealt with pest management and resistance, toxicity to target pests and non-target organisms, including bees and parasitic wasps, studies on food residues and human exposure, development of analytical methods, and removal of residues from surface waters. In addition to these, there were 27 other research papers on the topic published since 2005.

Given that the environmental aspects of NNIs have already been reviewed in recent years (Goulson 2013; van Lexmond *et al.* 2015; Bonmatin *et al.* 2021a), this chapter will deal only with the research on environmental issues coming from the Asia-Pacific region.

4.2 Environmental characteristics of neonicotinoids

4.2.1 Fate and transport

Sorption of imidacloprid and thiacloprid in soils in tropical Australia was not related to soil characteristics, such as organic carbon content, clay content, or pH; however, sorption was significantly correlated with the organic carbon in rice fields in the Philippines (Oliver *et al.* 2005). The dissipation of acetamiprid in soils amended with biochar (0.5% by mass) was retarded compared with that in soils without amendment, as biochar increased sorption of acetamiprid between 12% and 52%, depending on soil types (Yu *et al.* 2011). The same effect was observed with clothianidin, imidacloprid, and thiacloprid in soils amended with biochar (Zhang *et al.* 2018a). This effect of organic carbon on the sorption capacities is important, as it reduces leaching of these compounds through the soil profile. For instance, amendment of soils with humic acids and peat (0.5%) reduced leaching of paichongding by 17–42% (Xie *et al.* 2017).

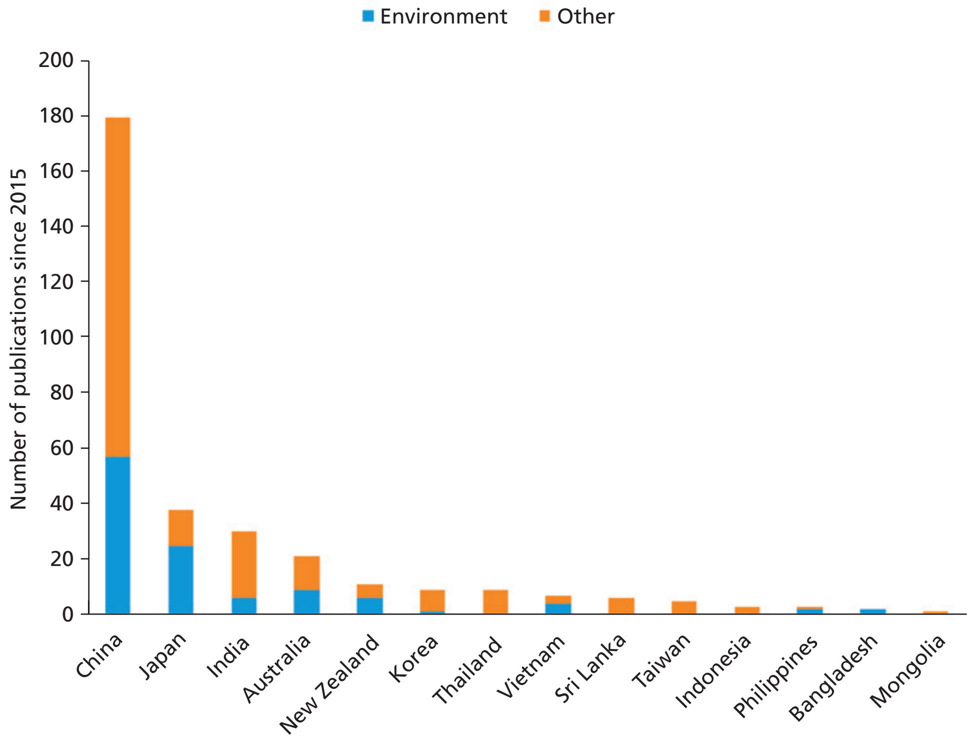


Figure 4.1 Research on NNIs in the Asia-Pacific region, with indication of the proportion of studies that focus on environmental issues (blue bars).

Mobility in paddy fields due to run-off and leaching depends greatly on the application method, namely whether at the nursing box before transplanting or at direct sowing (Thuyet *et al.* 2012a), since deposition and desorption rates are the major contributors to the variation of the predicted concentrations in paddy water and soil (Boulange *et al.* 2016; Zhang *et al.* 2018). Mobile compounds such as dinotefuran experience losses of 14–41% in the run-off from applied rice fields, thus contaminating nearby river waters (Yokoyama *et al.* 2015), whereas only 6% of imidacloprid applied to turf was washed off after rainfall events (Thuyet *et al.* 2012b). Modelling of outflows from paddy fields in northern Vietnam indicates that between 21% and 68% of imidacloprid applied goes into streams (La *et al.* 2015). In citrus orchards, clothianidin and imidacloprid losses in subsurface run-off accounted for up to 17% of total mass applied (Niu *et al.* 2022).

Leaching through the soil profile is a major pathway of NNI contamination in the environment. Since all NNIs are water soluble and stable in water (Table 4.1), leaching can be expected for the most mobile and persistent compounds (Thuyet *et al.* 2012a). For example, in experimental columns packed with different soil types, between 9% and 17% of paichongding was lost in the leachates (Xie *et al.* 2017), and about half of the clothianidin moved from the surface to the 5–25 cm soil layer after an equivalent 415 mm rainfall was applied, resulting in leachate concentrations up to 40 µg/L (Singh *et al.* 2018).

4.2.2 Persistence

All NNIs are stable in environmental waters, as this is an essential feature of systemic pesticides (Table 4.1). However, the nitroguanidine compounds undergo rapid photolysis in pure waters and produce several toxic metabolites (Simon-Delso *et al.* 2015), whereas acetamiprid and thiacloprid resist photodegradation (Chen *et al.* 2021).

Dissipation half-lives determined under field conditions represent the combined losses by crop plant uptake, soil biodegradation, leaching down the soil profile, and run-off after irrigation or rainfall events. Such dissipation does not mean the compounds are mineralised; rather, they are spread out in various environmental compartments. More accurate estimations of persistence in the environment are obtained in the laboratory, where half-lives in soil, water, or sediment are determined with the exclusion of other confounding factors.

Under laboratory conditions, residues of acetamiprid and thiacloprid in soil dissipated with half-lives of 25.1 and 19.1 days, respectively, under sunlight and slightly faster (11.1 and 12.8 days) under ultraviolet light (Gupta *et al.* 2008). Cycloxaprid also undergoes photolysis in

Table 4.1 Properties of NNIs relevant to environmental assessments*

	Acetamiprid	Clothianidin	Cycloxaprid	Dinotefuran	Imidacloprid	Imidaclothiz	Nitenpyram	Paichongding	Thiacloprid	Thiamethoxam
CAS RN	135410-20-7	210880-92-5	1203791-41-6	165252-70-0	138261-41-3	105843-36-5	150824-47-8	—	111988-49-9	153719-23-4
Molecular mass	222.7	249.0	322.8	202.2	255.7	261.7	270.7	366.9	252.7	291.7
Vapour pressure (Pa)	1.73×10^{-7}	2.8×10^{-11}	—	1.7×10^{-6}	4×10^{-10}	—	1.1×10^{-6}	—	3×10^{-10}	6.6×10^{-9}
Henry's law constant at 25 °C (Pa·m^3/mol)	5.3×10^{-8}	2.9×10^{-11}	—	8.7×10^{-9}	1.7×10^{-10}	—	3.54×10^{-13}	—	4.8×10^{-10}	4.7×10^{-10}
Water solubility (mg/L)	2,950	340	—	39,830	610	—	590,000	—	184	4,100
Dissociation (p*K*a)	0.7	11.1	—	12.6	NA	—	3.1	—	NA	NA
Log K_{OW}	0.8	0.905	—	–0.549	0.57	—	–0.66	—	1.26	–0.13
K_{OC} (L/kg)	200	123	—	26	328[a]	—	60	495–1629[b]	833[a]	56.2
GUS	0.94	3.74	—	4.85	3.69	—	2.01	—	1.1	3.58
BCF (L/kg)	—	—	—	—	0.61	—	—	—	—	—
Half-lives (days)										
Photolysis	34	0.1	—	0.2	0.2	—	—	—	Stable	2.7
Hydrolysis	Stable	Stable	—	Stable	Stable	—	Stable	—	Stable	Stable
Soil	2–20	277–1386	—	50–100	104–228	7 to >25[d]	8–15	365–433[e]	6–17	7–72
Water-sediment	—	56	5–7[c]	—	129	—	—	—	15	40
Comments	Low leachability	High leachability; persistent		Mobile; high leachability; moderately persistent	High leachability; persistent		Mobile; likely leaching	Leaching (b)	Low leachability	Mobile; high leachability

* Sources: http://sitem.herts.ac.uk/aeru/iupac/atoz.htm.
[a] Oliver *et al.* 2005.
[b] Xie *et al.* 2017.
[c] Cheng *et al.* 2022.
[d] Wu *et al.* 2010; Liu *et al.* 2011.
[e] Cai *et al.* 2016a.
Abbreviations: BCF, bioconcentration factor; CAS RN, Chemical Abstracts Service registry number; GUS, groundwater ubiquity score; K_{OW}, octanol–water partition coefficient; K_{OC}, adsorption coefficient; NA, not applicable; —, not available.

aqueous solutions under intense ultraviolet radiation (Hou *et al.* 2017).

Soil dissipation half-lives of clothianidin applied in granules to cabbage fields were determined from 4 to 10 days in three different Chinese provinces, and were not influenced by the application of manure, organic, and urea fertilisers (Zhang *et al.* 2018b). Similar dissipation half-lives (7–13 days) were determined in tomato fields from another three Chinese provinces (Li *et al.* 2012). However, longer soil half-lives (47–79 days) were determined for clothianidin in non-sterile soils incubated in the laboratory (Zhang *et al.* 2018c).

Dissipation half-lives of cycloxaprid in aerobic water-sediments have been estimated at 5–7 days (Cheng *et al.* 2022). Dissipation half-lives of dinotefuran were determined as 5.4 and 12 days in paddy water and soil, respectively (Yokoyama *et al.* 2015).

After application in nursery boxes and subsequent application to a rice field, dissipation half-lives of imidacloprid in paddy water were estimated as 1 or 2 days (Phong *et al.* 2009; Thuyet *et al.* 2011) and in surface soils as 12.5 days, with less than 0.1% lost in run-off (Phong *et al.* 2009). However, half-lives of 36–87 days were determined in four different soil types under laboratory conditions (Zhang *et al.* 2018c) and in rice paddy mesocosms (Sánchez-Bayo and Goka 2006a). In mushroom cultivation, dissipation half-lives of imidacloprid in casing soil and compost were estimated as 59–65 and 6–7 days, respectively (Zhang *et al.* 2020a).

Dissipation half-lives of imidaclothiz in soil from tea plantations were determined at 7.2 days for standard application rates and 18.2 days for double rates (Wu *et al.* 2020). However, under laboratory conditions, the estimated half-life in aerobic soil was more than 25 days (Liu *et al.* 2011).

Half-lives of paichongding in two loamy soils were in the range 90–173 days owing to biodegradation under aerobic conditions, whereas in sterile soils the novel compound was persistent, with half-lives in the range 365–433 days (Cai *et al.* 2016a).

Half-lives of thiacloprid in three types of soil varied between 9 and 24 days under aerobic conditions and 39–346 days under anaerobic conditions (Chen *et al.* 2021). The estimated half-life of thiamethoxam in soil of chive crops was 27.5 days, although the chemical was detected in soil for 210 days (Zhang *et al.* 2016), in agreement with the period of 26–45 days determined in four Chinese soil types, and 12–26 days in four Indian soils under laboratory conditions (Karmakar *et al.* 2006; Zhang *et al.* 2018b).

In greenhouse soils of Shouguang, east China, higher residues of seven NNIs were found in tomato and cucumber fields that had been treated for 8–9 years than in those that were cultivated for 2 years. This indicates a cumulative load of residues over time for most compounds, mainly imidacloprid, thiamethoxam, acetamiprid, and clothianidin in tomatoes (medians 1.89, 0.67, 0.46, and 0.18 µg/kg soil, respectively), and imidacloprid and dinotefuran (medians 0.46 and 0.30 µg/kg soil, respectively) in cucumbers (Wu *et al.* 2020). Evidence of residue accumulation in soils was obtained in citrus orchards after 10 and 20 years of cultivation, where total NNI loads were up to 25 µg/kg soil. At the same time, five NNIs migrated deeper into the soil profiles in orchards with a longer time since cultivation, particularly imidacloprid and thiamethoxam (Zheng *et al.* 2022).

4.2.3 Degradation

Degradation of NNIs in soils occurs mainly through nitrate reduction, cyano hydrolysis, and chloropyridinyl dechlorination (Zhang *et al.* 2018c), which results in the production of several metabolites, many of which are as toxic as the parent compounds (Cheng *et al.* 2022). The degradation of NNIs influences the soil nitrifying process, fostering Proteobacteria and Actinobacteria that are able to degrade these compounds (Zhang *et al.* 2018c).

The chemical degradation of NNIs seems to be mainly affected by pH and the cation exchange capacity of soils, rather than organic carbon content, as degradation of clothianidin, imidacloprid, and thiamethoxam was greatest in soils with the highest organic carbon. Abiotic removals of these three compounds after 60 days were in the range 32–40%, whereas biodegradation increased that proportion by 11–22% (Zhang *et al.* 2018c). Imidacloprid degradation in soil follows first-order kinetics and is not affected by moisture conditions (Baskaran *et al.* 1999). The main metabolites are urea-imidacloprid, olefin-imidacloprid and 6-chloronicotinic acid, which are as toxic as the parent compound (Simon-Delso *et al.* 2015) and have higher bioconcentration factors than imidacloprid (Zhang *et al.* 2020a).

Biodegradation by micro-organisms is evident, as more than 95% of acetamiprid and thiacloprid were degraded after 15 days incubation in aerobic soils, and 25% of imidaclothiz and 22.5% of imidacloprid after 25 days, while only 27% thiacloprid, 21% acetamiprid, 9% imidacloprid, and 0% imidaclothiz degradation were observed in sterile soils (Liu *et al.* 2011). Cycloxaprid can also be degraded in aerobic soils, with the fastest degradation rates in loamy soils and the slowest in acidic clay soils (Chen *et al.* 2017). Less than half of the cycloxaprid applied to a water-sediment was transformed into three metabolites, with 54% of the parent compound remaining as bound residues and only 0.015% mineralised after 56 days (Cheng *et al.* 2022). Unfortunately, its main metabolite, 2-chloro-5-

[(2-(nitromethylene)-1-imidazolidinyl)methyl]pyridine, is more toxic than the parent compound.

Substantial research into the identification of NNI-mineralising bacterial strains has been conducted to advance bioremediation efforts (Hussain *et al.* 2016). Micro-organisms, such as *Bacillus*, *Mycobacterium*, *Pseudoxanthomonas*, *Rhizobium*, *Rhodococcus*, *Actinomycetes*, and *Stenotrophomonas*, have been isolated and characterised (Pang *et al.* 2020). The oligotrophic bacterium *Hymenobacter latericoloratus* degrades imidacloprid in waters (Guo *et al.* 2020), acetamiprid can be degraded by *Phanerochaete chrysosporium* (Wang *et al.* 2019a), and the bacterium *Variovorax boronicumulans* has been identified as one of the microbes involved in the degradation of thiacloprid (Zhang *et al.* 2012) and acetamiprid (Sun *et al.* 2017), while *Pseudomonas putida* and *Bacillus aeromonas* degraded 38% and 45% of thiamethoxam, respectively, after incubation for 15 days (Rana *et al.* 2015; Lu *et al.* 2016). Up to 35% of paichongding can be degraded within 5 days by strains of the soil bacterium *Sphingobacterium* (Wang *et al.* 2016). At high concentrations, it seems that acetamiprid is slightly more toxic to soil microbes than imidacloprid (Wang *et al.* 2014).

Biochar amendments can change the soil bacterial community by modulating soil pH, dissolved organic matter, and nitrogen and phosphorus levels. For example, the biodegradation of thiacloprid was enhanced under amendments of 300-PT (pyrolysing temperature) biochars, but not with those of higher temperatures (Zhang *et al.* 2018d). Biodegradation of imidacloprid was also enhanced 5–9 times in bio-mixtures of rice straw, corn cobs, and peat, especially under high moisture conditions (Kumari *et al.* 2019).

4.2.4 Toxicity and toxicokinetics

Neonicotinoids are very toxic to all arthropods, and more specifically to terrestrial and aquatic insects. The variable sensitivity among taxa is primarily due to different subunits present in the target nicotinic acetylcholine receptors (nAChRs) and secondly to differences in detoxification mechanisms among individual species (Matsuda *et al.* 2020).

It has been recognised that acute toxicity tests with NNIs fail to gauge their real impacts on both terrestrial and aquatic organisms, as it is the chronic exposure to these systemic insecticides that really matters (Morrissey *et al.* 2015). Standard acute toxicity tests with bees and parasitoids, for example, are inadequate for NNIs and other pesticides that have systemic properties (Halm *et al.* 2006; Mommaerts *et al.* 2010). Results from such tests are misleading because they give the false impression of safety (Zhao *et al.* 2012); equally for acute tests with aquatic organisms (Hayasaka *et al.* 2013a). This handicap needs to be understood, as the toxicity of newly developed compounds, such as cycloxaprid to target and non-target species, continues to be evaluated using inadequate toxicological data (Singh and Leppanen 2020).

Importantly, the toxicity of NNIs is not only restricted to the parent compound but also includes the toxicity of metabolites that result from degradation processes in organisms, as well as soil and sediment bacteria and fungi. For example, dinotefuran applied to soil degrades quickly into two products that are as toxic to earthworms (*Eisenia fetida*) as dinotefuran (Liu *et al.* 2018). The increase in toxicity of NNIs over time has been attributed by some authors to the combined effect of the parent compound and its toxic metabolites, which remain bound to the receptors and confer a persistent toxic effect (Huang *et al.* 2021).

Although acute exposures are lethal to many aquatic organisms at levels above 1 µg/L (Sánchez-Bayo and Goka 2006a; Hayasaka *et al.* 2012), it is the chronic exposure to the low levels found in the environment (in the nanograms per litre range) that is of greater concern. This is because the toxicity of NNIs is cumulative over time, because of the irreversible binding to the target nAChRs and the equally irreversible damage inflicted on the affected neurons. This mode of action was known only for carcinogenic substances and mercurial compounds, but it has also been demonstrated with NNIs and a few other pesticides (Tennekes and Sánchez-Bayo 2012; Sánchez-Bayo and Tennekes 2020). It has also been confirmed in experimental mesocosms with nymphs of the mayfly *Deleatidium* spp. exposed chronically to relevant environmental concentrations of imidacloprid, clothianidin, and thiamethoxam (Macaulay *et al.* 2021a). Indeed, the 28-day lethal median concentrations (LC_{50} values), determined respectively as 0.28, 1.36, and greater than 4 µg/L (Macaulay *et al.* 2019), are two orders of magnitude lower than the acute LC_{50} values of other mayflies (Roessink *et al.* 2013). In toxicity mixtures such as those found in streams and rivers, imidacloprid toxicity dominates over that of other NNIs (Hunn *et al.* 2019; Macaulay *et al.* 2021b), and because all compounds in this chemical class have the same mode of action, it has become customary to indicate the overall toxicity of NNI mixtures as imidacloprid equivalents (Mahai *et al*. 2021a).

A few studies have investigated the sublethal toxicity of NNIs to *Daphnia*, an economically important taxon that is less sensitive to NNIs than insects and other crustaceans (Sánchez-Bayo and Goka 2006b). It took time to realise that the standard test species *Daphnia magna* is exceptionally tolerant to all NNIs in acute exposures (imidacloprid 48-hour LC_{50} = 85 mg/L). Such tolerance seems to be due to a high depuration rate, which avoids bioaccumulation in critical tissues. In spite of it, *Daphnia* experiences slow recovery (45 days) under chronic exposure to low concentrations of imidacloprid (Li *et al.* 2021). Differences in sensitivity to acetamiprid and clothianidin between the tolerant estuarine sand shrimp (*Crangon uritai*), the marine kuruma prawn (*Penaeus japonicus*), and a mysid (*Americamysis bahia*) were attributed to high levels of oxygenase enzyme in the shrimp, which metabolises the NNIs (Hano *et al.* 2020). Environmentally relevant concentrations of imidacloprid had sublethal effects on the eastern school prawn (*Metapenaeus macleaya*), such as changes in the fatty acid composition, which indicate a shift in lipid homeostasis (McLuckie *et al.* 2020). Imidacloprid causes oxidative stress, leading to reduced growth, tissue damage, and an impaired immune system in the Pacific white shrimp (*Litopenaeus vannamei*). Lack of immune response and the disturbance of circadian rhythm led to increasing abundance of gut pathogenic microbiota, resulting in several disorders (Fu *et al*. 2022).

Effects of NNIs in vertebrates have also been studied, in particular the metabolism and accumulation in the Mongolian racerunner lizard (*Eremias argus*). Dinotefuran was not easily metabolised; rather, it accumulated in the brain, whereas thiamethoxam and imidacloprid were rapidly absorbed and excreted in lizards, but did not accumulate in the brain. The toxic metabolite imidacloprid desnitro-olefin was produced in the brain, thus increasing the toxic effects of the parent imidacloprid (Wang *et al.* 2019b). Thiamethoxam was metabolised to the demethylated form and clothianidin, thus resulting in increased overall toxicity (Wang *et al.* 2018). Although parent NNIs and their metabolites, such as 6-chloropyridinyl acid, are excreted mainly through the urine, lizards can also transfer NNIs into the scales and eliminate them through moulting (Wang *et al.* 2019c). In another study, dinotefuran caused hepatic oxidative stress damage and decreased concentrations of plasma growth hormone, whereas thiamethoxam caused the least damage to the liver and had minimal impact on growth compared with dinotefuran and imidacloprid (Wang *et al.* 2019c).

The endocrine disruption of thiamethoxam and its metabolite clothianidin was also studied in the same lizards. Both NNIs can accumulate in the lizards' testes, producing a significant decrease in the amount of testosterone levels, while increasing the concentrations of 17-oestradiol in plasma, which causes androgen deficiency in the males. In female lizards, the two NNIs are accumulated in the ovaries, causing an increase in testosterone levels in the plasma and an upregulation of androgen receptor expression in the liver (Wang *et al.* 2019d).

A compilation of toxicity data on mammals has also been published (Wang *et al.* 2019e). This is useful in determining the risks to those animals and in understanding further the possible health risks to humans. A recent review indicates that exposure to NNI mixtures can result in reproductive and hormonal toxicity, genotoxicity, neurotoxicity, hepatotoxicity, and immunotoxicity in vertebrates (Zhao *et al.* 2020).

4.3 Monitoring studies

Numerous monitoring studies from other parts of the world have shown that NNIs are ubiquitous in aquatic systems (Starner and Goh 2012; Masiá *et al.* 2013) and soils (Jones *et al.* 2014; Schaafsma *et al.* 2015), not only in agricultural regions but also in urban areas (Hladik *et al.* 2014; Münze *et al.* 2017; Montiel-León *et al.* 2019). Most of the recent monitoring, however, has been from China and some Pacific countries, and these studies are discussed below.

4.3.1 Soils

Soil residue data are only available for China and the Philippines (Table 4.2). In China, total residue loads of NNIs vary greatly among regions, but not so much among land uses. A national survey found the highest residues during the spring season, when the chemicals are applied to crops. In Guangdong province, 95% of soils contained measurable residues of at least one NNI, with imidacloprid having the highest average concentrations (24 ng/g soil dry weight), and soils from vegetable farming having higher loads than rice paddies and fruit orchards (Yu *et al.* 2021). In Guangzhou, soils had the lowest average loads in the range 0.13–1.69 ng/g dry weight, with imidacloprid being the dominant compound (Zhang *et al.* 2020b). Residues of imidacloprid were also the highest in agricultural soils of Zhejiang province (49.6 ng/g dry weight), where total loads averaged 76 ng/g dry weight (Chen *et al.* 2022). Citrus orchards in Jiangxi had residues of clothianidin and imidacloprid in the range 820–5860 ng/g after application (Niu *et al.* 2022). In Tianjing, total NNI concentrations were highest in greenhouses during the spring season (average 459 ng/g dry weight, highest 5,060 ng/g dry weight), particularly in soils planted with watermelons, tomatoes, and peaches. Soils from other land uses had lower loads, averaging 138, 131, 102, and 84 ng/g dry weight in residential areas, orchards, parks, and farms, respectively, with individual compounds varying according to different land uses (Zhou *et al.* 2021).

Residues of at least one NNI were detected in all indoor dust samples collected from three large Chinese cities (Taiyuan, Wuhan, and Shenzhen). Average concentrations were 36 ng/g for all six NNIs detected (Wang *et al.* 2019f).

In the Philippines, residues of NNIs in the soil of agricultural crops were highest in banana and citrus plantations (average 3.8 ng/g dry weight), and lowest in

Table 4.2 Neonicotinoid concentrations (ng/g dry weight) in soils, sediments, and indoor dust

	Location	Land use	Period	ACE	CLO	DIN	IMI	IMZ	NIT	THC	TMX	ALL	Reference
Dust	China (three cities)	Indoor	2016–2018	2.28 (4227) 99%	0.41 (363) 30%	0.95 (476) 39%	4.44 (2823) 99%			0.25 (367) 34%	0.35 (92.1) 24%	36 (5277) 100%	Wang A 2019
Soil	Guangdong (China)	Agricultural	2014	2 (20) 49%	6.2 (70) 46%	0.36 (0.48) 5%	24 (370) 94%	1.1 (7) 3%				28 (390) 95%	Yu 2021
Soil	Tianjin (China)	Greenhouses	2016									459 (5060) 100%	Zhou 2021
Soil	Tianjin (China)	Residential, parks	2016									138 (952) 97%	Zhou 2021
Soil	Tianjin (China)	Orchards, farms	2016									131 (462) 100%	Zhou 2021
Soil	Guangzhou (China)	Agriculture	2017	0.06 (15.3) 98%	0.04 (95.7) 73%		0.58 (147) 75%			0.004 (0.076) 39%	0.02 (30.2) 42%	1.69 (229) 100%	Zhang C 2020
Soil	Guangzhou (China)	Industrial	2017	0.05 (0.29) 100%	0.003 (0.83) 33%		0.005 (5.76) 8%			0.009 (0.28) 58%	0.005 (0.81) 42%	0.13 (7.32) 100%	Zhang C 2020
Soil	Guangzhou (China)	Urban*	2017	0.07 (9.58) 100%	0.03 (2.08) 71%		0.13 (28.2) 50%			0.009 (0.12) 57%	0.005 (2.34) 43%	0.7 (32.8) 100%	Zhang C 2020
Soil	Luzon (Philippines)	Vegetable crops	2019	0.002 (0.002) 10%	0.02 (0.24) 100%		0.76 (39.56) 100%				0.005 (0.05) 90%	0.89 (39.6) 100%	Bonmatin 2021

Continues

Table 4.2 (continued)

	Location	Land use	Period	ACE	CLO	DIN	IMI	IMZ	NIT	THC	TMX	ALL	Reference
Soil	Marinduque (Philippines)	Rice	2019				0.013 (0.03) 29%				0.005 (0.01) 24%	0.02 (0.04) 29%	Bonmatin 2021
Soil	Mindanao (Philippines)	Banana, citrus	2019		1.43 (126.3) 100%		1.05 (903.3) 100%				0.28 (267.9) 100%	3.82 (1188) 100%	Bonmatin 2021
Soil	Zhejiang (China)	Agriculture	2017–2021	2.8 69%	6.62 100%	9.5 62%	49.6 92%		2.93 69%	1.1 85%	7.77 77%	75.8 100%	Chen 2022
Sediment	Guangzhou (China)	Agriculture, urban	2015	1.3 (9.23) 64%	0.35 (6.17) 98%	0.26 (3.25) 64%	1.79 (19.1) 86%			0.18 (0.78) 64%	0.34 (1.83) 74%	4.21 (23.8) 100%	Huang 2020
Sediment	Guangzhou (China)	Urban and rural	2017	0.33 (3.73) 100%	0.07 (4.81) 72%		0.009 (17) 31%			0.01 (0.15) 76%	0.06 (5.72) 72%	1.11 (31.3) 100%	Zhang C 2020
Sediment	Pearl river (China)	Agriculture, urban	2017	0.82 (2.07) 100%	0.06 (0.23) 89%		—			0.1 (0.38) 100%	0.11 (0.25) 78%	1.12 (2.59) 100%	Yi 2019
Sediment	Pearl river (China)	Agriculture		24.7 (67.6) 100%	18.1 (67.2) 100%		33.9 (162) 100%			0.9 (9.4) 75%	30.4 (102) 100%	98.7 (285) 100%	Zhang 2019
Sediment	Pearl river (China)	WWTP		12.3 (67.6) 100%	49.7 (103) 100%		36.5 (89.1) 96%			0.59 (2.38) 91%	79.5 (145) 100%	179 (350) 100%	Zhang 2019
Sediment	Harbin (China)	River	2019	0.75 (0.75) 9%	0.11 (0.22) 100%		1.2 (12.6) 100%				0.36 (1.58) 100%	1.76 (14.7) 100%	Liu 2021

* Includes educational, parks, residential, and traffic areas.
Numbers indicate the mean, the maximum in brackets, and the percentage of positive samples (greater than the limit of quantitation).
Abbreviations: ACE, acetamiprid; CLO, clothianidin; DIN, dinotefuran; IMI, imidacloprid; IMZ, imidaclothiz; NIT, nitenpyram; THC, thiacloprid; TMX, thiamethoxam; ALL, sum of NNIs.

rice crops (0.017 ng/g dry weight). Imidacloprid was the most common compound found in all fields, reaching the highest concentrations (1188 ng/g dry weight) in citrus orchards (Bonmatin *et al.* 2021b).

4.3.2 Sediments

Data on residues in sediments are only available from China. In sediments of the Pearl River (Guangzhou), total loads ranged between 0.40 and 2.59 ng/g dry weight, with a geometric mean of 1.12 ng/g dry weight; acetamiprid and thiacloprid were the most common NNIs found in sediments (Yi *et al.* 2019; Zhang *et al.* 2019). Another study found acetamiprid and thiamethoxam were the dominant compounds (Zhang *et al.* 2020b). Similar loads were measured in sediments of the Songhua River (Harbin, China), where total concentrations of three NNIs ranged from 0.61 to 14.7 ng/g dry weight (Liu *et al.* 2021).

In agricultural and urban areas of Guangzhou, total loads of NNIs in sediments reached a maximum of 23.8 ng/g dry weight, with an average of 4.2 ng/g dry weight. Clothianidin and imidacloprid were the dominant compounds in the rice-planting areas, whereas acetamiprid and thiacloprid were the most common in vegetable cropping areas. Total loads were highest in both vegetable crops and urban areas (Huang *et al.* 2020; Zhang *et al.* 2020c).

4.3.3 Surface waters

Neonicotinoids are the most frequent insecticides found in surface waters, not only in drains from agricultural areas but also in streams and rivers around cities, estuaries, and receiving seawaters (Sánchez-Bayo *et al.* 2016). Recent surveys have targeted not only the parent compounds but also some of the toxic metabolites (Hashimoto *et al.* 2020; Mahai *et al.* 2021a). Maximum levels and geometric means are reported in Table 4.3, together with the frequency of detection of each compound. In general, residue levels in the Asia-Pacific region are about the same or lower than those found in North America and Europe (Kreuger *et al.* 2010; Main *et al.* 2014; Ccanccapa *et al.* 2016; Hladik *et al.* 2018a; Montiel-León *et al.* 2019).

In agricultural areas, the highest NNI concentrations in water are found in flooded rice paddies. Measured concentrations depend mainly on the time lag between the application of the insecticide and the actual sampling. In Japan, direct aerial spray of dinotefuran to paddy fields in Niigata Prefecture resulted in paddy water concentrations as high as 290–720 µg/L, while levels of 2–10 µg/L in adjacent rivers were found owing to a combination of drift and run-off (Yokoyama *et al.* 2015). Following transplantation of rice seedlings treated with granular imidacloprid, the highest concentration of this chemical in waters of paddy mesocosms is in the range 39–239 µg/L (Sánchez-Bayo and Goka 2006a; Hayasaka *et al.* 2012; Kobashi *et al.* 2017), and those of dinotefuran were up to 10.5 µg/L (Kobashi *et al.* 2017). In the rice fields of northern Vietnam, imidacloprid levels in paddy water varied between 0.15 and 52.9 µg/L (Lamers *et al.* 2011; La *et al.* 2015). In the Philippines, drains from rice crops and plantations of banana and citrus had residues of imidacloprid and thiamethoxam in the range 0.15–5.22 ng/L, with the highest loads in the banana/citrus plantations (Bonmatin *et al.* 2021b). Draining ditches in rice paddies of Jiangxi (China) had total loads of 862 ng/L for six NNIs, with dinotefuran having the highest concentrations (Xiong *et al.* 2021).

Run-off discharges from agricultural land go directly into receiving streams, rivers, and lakes. Concentrations depend on usage patterns and application rates for the individual compounds in particular regions. In Japan, rivers adjacent to rice paddies had imidacloprid levels up to 410 ng/L, probably as a result of run-off (Hashimoto *et al.* 2020). The average range of concentrations of seven NNIs in Japanese river waters is between 2 and 180 ng/L, depending on the compounds and locations (Yamamoto *et al.* 2012; Hano *et al.* 2019; Hayashi *et al.* 2021; Terayama *et al.* 2021), with dinotefuran reaching the highest levels (Kamata *et al.* 2020). Lake Shinji had inputs of five NNIs in 44% of samples with total concentrations up to 489 ng/L (Doi *et al.* 2018). In the lakes and rivers of northern Vietnam, total NNI concentrations were between <1 and 49 ng/L (Wan *et al.* 2021), whereas in the Mekong River thiamethoxam was found at 950 ng/L (Chau *et al.* 2015). No residues of NNIs could be found in waters off the Cauvery Delta region in southern India, because of inadequate analytical sensitivity (Menon *et al.* 2021). In Australia, average residue levels of five compounds in rivers of the Sydney basin were 453 ng/L, with the highest being for imidacloprid in creeks that received drainage from turf farms (Sánchez-Bayo and Hyne 2014). Imidacloprid is the only NNI found in river catchments that drain towards the Great Barrier Reef (Smith *et al.* 2012; Hook *et al.* 2018). It is found mainly in areas with banana and sugar cane crops, but it can also be found in urban areas (Warne *et al.* 2020). Its water concentration has been increasing in the past decade, reaching an average 51 ng/L in 54% of the surface waters (Warne *et al.* 2022). The lowest levels of NNIs were found in New Zealand, where imidacloprid is the most common compound at levels below 1 ng/L (Hageman *et al.* 2019). The highest residue loads of NNIs are found in China's rivers, where there has been a flurry of local and national surveys in recent years. The Yangtze and Pearl Rivers and subtropical agricultural areas in Hainan Province contain the highest total loads of eight NNIs in the range 313–3240 ng/L (Chen *et al.* 2019a; Li *et al.* 2019; Xiong *et al.* 2019; Yi *et al.* 2019; Xu *et al.* 2020; Zhang *et al.* 2020b; Tan *et al.*

Table 4.3 Neonicotinoid concentrations (ng/L) in surface waters of the Asia-Pacific region

	Location	Year	ACE	CLO	DIN	IMI	IMZ	NIT	THC	TMX	ALL	Reference
Paddy mesocosm	Tsukuba (Japan)	2004–2005				3750 (239,200)						Sánchez-Bayo 2006, 2007
Paddy water	North Vietnam	2008				151 (190)						Lamers 2011
Paddy water	North Vietnam	2008				2773 (52,900)						La 2015
Paddy mesocosm	Tsukuba (Japan)	2010–2011				39,000–49,000						Hayasaka 2012a 2012b
Paddy mesocosm	Nara (Japan)	2014			486 (10,540)	136 (157,500)						Kobashi 2017
Paddy water	Niigata (Japan)	2013			76,443 (720,000)							Yokoyama 2015
Paddy water	Philippines	2019				1.3 (3) 50%				0.2 (0.2) 17%	1.3 (3.2) 50%	Bonmatin 2021
Paddy ditch	Jiangxi (China)	2019	8.76	6.53	799	12.8		18.8		15.3	862	Xiong 2021
Rivers	Mekong (Vietnam)									630 (950) 4%		Chau 2015
Rivers	Hainan (China)		103 (3,420) 41%			150 (8,630) 67%						Tan 2021
Rivers	Guangzhou (China)		17.5 (67.6) 100%	18.4 (67.2) 100%		30.4 (162) 99%			0.66 (9.35) 68%	31.7 (102) 100%		Zhang 2019
Rivers	Osaka (Japan)	2009		3.5 (12) 83%	30 (100) 100%	2.5 (7) 67%				1.5 (3.2) 75%		Yamamoto 2012
Rivers	Kanagawa (Japan)	2009	(60)			(420)						Terayama 2020
Rivers	Queensland (Australia)	2009–2019				51 (1,300) 54%						Warne 2020, 2022
Rivers	Osaka (Japan)	2010	1.4 (1.4) 10%	2.9 (7.8) 100%	9.9 (31) 100%	8.6 (25) 95%				3.8 (11) 100%		Yamamoto 2012

Continues

Table 4.3 (continued)

	Location	Year	ACE	CLO	DIN	IMI	IMZ	NIT	THC	TMX	ALL	Reference
Rivers and lake	Japan	2012–2017		20%	(3700) 10%	26%						Kamata 2020
Rivers	Sydney (Australia)	2013	80 (383) 73%	56 (425) 60%		201 (4,560) 93%			145 (1,370) 80%	102 (200) 27%	453 (5,927) 100%	Sánchez-Bayo 2014
Rivers	Niigata (Japan)	2013			1198 (10,000)							Yokoyama 2015
Rivers	Kanagawa (Japan)	2015	(23)	(85)	(48)	(104)			(2)	(202)		Terayama 2020
Rivers	Japan (35 locations)	2015–2016		(287) 45%	(6,031) 57%	(43.8) 33%		(3.5) 3%		(163) 19%		Furihata 2019
River mouth	Seto Island (Japan)	2015–2018	1 (2) 21%	9 (57) 96%	142 (866) 100%	36 (267) 100%				7 (20) 92%		Hano 2019
Rivers	Queensland (Australia)	2016				14 (345) 71%						Hook 2018
Rivers	Yangzte (China)	2016	8.1 (22.9) 88%	11.6 (81.3) 75%	65.8 (1,730) 92%	13.1 (71.4) 100%	15.6 (85) 100%	105.9 (1,250) 96%	1.6 (7.8) 67%	11.7 (157) 96%	296 (3,240) 100%	Chen 2019a
Rivers	Guangzhou (China)	2016	56.1 (278) 100%	15.8 (47.6) 100%		53.4 (249) 100%				7.3 (52.4) 84%	139 (401) 100%	Xiong 2019
Rivers	Kagoshima (Japan)	2016				— (409) 90%						Hashimoto 2020
Rivers	Gifu (Japan)	2016	2.8 (2.8) 1%	6.8 (62.2) 47%	12.5 (239) 82%	4.6 (23) 22%				4.4 (22.3) 19%	18 (250.9) 84%	Hayashi 2021
Rivers	New Zealand	2017		8%		0.2 (0.5) 22%				3%		Hageman 2019
Rivers	Guangzhou (China)	2017	24.6 (77.1) 100%	24.6 (38) 100%		70.7 (154) 100%			1.0 (2.97) 100%	48.8 (70.2) 100%	174 (321) 100%	Yi 2019

Continues

Table 4.3 (continued)

	Location	Year	ACE	CLO	DIN	IMI	IMZ	NIT	THC	TMX	ALL	Reference
Rivers	Hangzhou (China)	2017	17.6 (34.4) 88%	7.6 (29.5) 94%	3 (20.1) 25%	11.9 (31.7) 100%		3.8 (16.7) 40%		4.8 (29.6) 44%	128 (540) 100%	Lu 2020
Rivers	Kanagawa (Japan)	2017	(779)	(482)	(373)	(836)			(6)	(29)		Terayama 2020
Rivers	Tokyo (Japan)	2017	(1.7)	(6.3)	(16)	(7)		(1.6)	(0.5)	(7.9)		Terayama 2020
Rivers	Nagoya (Japan)	2017	(24)	(210)	(840)	(25)		(11)	(5)	(370)		Terayama 2020
Rivers	Osaka (Japan)	2017	(30)	(30)	(1900)	(500)				(10)		Terayama 2020
Rivers (urban, rural)	Guangzhou (China)	2017	25 (189) 100%	22.4 (68.7) 100%		29.1 (273) 93%			0.15 (3.74) 76%	43.8 (156) 100%	153 (636) 100%	Zhang C 2020
Rivers	Jiaozhou Bay (China)	2018	490 (490) 13%	2,800 (2,800) 13%		510 (650) 25%				600 (720) 25%	628 (2,050) 75%	Li 2019
Rivers	Fukui (Japan)	2018	(1.2)	(130)	(270)	(55)			(1.2)	(76)		Terayama 2020
Rivers	Bohai sea (China)	2018	16 (128) 100%	4.9 (55.2) 100%	17.2 (17.2) 47%	12.9 (104) 100%		1.77 (1.77) 11%	5.44 (5.44) 42%	9.2 (99.8) 100%		Naumann 2022
Rivers and lake	Lake Taihu (China)	2018	6 (38) 90%			39 (438) 88%				5 (53) 31%		Zhou 2020
Rivers	Shanghai (China)	2018–2019	119 (443) 98%			318 (1,702) 98%				97 (1,567) 100%		Xu 2020
Rivers	Harbin (China)	2019	0.85 (10.8) 100%	2.75 (13.1) 100%	1.69 (5.91) 23%	16.9 (83.5) 100%			0.69 (1.21) 15%	28.4 (83.5) 100%	52.3 (135) 100%	Liu 2021

Continues

Table 4.3 (continued)

	Location	Year	ACE	CLO	DIN	IMI	IMZ	NIT	THC	TMX	ALL	Reference
Rivers	Jiangxi (China)	2019	2.2 (9.9) 100%	2.2 (12.1) 93%	31 (802) 97%	7.2 (47.4) 100%		3.4 (16.4) 14%	0.6 (4.2) 69%	4.6 (28.5) 86%	54 (866) 100%	Xiong 2021
Rivers	Fukuoka (Japan)	2019	(20)	(120)	(430)	(110)		(<10)	(<10)	(30)		Terayama 2020
Rivers	North Vietnam	2019	0.25			0.29				0.23	0.98	Wan 2021
Lakes	Lake Shinji (Japan)	2017	280 (280) 11%	10.8 (19) 44%	12 (32) 44%	31.1 (149) 44%				11.4 (31) 44%	128 (489) 44%	Doi 2018
Lakes	North Vietnam	2019	5.37 (18.6)			2.13 (5.43)				0.81 (1.77)	17.2 (49.1)	Wan 2021
Estuaries	Seto Island (Japan)	2015–2018	1 (4) 11%	2 (61) 27%	17 (1,055) 98%	6 (213) 26%				3 (13) 9%		Hano 2019
Estuaries*	Yangtze (China)	2017	0.9 (1.4) 20%		0.6 (2.7) 67%	3.7 (7.2) 47%		2 (2.4) 40%	0.3 (0.4) 20%	0.7 (1.8) 80%	2.4 (12.5) 87%	Pan 2020
Estuaries	Jiaozhou Bay (China)	2018	3.1 (12.3) 57%	0.4 (2.8) 22%	0.88 (6.77) 22%	13.3 (51.4) 86%		14%	0.92 (3.98) 50%	10.2 (125.3) 78%	28 (183) 100%	He 2021b
Seawater	Jiaozhou Bay (China)	2018	300 (310) 13%			357 (510) 40%		353 (500) 53%			575 (1180) 87%	Li 2019
Seawater	Jiaozhou Bay (China)	2018	0.54 (1.26) 92%	(0.7) 7%		0.67 (2.83) 93%	(1.78) 7%	0.32 (0.5) 67%	0.25 (1.43) 50%	(0.27) 47%	1.2 (4.21) 100%	He 2021b
Seawater	Bohai sea (China)	2018	0.37 (0.94) 100%	(1.1) 6%		(0.75) 6%			(0.14) 85%	(0.55) 30%		Naumann 2022
Urban run-off	Pearl river (China)	2018	6 (18)	11 (41)		18 (170)					Not determined	Zhang XP 2020
WWTP effluent	Guangzhou (China)		12.3 (67.6) 100%	49.7 (103) 100%		36.5 (89.1) 83%			0.59 (1.35) 67%	79.5 (145) 100%	179 (350) 100%	Zhang 2019

* Excluding open-sea samples (n=15).
Numbers indicate the geometric mean, the maximum in brackets, and the percentage of positive samples (greater than the limit of quantitation).
Abbreviations: ACE, acetamiprid; CLO, clothianidin; DIN, dinotefuran; IMI, imidacloprid; IMZ, imidaclothiz; NIT, nitenpyram; THC, thiacloprid; TMX, thiamethoxam; ALL, sum of NNIs; n, number of samples.

2021;). Up to seven toxic metabolites have also been measured in rivers in the Jiangxi region (Xiong *et al.* 2021). Overall, residue levels are higher in summer, as higher precipitation results in increased run-off and, hence, higher residue loads (Mahai *et al.* 2019). They are lower in receiving lakes (Zhou *et al.* 2020), rivers of industrial regions (Liu *et al.* 2021), and urban areas (Zhang *et al.* 2020b). Effluents from water treatment plants seem to have similar residue levels (179–350 ng/L) as the influents (Zhang *et al.* 2019), since most NNIs are recalcitrant to conventional remediation technologies (Sadaria *et al.* 2017; Kim *et al.* 2021).

Concentrations are diluted as the residues move from agricultural fields to the mouths of rivers (Xiong *et al.* 2021; Naumann *et al.* 2022). Estuaries typically contain the lowest residues, with total loads of up to 12 ng/L in the Yangtze estuary of China (Pan *et al.* 2020) and 183 ng/L in China's Jiaozhou Bay (He *et al.* 2021a). The exception is the estuary off Seto Island in the Japan Sea, where maximum concentrations of dinotefuran and imidacloprid were measured at 1050 and 213 ng/L, respectively (Hano *et al.* 2019).

4.3.4 Drinking water

Drinking water has also attracted attention, because of the widespread contamination observed in the natural environment (Table 4.4). The main sources of drinking water are groundwaters and rivers (raw water), although many cities rely on treated water facilities for supplying tap water to their citizens. In general, residue levels in drinking water from the Asia-Pacific region are similar to those in other regions of the world (Klarich *et al.* 2017; Sultana *et al.* 2018).

Imidacloprid residues as high as 1530 ng/L were found in groundwater from 59% of wells in a rural region in northern Vietnam (Lamers *et al.* 2011), while loads of up to 8,622 ng/L of eight NNIs were measured in groundwaters in a national survey of 32 Chinese cities, where thiamethoxam and clothianidin reached the highest concentrations (Mhai *et al.* 2021b).

Tap water has much lower residue levels: in northern Vietnam, total loads are usually below 1 ng/L, with a maximum of 8 ng/L (Wan *et al.* 2021). Drinking water in South Korea had total NNI levels of 108 and 27 ng/L in raw and treated waters, respectively, because of their poor removal efficiencies, with dinotefuran reaching the highest concentrations (Kim *et al.* 2021). For the same reason, treated waters of 12 cities in Japan had dinotefuran residues up to 2200 ng/L (Kamata *et al.* 2020). Drinking water from a variety of Chinese cities had average loads of eight NNIs and their toxic metabolites were in the range 5–168 ng/L for raw water and 17–173 ng/L for tap water (Wan *et al*. 2019; Lu *et al*. 2020; Wan *et al*. 2020; He *et al*. 2021b; Mahai *et al*. 2021a; Zhang *et al*. 2021), with maximum totals of 3,233 ng/L (Mahai *et al.* 2021b). Treated water in the city of Wuhan had total residue loads between 20 and 119 ng/L for seven NNIs (Wan *et al.* 2019), indicating that treatment does not help reduce the total toxic load of these insecticides.

Such contamination of drinking waters with multiple NNI compounds should be a concern for human health, since they result in chronic exposure, even though research on their human impacts is still in its infancy (Taira 2014; Thompson *et al.* 2020).

4.4 Ecological impacts

Impacts of NNIs on the environment depend, to a large extent, on the length of exposure in specific taxa, as the chronic effects of these insecticides are more important than their acute ones (Sánchez-Bayo *et al.* 2016). Such exposure differs markedly between terrestrial and aquatic organisms, so they are treated separately here.

4.4.1 Terrestrial ecosystems

Exposure of most terrestrial organisms to systemic NNIs is sporadic, as it occurs mainly through ingestion of contaminated food, such as treated vegetation, fruits, pollen, and nectar (Hladik *et al.* 2018b). Direct exposure to spray drift is rare in the case of NNIs, as these insecticides are commonly applied through coated seeds, granules, and soil drenches.

The impact of NNIs on microbial soil communities has been studied in China. Naturally, some micro-organisms can use insecticides as an additional energy source, so bacteria and fungi, such as Proteobacteria, Firmicutes, Planctomycetes, Chloroflexi, Armatimonadetes, and Chlorobi, increased markedly after application of paichonding, while the phyla of Bacteroidetes, Actinobacteria, and Acidobacteria decreased (Cai *et al.* 2016a; 2016b). Wheat seeds coated with imidacloprid and clothianidin also changed the microbial community of the crop soils, as species richness of the bacterial and fungal community was suppressed during the seedling stage, but recovered at the end of the wheat planting season following the dissipation of soil residues (Li *et al.* 2018). It seems the presence of imidacloprid in plant tissues fosters the growth of certain bacteria and fungi, which become dominant and change the microbial phyllosphere environment (Jing *et al.* 2011).

Impacts on earthworms have not been assessed, but considering these organisms are chronically exposed to soil residues both by direct absorption through the epidermis and by ingestion of soil particulates, the current NNI loads in 95% of agricultural soils in southern China are concerning (Yu *et al.* 2021). In Australia, there is concern about the impact of NNI residues in soil on larvae of the migratory Bogong moths (*Agrotis infusa*), which have experienced a marked

Table 4.4 Neonicotinoid concentrations (ng/L) in drinking waters of the Asia-Pacific region

	Location	Year	ACE	CLO	DIN	IMI	IMZ	NIT	THC	TMX	ALL	Reference
Groundwater	North Vietnam	2008				28.5 (1530) 59%						Lamers 2011
Groundwater	China (32 cities)	2019	0.22 (3.38) 86%	2.11 (137) 54%	0.23 (5.64) 11%	1.88 (20) 73%	0.002 (0.18) 1%	0.03 (0.1) 2%	0.03 (1.46) 26%	9.1 (807) 44%	129 (8,622) 100%	Mahai 2021
Raw water	Wuhan-Yangtze (China)	2018	5.03 (9.98) 100%	1.19 (4.2) 100%	0.19 (1.2) 64%	9.66 (28.5) 100%		0.89 (8.7) 100%	0.05 (0.16) 100%	4.93 (19.8) 100%	23.9 (65.3) 100%	Wan 2019
Raw water	Wuhan-Han (China)	2018	11.6 (22.7) 100%	2.89 (10.5) 100%	0.8 (3.02) 83%	24.7 (82.4) 100%		0.07 (1.66) 100%	0.44 (0.28) 100%	15.3 (64.8) 100%	59.1 (186) 100%	Wan 2019
Raw water	China	2018	0.27 (15.5) 78%	0.51 (109) 80%		1.07 (55) 84%			0.01 (3.11) 24%	0.43 (88.5) 63%	4.77 (188) 95%	Zhang 2021
Raw water	South Korea	2020	1.45 (2.57) 80%	17 (26.5) 100%	70.8 (115) 100%	6.19 (9.34) 100%			1.88 (4.62) 87%	10.5 (15) 100%	108 (166) 100%	Kim 2021
Tap water	Hangzhou (China)	2017	5.8 (!5.5) 83%	0.6 (5.7) 15%	1.8 (25) 17%	4 (10.6) 82%		2.5 (22.6) 31%			17 (105) 96%	Lu 2020
Tap water	China (38 cities)	2017–2019	1.81 (69.2) 94%	2.24 (104) 92%	3.92 (312) 90%	4.6 (68.3) 99%			0.48 (74.2) 86%	2.15 (214) 87%	24 (607) 100%	He 2021a
Tap water	China (32 cities)	2019	1.75 (182) 94%	3.22 (98.8) 70%	0.72 (13.7) 26%	5.63 (233) 84%	0.01 (0.62) 5%	0.05 (1.62) 5%	0.08 (7.18) 42%	6.68 (232) 68%	173 (3,233) 100%	Mahai 2021
Tap water	Wuhan (China)	2019				4.1 (32) 100%						Wan 2020
Tap water	North Vietnam	2019	0.07 (0.74)	0.04 (0.46)		0.15 (2.84)				0.19 (2.86)	0.7 (7.98)	Wan 2021
Treated water	Japan (12 cities)	2012–2017		20%	>100 (2,200) 4%	>100 3%						Kamata 2020
Treated water	Wuhan-Yangtze (China)	2018	2.86 (9.18)	1.18 (3.76)	0.16 (1.56)	8.1 (27.0)		0.98 (12.9)	0.04 (0.14)	4.69 (18.7)	20 (60.6)	Wan 2019
Treated water	Wuhan-Han (China)	2018	10.6 (17.2)	1.16 (1.64)	0.54 (1.34)	21.7 (51.5)		0.06 (0.18)	0.4 (0.8)	2.86 (7.48)	53.5 (119)	Wan 2019
Treated water	South Korea	2020	0.13 (0.69) 33%		23.5 (85.3) 100%	0.58 (1.69) 73%			0.008 (0.12) 7%	2.65 (9.07) 93%	26.8 (94.7) 100%	Kim 2021

Numbers indicate the geometric mean, the maximum in brackets, and the percentage of positive samples (greater than the limit of quantitation).
Abbreviations: ACE, acetamiprid; CLO, clothianidin; DIN, dinotefuran; IMI, imidacloprid; IMZ, imidaclothiz; NIT, nitenpyram; THC, thiacloprid; TMX, thiamethoxam; ALL, sum of NNIs.

decline in abundance since the mid-1990s, when these insecticides were first introduced in the agricultural areas where the moths breed (Mansergh and Heinze 2019).

Changes in abundance and composition of insect communities of an eggplant crop treated with granular imidacloprid were evident a few weeks after application. However, these effects diminished afterwards, owing to the dilution of concentrations in plant tissues and the dissipation of soil residues through leaching and run-off (Sánchez-Bayo *et al.* 2007). Apart from this study, no other work on the impacts of NNIs on terrestrial arthropod communities has been done in the Asia-Pacific region, even if this is perhaps the main issue concerning this class of insecticides: see reviews by Pisa *et al.* (2015; 2021). Impacts on pollinators are not discussed here because they are reported in a separate document.

4.4.2 Aquatic ecosystems

Aquatic organisms are continuously exposed to chemical residues in their environment, and when such residues linger in water and sediments for weeks and months, as is the case of NNIs, their impacts are determined by their chronic toxicity, not their short acute effects. Despite this, some risk assessments still evaluate the impacts of NNIs against the acute endpoints (Nagai and Yokoyama 2012; Furihata *et al.* 2019), thus underestimating the real impacts observed in mesocosms and monitoring studies.

Ecological impacts have been determined in controlled mesocosm studies with individual compounds. In Japan, rice paddy mesocosms treated with imidacloprid revealed the disappearance of midges (*Chironomus* sp.) and epibenthic crustaceans such as seed-shrimps (Ostracoda), and decreased the abundance of mayflies (Ephemeroptera), water bugs (Hemiptera), water beetles (Coleoptera), and damselflies (Odonata), when concentrations were above 1 µg/L (Sánchez-Bayo and Goka 2006a; Hayasaka *et al.* 2012). Effects were more severe after a subsequent treatment the following season (Hayasaka *et al.* 2013b). Similarly, imidacloprid decreased the abundance and emergence of mayflies and decreased survival of chironomid species (Tanypodinae and Orthocladiinae) in lentic microcosms at time-weighted average concentrations of 2.3 µg/L; at the same time, water snails (*Radix* sp.) increased in numbers, owing to a lack of competition for food sources (Colombo *et al.* 2013). In Bangladesh, freshwater microcosms were treated with variable concentrations of imidacloprid up to 3 µg/L. Significant effects were observed on the zooplankton and macroinvertebrate communities, some individual phytoplankton taxa, and water quality variables; mayflies (*Cloeon* sp.), copepods (*Diaptomus* sp.), and rotifers (*Keratella* sp.) were the species most affected (Sumon *et al.* 2018). In New Zealand, stream mesocosms were treated with imidacloprid up to 4.6 µg/L in several pulses in combination with variable water temperatures. Both the insecticide and high temperatures decreased the abundance of insects, in particular the most sensitive EPT (Ephemeroptera, Plecoptera and Trichoptera) species, while increasing the proportion of tolerant EPT species, snails, and oligochaete worms; the impacts of imidacloprid were more obvious in cold waters (Macaulay *et al.* 2021c). Similar findings have been reported in other parts of the world with other stressors (Rico *et al.* 2018; Barmentlo *et al.* 2019).

Interactions between imidacloprid and food scarcity have also demonstrated that the impact of both stressors combined amplify the already negative effect of the insecticide alone (Haan *et al.* 2019). It is known that one of the sublethal effects of imidacloprid on crustaceans is feeding inhibition (Nyman *et al.* 2013), and since this effect occurs at concentrations well below the levels for survival, it affects the growth and development of shrimp. In fact, imidacloprid exposure reduced the ability of post-larval shrimp (*Penaeus monodon*) to capture live prey at environmentally realistic concentrations, such as those found in some aquaculture shrimp farms in Australia (Hook *et al.* 2018).

The impacts of imidacloprid on functional freshwater invertebrate communities in the urban and rice-paddy-dominated regions of Japan have been analysed. Significant reductions in the abundance of six functional groups (shredders, filter feeders, collectors, grazers, predators, and scavengers) were associated with imidacloprid concentrations, whereas dinotefuran concentrations did not seem to have any consistent pattern (Takeshita *et al.* 2020). The pronounced decline of iconic *Sympetrum* dragonflies (akatombo) in Japan in the past two decades has been linked to the use of NNIs and fipronil insecticides in rice farming (Ueda and Jinguji 2013; Nakanishi *et al.* 2018; Nakanishi *et al.* 2020). It is clear that both insecticides are extremely toxic to nymphs of dragonflies (Jinguji *et al.* 2013), while paddies that do not apply them have greater abundances of adults and nymphs than conventionally treated paddies (Baba *et al.* 2019). The NNIs clothianidin, dinotefuran, and thiamethoxam showed significant negative impacts on the abundance of lentic and benthic dragonfly nymphs in rural and urban areas of Kyushu (Japan), although residues of two other insecticides also showed negative relationships (Tazunoki *et al.* 2022). Mixtures of NNIs in Lake Shinji are also blamed for the decline of the planktonic and macrobenthic fauna that provides food to fish, since other pesticides and environmental factors were unrelated to the declines. Indeed, since the introduction of imidacloprid to rice paddies in the mid-1990s, the marked reduction of invertebrates has been followed by the disappearance of smelt fish (*Hypomesus nipponensis*) and declines in other two fish species (eel and ice fish) to levels that make fishing uneconomical; obviously, the indirect effect on the fish is due to the starvation that results from scarcity of their main food source (Yamamuro *et al.* 2019).

Imidacloprid was identified as having the highest chronic risk to aquatic organisms among 12 pesticides used in rice-prawn farming in Bangladesh, while the risk of thiamethoxam could not be determined owing to a lack of relevant toxicity data (Sumon *et al.* 2016). Only imidacloprid detected in brackish waters (salinity < 10‰) in Japan exceeded the benchmarks for freshwater invertebrates and, therefore, represented a risk to crustaceans and other invertebrates (Hano *et al.* 2019). Equally, imidacloprid was the only NNI that exceeded the benchmark for environmental protection in the Yangzte River basin (Mahai *et al.* 2019). It was the only insecticide that approached a risk quotient of 1 in surface waters in Queensland (Australia), which accounted for 26% of the total mixture toxicity (Spilsbury *et al.* 2020). However, the risk posed by imidacloprid in the rivers of that region was estimated as low, with 74% of samples protecting at least 99% of aquatic species. Nevertheless, it was estimated that up to 42% of species would experience harmful chronic effects, especially when residue levels are increasing (Warne *et al.* 2022).

An ecological risk assessment of the exposure to current environmental concentrations of imidacloprid and five other NNIs in the Pearl River system suggests a threat to sensitive non-target invertebrates, such as aquatic insects, since 67% to 87% of waters exceeded the chronic thresholds (Zhang *et al.* 2019; 2020b). In the Yangtze River system, 27% and 84% of samples exceeded the thresholds for acute (362 ng/L) and chronic (58 ng/L) ecological protection (Chen *et al.* 2019b). In a survey of rivers throughout Japan, only dinotefuran exceeded the acute ecological threshold of 3.8 µg/L (Furihata *et al.* 2019). In China, a national study has indicated that losses of agricultural pesticides to the aquatic environment increased by 30% between 2004 and 2013, while more than 50% of farmland areas were identified as high risk (Sun *et al.* 2019). This has prompted the authorities to establish guidelines for NNIs, to protect aquatic ecosystems against their clear and present threat (Wang *et al.* 2022).

4.5 Conclusion

In the Asia-Pacific region, research on the environmental impacts of NNIs has followed the research done in other parts of the world. The physico-chemical characteristics of newly developed compounds within this class, namely cycloxaprid and paichongding, are still lacking and require further research, more specifically their acute and chronic toxicological features, their fate and transport, including persistence and biodegradability, and the toxicity of their metabolites.

Monitoring studies of the residues in soil, water, and sediments are, on the contrary, numerous and comprehensive, and help us to understand the sources and extent of the contamination with these soluble and mobile pesticides. In general, the findings are consistent with the known widespread contamination of surface waters in North America and Europe.

As NNIs were first developed in Japan, it is not surprising that the first studies on ecological impacts of this new class of systemic insecticides on aquatic ecosystems were performed in this country. In fact, such studies prompted similar research in Canada and Germany (Kreutzweiser *et al.* 2007; Beketov *et al.* 2008; Pestana *et al.* 2009), and later in other countries (Rico *et al.* 2018; Barmentlo *et al.* 2021; Macaulay *et al.* 2021a) as efforts intensified worldwide to comprehend the ecological damage inflicted on aquatic communities. Indeed, impacts on the aquatic environment were underestimated, and unlike the impacts of NNIs on bees, they were often dismissed as irrelevant until the collapse of fisheries in certain parts of Japan (Yamamuro *et al.* 2019) brought them to the world's attention.

Current contamination levels are expected to increase, owing to continuous use in all kinds of crops and urban settings, and the persistence of parent compounds and their toxic metabolites. Consequently, the environmental risks of NNIs, particularly those to the aquatic environment, will only get worse, unless restrictions are applied.

5 Risk assessment of neonicotinoids on pollinator populations

Chapter summary

Bees are important pollinators for about 70% of cultivated crops, accounting for 30% of the total food production of the world. Exposure to neonicotinoid insecticides (NNIs), a group of synthetic pesticides, seems to have a negative effect on honeybee health and has resulted colony loss in several regions of the world, especially Europe and North America during the past two decades. To understand the current situation of the effect of NNIs on pollinator populations in Asia, we have systematically reviewed the available scientific evidence from Asian and Oceania countries. In the context of honeybee colonies in Asian countries, two different trends have been observed. Countries such as China, Korea, India, Iran, Israel, Myanmar, Pakistan, Turkey, and Vietnam have shown increasing honeybee hive numbers during the past three decades. In contrast, Australia, Japan, Mongolia, Taiwan, and Uzbekistan have shown either decreasing trends or no change. This review in this chapter represents the diverse situation of Asian honeybees in relation to population dynamics and possible NNI exposure, NNI toxicity to honeybees and other pollinators such as bumblebees and solitary bees, geographical and environmental conditions, and patterns of insecticide use.

5.1 Introduction

Bees are the most important pollinators for about 70% of cultivated crops, accounting for 30% of the total food production of the world (Nabhan and Buchmann 1997; Klein *et al.* 2007; Ramírez *et al.* 2018). Although thousands of species, primarily bees, participate in crop pollination, only a few (honeybees, bumblebees) have been commercialised as pollinators. The most widely used managed pollinator, being a generalist and with the longest history of domestication, is the honeybee (*Apis* spp.). Apart from nutritional stress due to habitat loss, pest and pathogen burden, pesticide (including neonicotinoid) exposure has also been a major cause of honeybee colony loss in several regions of the world. Exposure to neonicotinoid insecticides (NNIs) has been reported to disrupt the nest behaviour of bumblebees and affect their thermoregulation and social network (Crall *et al.* 2018). NNIs, being a group of synthetic insecticides, are also environmental pollutants and, more importantly, non-target toxic agents (Sánchez-Bayo and Goka 2014; Sánchez-Bayo *et al.* 2016; Pagano *et al.* 2020; Zhao *et al.* 2020). The use of this class of insecticides is of particular concern because of their potential non-target effect primarily on insect pollinators, in particular bees. At first, information came out about huge bee-colony losses in 2006 in the USA which drew the attention of honeybee researchers. Since the approval and initial marketing of NNIs, three decades have been passed. During this period, many investigations have provided evidence of the toxic effect of NNIs not only on bees but also on many different terrestrial and aquatic animals (Sánchez-Bayo 2014). Other concerns include the development of resistance in some target pests (Özdemir and Yorulmaz 2021) and the long-term persistence of NNIs in the environment (Frank and Tooker 2020). Despite having a voluminous dataset on the lethal and sublethal effects of NNIs on bees and other terrestrial and aquatic organisms, information on the actual field-based use of these insecticides is limited. Concern has been reflected by the increasing number of publications on 'NNIs and honeybees' over recent years (Figure 5.1a). As a responsive policy, a few countries have imposed time-limited restrictions on the use of three NNIs: clothianidin, thiamethoxam, and imidacloprid.

Following the reports published on NNI use and their effects on agriculture, associated ecosystems, and the environment in Europe and Africa (EASAC 2015; NASAC 2019), on the basis of available scientific evidence, we need to look at the context of Asia and Oceania. Environmental conditions, agricultural practices, crop patterns, harvesting methods, insecticide application, etc. are remarkably different in Asia from European, American, or African counterparts. To focus on agricultural practice, in Asia most of farmers are smallholders unlike in Europe and North America. The foliar application of NNIs is mainly used here whereas seed treatment predominates in Europe and North America. There is increasing attention being paid to NNIs in Asian and Oceanian countries, reflected by the number of publications in different countries from 2015 to 2022 (Figure 5.1b). In this chapter, we focus on the concerns about NNIs as they relate to honeybees and other pollinators.

5.2 Trend analysis of honeybee hives in Asian countries

Table 5.1 shows the trend in honeybee hive numbers from 1990 (the year NNIs were introduced to the market) to 2020 in different Asian countries on the basis of the available data from FAOSTAT. Two different trends can be observed. Countries such as China, Korea India, Iran, Israel, Myanmar, Pakistan, Turkey, and Vietnam have shown an increasing trend in honeybee hive numbers during the period. In contrast, Australia, Japan, Mongolia, Taiwan, and Uzbekistan followed either a decreasing trend or showed no change. As

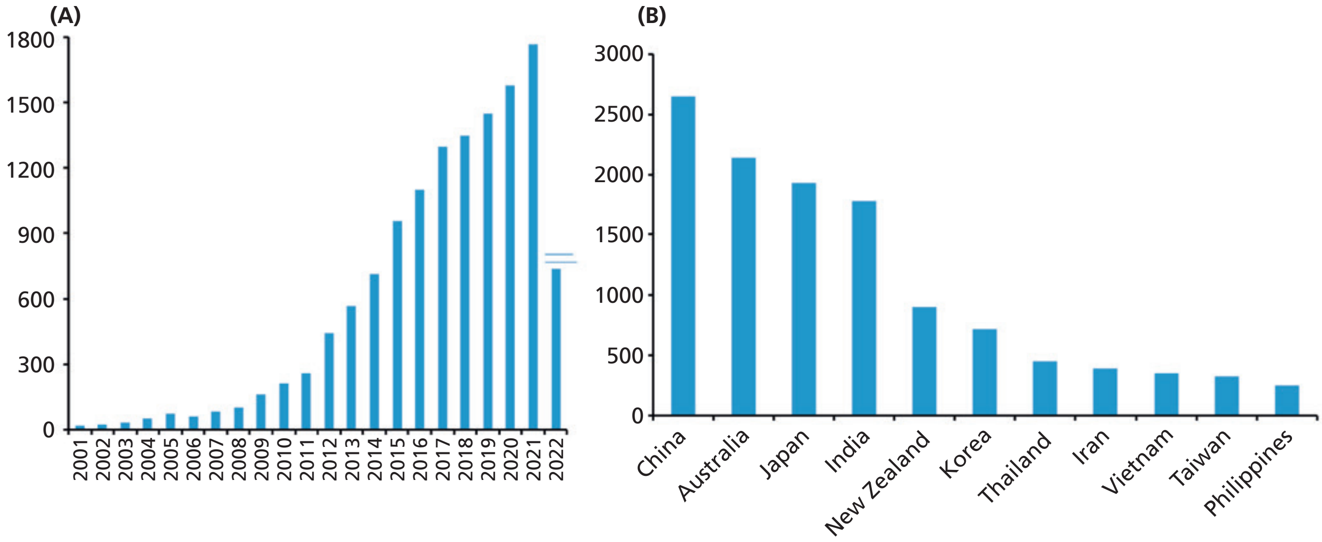


Figure 5.1 (A) Number of hits in Google Scholar on 'neonicotinoids and honeybees' by selected year; (B) number of hits by country in Google Scholar on 'risk of neonicotinoids on pollinator honeybees' from 2015 to 2022.

Table 5.1 Changes in beehive numbers from 1990 to 2020 in Asian countries (data adopted from FAOSTAT)

Country	Trend line equation	Coefficient (R^2)
Australia	$y = 2785.9x + 367814$	0.0788
China	$y = 93996x + 7\times10^6$	0.8138
India	$y = 103689x + 9\times10^6$	0.8197
Iran	$y = 204597x + 836855$	0.8477
Israel	$y = 1523.3x + 64021$	0.8676
Japan	$y = -1216.3x + 216040$	0.3953
Mongolia	$y = 239.63x - 158.55$	0.4475
Myanmar	$y = 2705.3x - 11391$	0.8376
Pakistan	$y = 14122x + 32296$	0.8384
South Korea	$y = 58524x + 588308$	0.8076
Taiwan	$y = -26.252x + 118679$	8×10^{-5}
Turkey	$y = 171080x + 3\times10^6$	0.8976
Uzbekistan	$y = 9078.7x + 132447$	0.2614
Vietnam	$y = 6100.6x + 96870$	0.9005

mentioned earlier, the losses of honeybee colonies are linked to several factors such as habitat loss, pests, parasites, and pesticides (Moritz and Erler 2016) and for the simple reason that the magnitude of the factors are not same everywhere. The global scale analysis did not show any general colony decline. Western Europe and the USA suffered colony declines whereas other regions showed an increase (Moritz and Erler 2016). This diverse situation may be attributed to different commercial honeybee species and their different responses to NNIs, geographical and environmental conditions, and of course patterns of insecticide use (Li *et al.* 2017; Yue *et al.* 2018; Gao *et al.* 2020).

Apis mellifera is generally reared in Europe and Africa. In Asia two species, *Apis mellifera* and *Apis cerana*, are used in commercial apiaries. However, a clear decline in the population of *A. cerana* has been observed over the past few decades (Theisen-Jones and Bienefeld 2016). Presumably, the *A. cerana* colonies have been replaced by *A. mellifera* as the latter produces more honey, and apiaries are generally focused on honey production. Jung and Cho (2015) reported the changing pattern of beekeeping using *A. cerana* and *A. mellifera* over time. In Vietnam, *A. mellifera* colonies increased by approximately 33 times from 1990, and currently account for 76.7% of all hives in the country (Thai *et al.* 2018). Although the absence of a database on colony numbers based on honeybee species for most countries in Asia is a limitation, the different species involved in commercial beekeeping might have different responses to the NNIs which could be one reason for the two different trends.

5.3 Mechanism of neonicotinoid toxicities to honeybees

NNIs are neurotoxins affecting the nervous system of target insects as well as non-target honeybees. Nicotinic acetylcholine receptors (nAChRs) belong to the cys-loop superfamily of ligand-gated ion channels which is 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. NNIs are recognised as nAChR agonists, namely a substance that initiates a physiological response when combined with a receptor. Upon prolonged and repeated exposure to NNIs, desensitisation occurs at the receptors resulting in the initial opening of the ion channel, ion exchange across the cell membrane, followed by rapid channel closure, effectively inhibiting neurotransmission (LaLone *et al.* 2017). The alterations in gene transcripts such as increasing abundance of the nAChR α1-subunit in the brain, vitellogenin, and genes related to immune and memory formation may occur in response to NNIs (Christen *et al.* 2017).

5.4 Acute toxicity to pollinators

Table 5.2 represents the LC_{50} (the concentration lethal to 50% of animals tested) and LD_{50} (the dose lethal to 50% of animals tested) values for oral as well as contact exposure to different NNIs of honeybees obtained from the available scientific literature. The wide range of toxicity values is attributed not only to different bee species but also to several variables such as the developmental stage of insects, the route of insecticide exposure, the genetic makeup of the insect, the environment, etc.

5.4.1 *Apis mellifera*

Apis mellifera is the bee that has been studied most widely of all pollinators in the world. In the context of

Table 5.2 Toxicity (LC_{50} and LD_{50}) of different classes of neonicotinoid on pollinator populations in Asia

A.I. (%)	Target organism (scientific name)	Stage	LC_{50} (p.p.m.)		LD_{50} (µg/insect)		Check time (hour)	Country	Reference
			Contact	Oral	Contact	Oral			
Imidacloprid									
98	*Apis cerana japonica*	A (NE)			0.008		24	Japan	Yasuda *et al.* 2017
98	*Apis cerana japonica*	A (NE)			0.004		48		
99	*Bombus terrestris*	A			0	–	4	Korea	Kim *et al.* 2020
99	*Bombus terrestris*	A			11.27	0.815	24		
99	*Bombus terrestris*	A			3.967	0.325	48		
99	*Bombus terrestris*	A			3.288	0.245	72		
99	*Bombus terrestris*	A			1.643	0.168	96		
10	*Apis mellifera*	A		0.01			12		Lee *et al.* 2016
10	*Apis cerana*	A		0			12		
10	*Apis florea*	A		2.95			12		
10	*Apis dorsata*	A		1.92			12		
10	*Apis mellifera*	A			1.79	0.109	24		Begna and Jung 2021
10	*Apis mellifera*	A			0.82	0.001	48		
	Apis mellifera	A				0.0086	24	China	Li *et al.* 2017
	Apis cerana	A				0.0027	24		
97	*Apis mellifera*	A (F)		8.23			48		Yue *et al.* 2018
97	*Apis cerana*	A (F)		16.5			48		
	Trigona iridipennis	A			0.002		24	India	Kumar *et al.* 2005
	Apis cerana	A			0.003		24		
	Apis florea	A			0.002		24		
	Apis mellifera	A			0.0026		24		
17.8	*Apis cerana indica*	A			0.027		24		Jeyalakshmi *et al.* 2011
99.9	*Apis mellifera*	A			0.037		24		Ram *et al.* 2017
17.8	*Apis mellifera mellifera*	A	0.007				24		Pashte and Patil 2018
17.8	*Apis cerana indica*	A	32.26				24		Vinothkumar *et al.* 2020
99	*Apis mellifera*	A		0.6			240	Israel	Bommuraj *et al.* 2021
99	*Apis mellifera*	A		2.2			48		

Continues

Table 5.2 (continued)

A.I. (%)	Target organism (scientific name)	Stage	LC$_{50}$ (p.p.m.)		LD$_{50}$ (µg/insect)		Check time (hour)	Country	Reference
			Contact	Oral	Contact	Oral			
Thiamethoxam									
99	*Apis cerana japonica*	A (NE)			0.003		24	Japan	Yasuda *et al.* 2017
99	*Apis cerana japonica*	A (NE)			0.0024		48		
10	*Apis mellifera*	A		0.006			12	Korea	Lee *et al.* 2016
10	*Apis cerana*	A		0.003			12		
10	*Apis florea*	A		0.031			12		
10	*Apis dorsata*	A		0.088			12		
99	*Bombus terrestris*	A			17.749	0.047	4		Kim *et al.* 2020
99	*Bombus terrestris*	A			0.78	0.018	24		
99	*Bombus terrestris*	A			0.747	0.018	48		
10	*Apis mellifera*	A				0.019	24		Begna and Jung 2021
10	*Apis mellifera*	A				0.034	48		
96	*Apis mellifera*	A (F)		24.09			48	China	Yue *et al.* 2018
96	*Apis cerana*	A (F)		37.34			48		
	Apis mellifera	A			0.0061		24	India	Kumar *et al.* 2005
	Trigona iridipennis	A			0.0051		24		
	Apis cerana	A			0.0056		24		
	Apis florea	A			0.0056		24		
25	*Apis cerana*	A			0.026		24		Jeyalakshmi *et al.* 2011
25	*Apis cerana indica*	A	30.61				24		Vinothkumar *et al.* 2020
	Apis mellifera lamarckii	A (F)	8.05	0.22			48	Turkey	Shah *et al.* 2020
Clothianidin									
99	*Apis cerana japonica*	A (NE)			0.004		24	Japan	Yasuda *et al.* 2017
99	*Apis cerana japonica*	A (NE)			0.003		48		
8	*Apis mellifera*	A		0.007			12	Korea	Lee *et al.* 2016
8	*Apis cerana*	A		0.083			12		
8	*Apis florea*	A		0.036			12		
8	*Apis dorsata*	A		0.22			12		
99	*Bombus terrestris*	A			0.467	0.005	48		Kim *et al.* 2020
8	*Apis mellifera*	A				0.003	24		Begna and Jung 2021
>98	*Apis mellifera*	A				0.002	24	China	Li *et al.* 2017
	Apis cerana	A				0.0005	24		
50	*Apis cerana indica*	A			0.014		24	India	Jeyalakshmi *et al.* 2011
50	*Apis cerana indica*	A	40.31				24		Vinothkumar *et al.* 2020
Nitenpyram									
95.9	*Apis mellifera*	A (F)		52.14			48	China	Yue *et al.* 2018
95.9	*Apis cerana*	A (F)		84.48			48	China	Yue *et al.* 2018

Continues

Table 5.2 (continued)

A.I. (%)	Target organism (scientific name)	Stage	LC_{50} (p.p.m.)		LD_{50} (µg/insect)		Check time (hour)	Country	Reference
			Contact	Oral	Contact	Oral			
Dinotefuran									
99	*Apis cerana japonica*	A (NE)			0.0014		48	Japan	Yasuda *et al.* 2017
20	*Apis mellifera*	A			0.138		24	Korea	Ulziibayar and Jung 2019
20	*Apis mellifera*	A			0.14		48		
98	*Bombus terrestris*	A			10.456	1.523	4		Kim *et al.* 2020
98	*Bombus terrestris*	A			3.867	0.058	24		
98	*Bombus terrestris*	A			3.741	0.056	48		
95	*Apis mellifera*	A (F)		48.48			48	China	Yue *et al.* 2018
95	*Apis cerana*	A (F)		50.69			48		
99	*Apis cerana japonica*	A (NE)			0.023		24		
20	*Apis cerana indica*	A	103.28				24	India	Vinothkumar *et al.* 2020
Acetamiprid									
97	*Apis cerana japonica*	A (NE)			0.22		24	Japan	Yasuda *et al.* 2017
97	*Apis cerana japonica*	A (NE)			0.278		48		
8	*Apis mellifera*	A	>500				48	Korea	Begna and Jung 2021
96	*Apis mellifera*	A (F)		353.4			48	China	Yue *et al.* 2018
96	*Apis cerana*	A (F)		285.3			48		
97.4	*Apis mellifera ligustica*	A			26		48		Chen *et al.* 2019
5	*Apis mellifera ligustica*	A			>1000		48		
5	*Apis mellifera ligustica*	A			357		48		
5	*Apis mellifera ligustica*	A			1436		48		
70	*Apis mellifera*	A (NE)		365.5			48		Wang *et al.* 2020
70	*Apis mellifera*	A (NE)		251.9			96		
70	*Apis mellifera*	A (NE)		187.6			168		
99.7	*Apis mellifera*	A		188.5		5.65	72		Yang *et al.* 2020
20	*Apis cerana indica*	A	54.27				24	India	Vinothkumar *et al.* 2020
99	*Apis mellifera*	A		98.9			240	Israel	Bommuraj *et al.* 2021
99	*Apis mellifera*	A		277.3			48		
Thiacloprid									
10	*Apis mellifera*	A			6.109		24	Korea	Ulziibayar and Jung 2019
10	*Apis mellifera*	A			33.1		48		
21.8	*Apis cerana indica*	A	96.86				24	India	Vinothkumar *et al.* 2020
99	*Apis mellifera*	A		58.4			240	Israel	Bommuraj *et al.* 2021
99	*Apis mellifera*	A		194			48		

Abbreviations: A, adult; F, forager; NE, newly emerged.

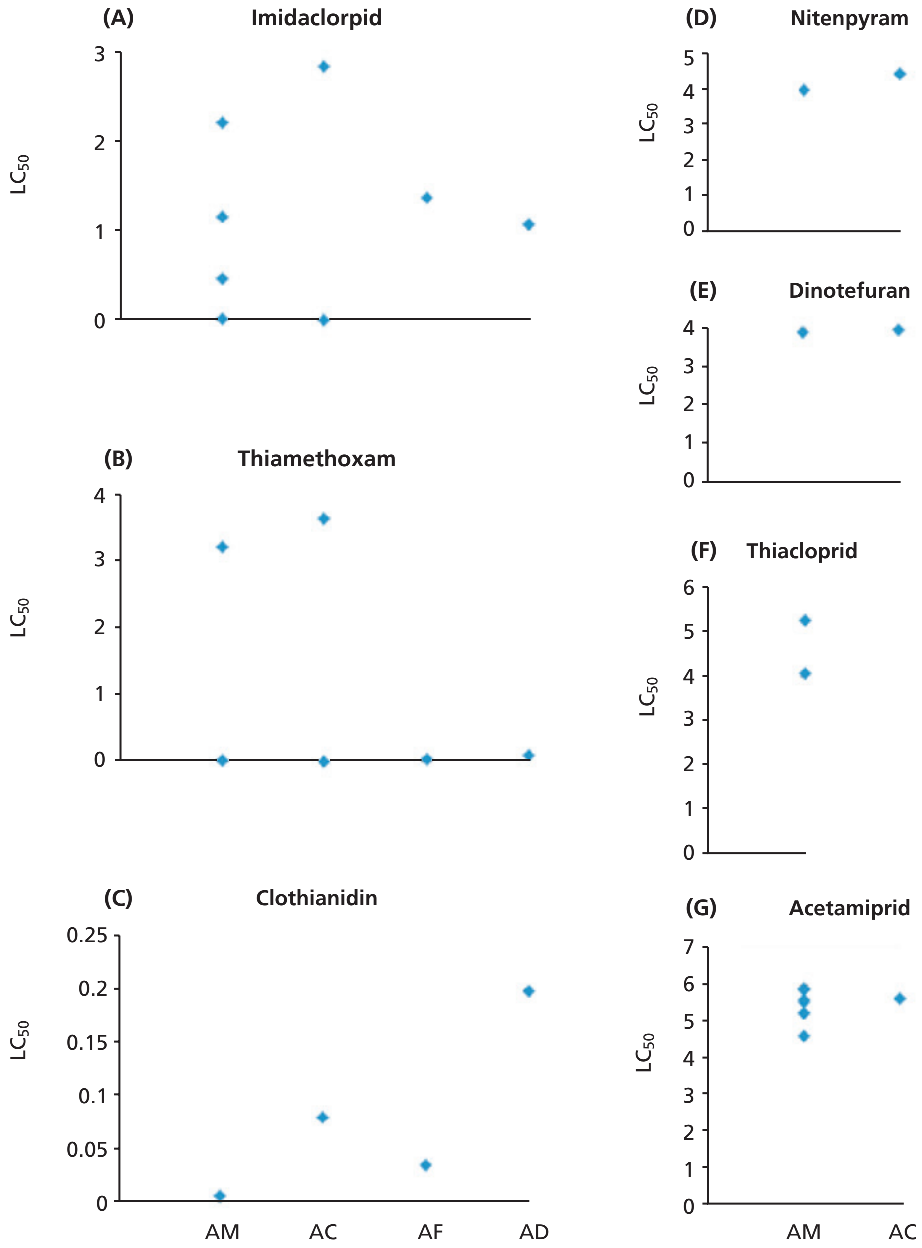


Figure 5.2 LC_{50} oral toxicity of NNIs to different honeybee species at 12–240 hrs after treatment. Studies considered in the graphical representation were conducted in Asia. LC_{50} values were obtained from Lee et al. (2016), Yue et al. (2018), Yasuda et al. (2017), Yang et al. (2020), Bommuraj et al. (2021), and transformed into ln (n+1). AM, Apis mellifera; AC, Apis cerana; AF, Apis florea; AD, Apis dorsata.

Asia there are two honeybee species in commercial beekeeping: *Apis mellifera* and *Apis cerana*. The trend in the results of oral LC_{50} values obtained from laboratory tests reveals that clothianidin, thiamethoxam, and imidacloprid are highly toxic to honeybees (Table 5.2). Acetamiprid and thiacloprid, being structurally different, are less toxic. The higher toxicities of clothianidin, thiamethoxam, and imidacloprid are also reflected by the LC_{50} and LD_{50} values. Figure 5.2 represents a comparative toxicity (oral LC_{50}) of different NNIs to different honeybee species on the basis of the available literature from Asia. Although the values differ, presumably because of different species' physiological and methodological variations, overall imidacloprid, thiamethoxam, and clothianidin are found to be highly toxic compared with nitenpyram, dinotefuran, acetamiprid, and thiacloprid. Similarly, the LD_{50} oral toxicities of imidacloprid, thiamethoxam, and clothianidin to *A. mellifera* at 24 hours after treatment, as shown in Figure 5.3, are the highest of these NNIs.

5.4.2 *Apis cerana*

The Asian honeybee *Apis cerana* has been raised as the primary honeybee species in indigenous culture across Asia. Even so, the species is also found in Australia,

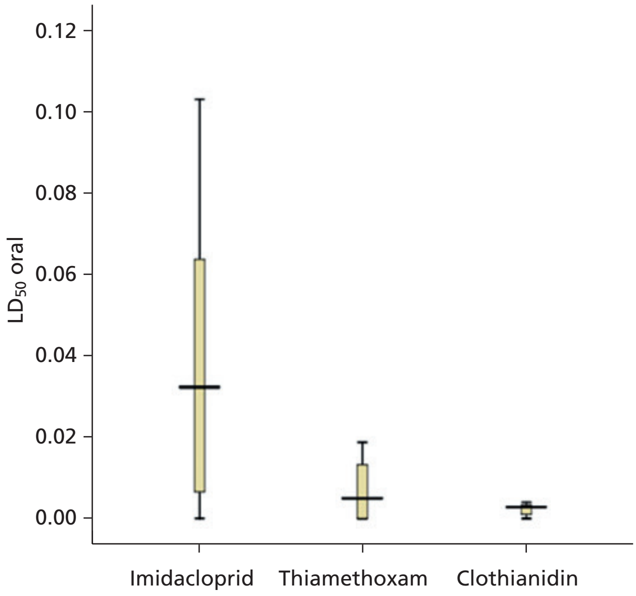


Figure 5.3 LD_{50} oral toxicity of imidacloprid, thiamethoxam, and clothianidin to Apis mellifera at 24 hours after treatment. LD_{50} values were obtained from Colin et al. (2004), Decourty et al. (2004), Prabhaker et al. (2007), Rafael Valdovinos-Nunez et al. (2009), Laurino et al. (2011), Akeju (2014), Retschnig et al. (2015), Lewis et al. (2016), Wu-Smart and Spivak (2016), Baines et al. (2017), Samson-Robert et al. (2017), Li et al. (2017), Abbo et al. (2017), Uliziibayar and Jung (2019), Taleh et al. (2020), Mazi et al. (2020), Reid et al. (2020), Begna and Jung (2021), and transformed to ln (n+1).

Papua New Guinea, and some other Pacific islands such as Solomon Islands where it has been introduced (Koetz 2013, *cf.* Theisen-Jones and Bienefeld 2016). However, the decline of the species has been accelerated by several factors. From the commercial point of view, the western honeybee has been promoted and introduced in almost in every part of the world except Antarctica. With the increasing overemphasis and promotion of European *A. mellifera*, less attention was paid to *A. cerana*. A study by Theisen-Jones and Bienefeld (2016) demonstrated, on the basis of respondents' estimations to a survey across Asian countries, that populations (commercial colonies) of *A. cerana* decreased by 55%. However, the decrement values vary in different countries, roughly 5% in Bhutan to 95% or higher in Thailand, and trend of decreasing populations of *A. cerana* continues (Theisen-Jones and Bienefeld 2016). Jung and Cho (2015), in their detailed analysis of commercial honeybee populations, showed an increasing trend for *A. mellifera* and a decreasing trend for *A. cerana* over time in Korea.

In the context of ecotoxicity to *A. cerana*, in a study (Li *et al.* 2017) comparing *A. mellifera* and *A. cerana*, it was found that *A. cerana* was more sensitive to imidacloprid and clothianidin. The acute oral LD_{50} values of imidacloprid and clothianidin for *A. mellifera* were 8.6 and 2.0 ng per bee, respectively, whereas the corresponding values for *A. cerana* were 2.7 and 0.5 ng per bee. Lee *et al.* (2016) found that the LC_{50} values for *A. mellifera* and *A. cerana* were found to be significantly lower than those for *Apis florea* and *Apis dorsata*. However, between *A. mellifera* and *A. cerana*, the latter was found to be more sensitive, having lower LC_{50} values than the former. Yasuda *et al.* (2017) showed that the LD_{50} values for insecticides including NNIs were lower for *A. cerana japonica* than those previously reported for *A. mellifera*. Among the NNIs tested in the experiment, on the basis of the 48-hour LD_{50} value, *A. cerana japonica* was found to be more sensitive to dinotefuran followed by thiamethoxam. On the contrary, Yue *et al.* (2018) demonstrated the higher sensitivity of *A. cerana* for dinotefuran, nitenpyram, imidacloprid, and thiamethoxam as lower LC_{50} values were obtained compared with *A. mellifera*. Tan *et al.* (2015) reported that imidacloprid impaired olfactory learning in *A. cerana*. The study revealed that honeybee larvae exposed to a dose of 0.24 ng per bee did not show any reduced survival to adulthood but exhibited significantly impaired olfactory learning in the adult stage (Tan *et al.* 2015). Studies conducted by Matsumoto (2013a,b) indicated that clothianidin spraying in rice fields increased the mortality of honeybees but did not always cause colony collapse. Saleem *et al.* (2020) demonstrated that honeybees (*A. mellifera*) were more sensitive to NNIs, namely imidacloprid and thiamethoxam, at a constant temperature of 24 °C or at a varying temperature of 13 °C at night and 24 °C during the day than at 35 °C. Therefore, honeybee colonies during winter may be more susceptible to NNIs than in summer.

5.4.3 Possible causes of the response difference

Honeybee species possess distinct mechanisms to mount an innate immune response against NNI exposure. To generalise, the different body sizes of insects, especially the ratio of surface area to volume, may contribute to different sensitivities towards NNIs. The body size and weight of *A. mellifera* are 12–14 mm and 70–120 mg, respectively, whereas the corresponding values for *A. cerana* are 10–13 mm and 60–90 mg, and this difference could presumably be a cause of their different response to pesticides. Yue *et al.* (2018) suggested that the sensitivity of different species of honeybees such as *A. mellifera* and *A. cerana* is closely associated with the chemical structure of pesticides. Another possible cause for different sensitivities between species is the differences in the expression of genes related to detoxification and antioxidants. In contrast to upregulation of carboxylesterase, prophenoloxidase, and acetylcholinesterase activities in *A. cerana*, they were significantly downregulated in *A. mellifera* after 48 hours of imidacloprid treatment whereas during clothianidin exposure acetylcholinesterase was downregulated, and glutathione *S*-transferase activity was upregulated in both species. A different response was observed in different developmental stages of the honeybee: increasing activities of glutathione *S*-transferase and carboxylesterase *para* were found in the pupal stage in response to thiamethoxam exposure (Tavares *et al.* 2017). Strong downregulation of gene coding for major jelly proteins was observed in response to imidacloprid exposure which weakens bee colonies (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), the currency of biological energy. Further, mitochondria are also associated with several processes such as Ca^{2+} storage and release for cell signalling, heat production, mediating cell growth and cell death. Thus mitochondrial dysfunction manifests perturbations in these processes. However, uncertainty exists as to the presence of the nicotinic acetylcholine receptor (nAChR) in invertebrate mitochondria (LaLone *et al.* 2017).

5.4.4 Acute toxicity to bumblebees and solitary bees

Studies with honeybees and bumblebees (*Bombus terrestris*) demonstrated the adverse impacts on mitochondria while exposed to nAChR agonists (Nicodemo *et al.* 2014; Moffat *et al.* 2015). Moffat *et al.* (2015) described mitochondrial depolarisation (i.e. loss of membrane potential) in bumblebee neurons upon exposure to 1 nM imidacloprid for 48 hours. A change in the foraging behaviour of bumblebees in response to clothianidin has been confirmed by one recent study (Arce *et al.* 2017). The sublethal effects of NNIs could also scale up, causing loss of wild bee diversity (Woodcock *et al.* 2016). Kim *et al.* (2020) investigated the toxicity of NNIs on *B. terrestris* in Korea. However, toxicity data on solitary bees are largely absent in Asia. It is, therefore, of utmost importance to conduct toxicology studies on solitary or wild bees. Irrespective of the pollinator species, NNIs such as imidacloprid, thiamethoxam, and clothianidin show more toxicity than acetamiprid and thiacloprid (Figure 5.4). Figure 5.5 shows the LD_{50} contact toxicity values of NNIs to pollinator groups such as honeybees, bumblebees, and wild bees. Similarly, imidacloprid, thiamethoxam, and clothianidin were comparatively more toxic than acetamiprid and thiacloprid, and it has been shown that honeybees are more sensitive than bumblebees. Although studies on solitary or wild bees are limited, the analysis shows that solitary bees are more highly sensitive than honeybees or bumblebees.

5.5 Sublethal exposure to pollinators

Table 5.3 presents the sublethal effects of NNIs on different pollinator populations. Although most of the field or semi-field studies were conducted outside Asia, they could be a suitable guide to understanding the sublethal effects on pollinator populations. Overall, sublethal effects include behavioural ones such as foraging and hygienic behaviour, olfactory learning and memory, physiological ones such as weakening immune response, downregulating genes related to metabolism, developing ataxia and hyperactivity, etc.

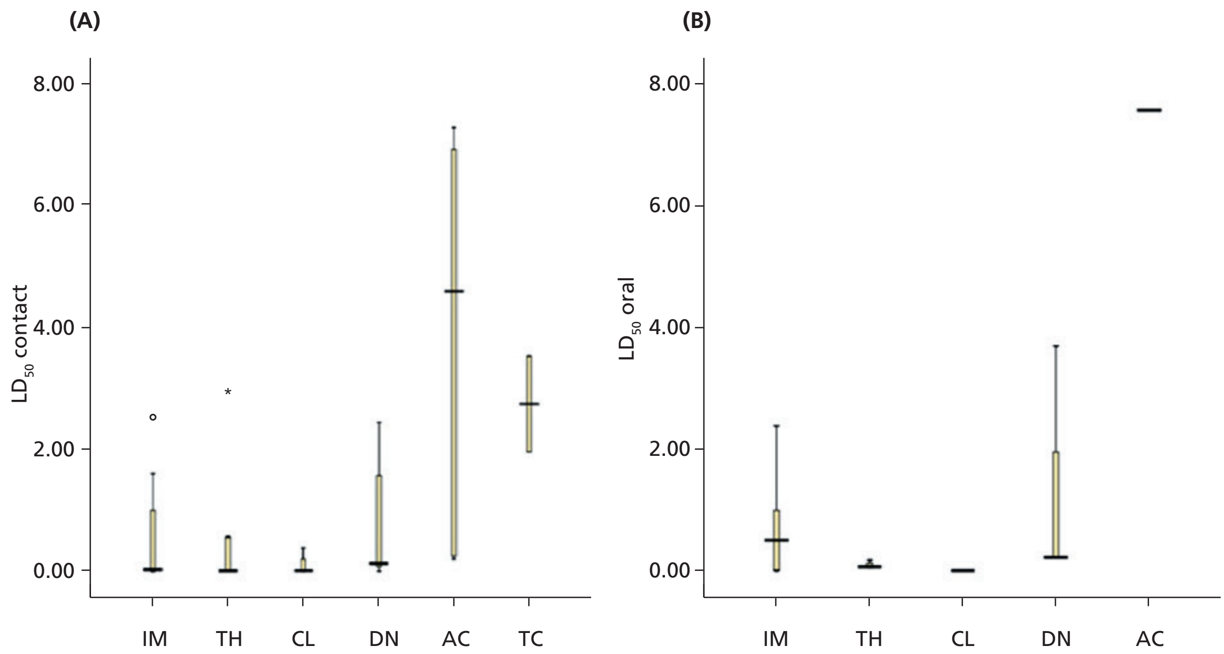


Figure 5.4 Toxicity values of (A) LD_{50} contact and (B) LD_{50} oral to pollinators including honeybees, bumblebees, and wild or solitary bees at 12–240 hours after treatment. LD_{50} values were obtained from Kumar et al. (2005), Jayalakshmi et al. (2011), Yasuda et al. (2017), Yue et al. (2018), Chen et al. (2019), Ulziibayar and Jung (2019), Kim et al. (2020), Begna and Jung (2021), and transformed into ln (n+1).

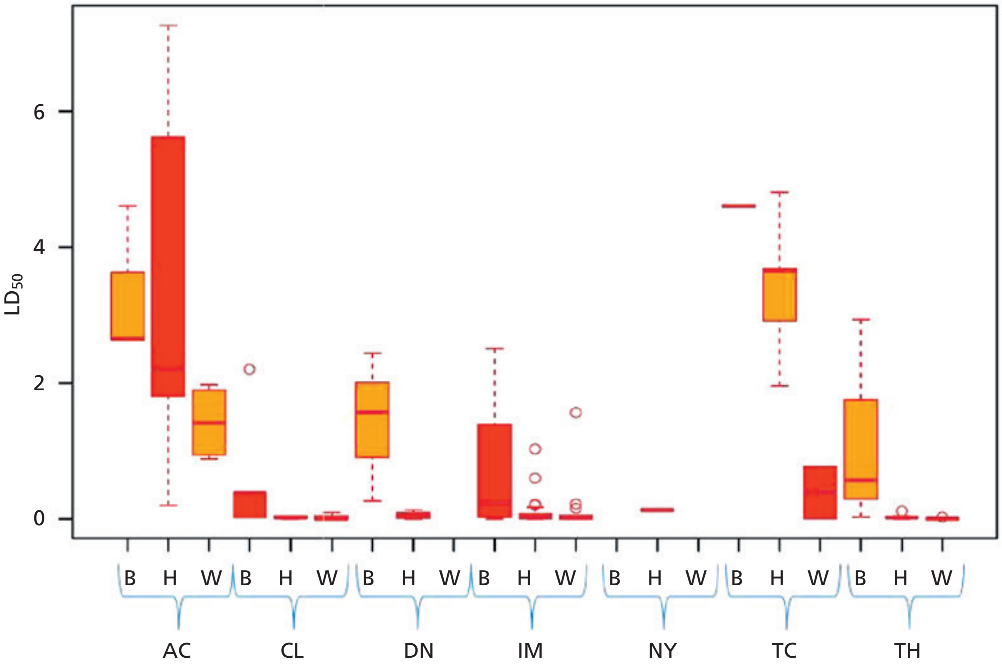


Figure 5.5 LD_{50} contact toxicity values of neonicotinoids to the pollinator groups at 12-240 hours after treatment. B, bumblebee; H, honeybee; W, wild bee; AC, acetamiprid; CL, clothianidin; DN, dinotefuran; IM, imidacloprid; NY, nitenpyram; TC, thiacloprid; TH, thiamethoxam. Data were obtained from Suchail et al. (2000), Schmuck et al. (2001), Nauen et al. (2001), Suchail et al. (2001), Armengaud et al. (2002), Bortolotti et al. (2002), Iwasa et al. (2004), Kumar et al. (2005), Rafael Valdovinos-Nunez et al. (2009), Jeyalakshmi et al. (2011), Bodiou-Bénéteau et al. (2012), DiPrisco et al. (2013), EFSA (2013), Stoner and Eitzer (2013), Chahbar et al. (2014), Poquet et al. (2014), Sanchez-Bayo and Goka (2014), Gill and Raine (2014), Thompson et al. (2014), da Costa et al. (2015), Soares et al. (2015), Zhu et al. (2015), Alptekin et al. (2016), Lewis et al. (2016), Powner et al. (2016), Auteri et al. (2017), Heard et al. (2017), Hussain et al. (2017), Samson-Robert et al. (2017), Stanley et al. (2017),), Yasuda et al. (2017), Bovi et al. (2018), Santos et al. (2018), Uhl et al. (2018), Yue et al. (2018), Chen et al. (2019), Ulziibayar and Jung (2019), Abbassey et al. (2020), Kim et al. (2020), Li et al. (2020), Piovesan et al. (2020), Reid et al. (2020), Vinothkumar et al. (2020), Begna and Jung (2021), Peterson et al. (2021), and transformed into ln (n+1).

Table 5.3 Sublethal effect of different neonicotinoids to different pollinator species

Pollinator	Stage	Sublethal exposure			Note	
		Dose (µg per insect)	Concentration (p.p.m.)	Sublethal effects	Field/ laboratory	Reference
Imidacloprid						
Apis mellifera	A		0.5, 1	Homing rate and foraging activity	Field	Bortolotti *et al.* 2003
	A (F)		0.006	Decreased activity of bees	Field	Colin *et al.* 2004
	A	0.012, 0.00012		Olfactory learning performances and increase cytochrome oxidase	Laboratory	Decourtye *et al.* 2004
	A		0.006–0.012	Learning abilities	Laboratory	Decourtye *et al.* 2003
	L	0.00004, 0.0004		Impaired olfactory associative behaviour	Field	Yang *et al.* 2012
	L	24–8000		Brood-capped rates	Field	Yang *et al.* 2012
	A (F)		0.05–6	Abnormal behaviour and affect foraging behaviour	Laboratory	Yang *et al.* 2008

Continues

Table 5.3 (continued)

Pollinator	Stage	Sublethal exposure			Note	
		Dose (µg per insect)	Concentration (p.p.m.)	Sublethal effects	Field/ laboratory	Reference
	A (F)		0.05, 0.5	Spent significantly more time near the food	Field	Teeters *et al.* 2012
	A	0.809, 8.09, 40.4		Cytotoxic activity in the malpighian tubules	Laboratory	Li *et al.* 2020
	A (NE)		0.001, 0.01	Reduced haemocyte density, encapsulation response, and antimicrobial activity	Laboratory	Brandt *et al.* 2016
	A		4.3	Feeding suppression	Field	Zhu *et al.* 2017
	A		0.01, 0.02, 0.05, 0.1	Egg-laying and locomotor activity, foraging and hygienic activities	Field	Wu-Smart and Spivak 2016
	L		0.002	Gene expression changes in the brain of adults	Field/ laboratory	Wu *et al.* 2017
	A (NE)		0.05	Health and survival of honeybees	Laboratory	Abbo *et al.* 2017
	A (NE)			Develop ataxia	Laboratory	Baines *et al.* 2017
	L		0.002	Downregulation of genes in glycolytic and sugar-metabolising pathways	Field	Derecka *et al.* 2013
	A			Reduced immune defense and promotes the replication of the deformed wing virus	Laboratory	DiPrisco *et al.* 2013
	L		1–100	Weakening effect on phagocytosis with but also without LPS activation	Laboratory	Wade *et al.* 2019
	A		10 nM	Affected short-term memory	Laboratory	Wright *et al.* 2015
	A		0.003, 0.03, 0.3	Affected foraging activity and decrease long-term memory formation	Laboratory	Christen *et al.* 2016
	A		0.05, 0.2, 0.5	Increased mortality in honeybees, but this mortality does not seem to be linked to the microbiome	Field/ laboratory	Raymann *et al.* 2018
	A (F)		10 µM	Disruption in odour coding and olfactory discrimination ability	Laboratory	Mengoni Goñalons and Farina 2015
	A (F)	0.00025, 0.0005		Gustatory responsiveness and impairment of learning and memory	Laboratory	Stanley *et al.* 2015
	A	0.00125, 0.0025, 0.005		Facilitated or inhibited neural metabolism	Laboratory	Armengaud *et al.* 2002
	A		0.0007, 0.007, 0.07	Weakened honeybee immune parameters with *Nosema* infection	Laboratory	Alaux *et al.* 2010
	A (F)		0.2	Short-term exposure to imidacloprid adversely affect honeybee foragers	Laboratory	Delkash-Roudsari *et al.* 2020
	A	0.0001, 0.001, 0.01		Altered the number of trials needed to habituate response to multiple sucrose stimulation	Laboratory	Guez *et al.* 2001

Continues

Table 5.3 (continued)

Pollinator	Stage	Sublethal exposure			Note	
		Dose (µg per insect)	Concentration (p.p.m.)	Sublethal effects	Field/ laboratory	Reference
	A	0.00125		Low dose can facilitate a simple form of learning in the honeybee	SField	Lambin *et al.* 2001
	A	0.0025, 0.005, 0.01, 0.02		Ability of the honeybee to move in an open-field-like apparatus is impaired	SField	Lambin *et al.* 2001
	A		0.1	Lower adult bee populations, brood surface areas and average frame weights, and reduced temperature control	Field	Meikle *et al.* 2016
	A		0.5	Affected syrup consumption and foraging activity	Field	Ramírez-Romero *et al.* 2005
	A		0.072, 0.038	Affected behaviours such as trophallaxis, grooming, avoidance	Laboratory	Santos *et al.* 2018
	A	0.00074		Abandoned their hives, and were eventually dead with symptoms	Field	Chensheng *et al.* 2014
	A	0.0043		Carboxylesterase, prophenol oxidase and acetylcholinesterase down regulate	Laboratory	Li *et al.* 2017
	A	0.0308, 0.108		Motor function impaired	Laboratory	
Bombus terrestris	A			Reduction in bumblebee foraging activity, locomotion, and foraging rhythmicity	Field	Kiah Tasman *et al.* 2020
	A		0.0005–0.125	Decrease in feeding rate	Laboratory	Cresswell *et al.* 2012
	A		0.00008–0.125	Reduced fecundity with a dose dependence	Laboratory	Laycock *et al.* 2012
	A		0.006	Affected pollen foraging ability	Field	Feltham *et al.* 2014
	A		0.01	Affected foraging behaviour and preferences	Field	Andrione *et al.* 2016
Tetragonisca angustula	A (F)			Reduced locomotion activity	Laboratory	de Oliveira Jacob *et al.* 2019
	A (F)		1.7	Impaired locomotion	Laboratory	de Oliveira Jacob *et al.* 2019
Osmia lignaria	L		0.03, 0.3	Affected larval developmental time	Field/ laboratory	Abbott *et al.* 2008
Eristalis tenax, Episyrphus balteatus	A		0.001	Avoidance of traps	Field	Easton and Goulson 2013
Melipona quadrifasciata anthidioides	L	0.0056–56		Impaired brain development and mobility at the young adult stage	Laboratory	de Almeida Rossi *et al.* 2013
Scaptotrigona postica	A (F)		89.11	Affected the speed, distance travelled, duration, and frequency of resting, and continuous mobility	Laboratory	Jacob *et al.* 2019

Continues

Table 5.3 (continued)

Pollinator	Stage	Sublethal exposure			Note	
		Dose (µg per insect)	Concentration (p.p.m.)	Sublethal effects	Field/ laboratory	Reference
Thiamethoxam						
Apis mellifera	A (NE)	0.0001, 0.001		Learning and memory	Laboratory	Aliouane *et al.* 2009
	A (NE)	0.0038		Locomotor deficit	Field/ laboratory	Charreton *et al.* 2015
	A (NE)		0.0428	Condensed cells in the mushroom bodies and optical lobes	Laboratory	Oliveira *et al.* 2014
	A		0.004	Impact synergistically with mites	Laboratory	Straub *et al.* 2019
	L		1.43	Showed condensed cells and early cell death in the optic lobes	Laboratory	Tavares *et al.* 2015
	A (NE)			Developed ataxia	Laboratory	Baines *et al.* 2017
	A		0.005	Decreased colony performance and productivity, colony growth	Field	Sandrock *et al.* 2014
	A (NE)		0.01	Seven miRNAs were differentially expressed	Laboratory	Shi *et al.* 2017b
	A (NE)		0.01	Transcriptome of honeybees	Laboratory	Shi *et al.* 2017a
	L	0.00856		Decrease in the number of sperm within honeybee queen spermatheca	Field/ laboratory	Gajger *et al.* 2017
	A (NE)		0.01	Downregulation of almost all tested detoxification and immune gene	Field/ laboratory	Tesovnik *et al.* 2020
	A (F)	0.00134		Increased flight duration	Laboratory	Tosi *et al.* 2017a,b
	A (Q)		0.004	Severely affected honeybee queens	Field	Wade *et al.* 2019
	A		10, 100 nM	Affect normal function in a field setting	Laboratory	Williamson *et al.* 2014
	A		10 nM	Affected short-term memory	Laboratory	Wright *et al.* 2015
	A		0.0001, 0.0005, 0.001, 0.01	Affected foraging activity and decrease long-term memory formation	Laboratory	Christen *et al.* 2016
	A	0.0038		Locomotor deficit in honeybees	Laboratory	Charreton *et al.* 2015
	L	0.0006		Impaired learning and memory in adult bees fed thiamethoxam larvae stage	Laboratory	Papach *et al.* 2017
Apis mellifera ligustica	A (NE)		0.01, 0.04	Food quality, considered as nutritional value and pesticide contamination, for the physiological development of honeybees	Laboratory	Renzi *et al.* 2016
		0.0002, 0.001		Altered bee survival, food consumption and haemolymph sugar levels	Laboratory	Tosi *et al.* 2017a,b

Continues

Table 5.3 (continued)

Pollinator	Stage	Sublethal exposure			Note	
		Dose (µg per insect)	Concentration (p.p.m.)	Sublethal effects	Field/ laboratory	Reference
Apis mellifera carnica	L		0.01	Immune-related genes were upregulated only in brown-eyed pupae, while in white-eyed pupae they were downregulated	Field/ laboratory	Tesovnik *et al.* 2017
	A		0.004	Reduced the mating frequency of queens	Field	Forfert *et al.* 2017
Bombus terrestris	A		0.001, 0.01	Reductions in the intake, store of artificial nectar and, reproduction	Laboratory	Elston *et al.* 2013
	A	0.0025		Impaired spatial working memory impairment	Field	Samuelson *et al.* 2016
	A	0.0024		Affected both foraging ability and homing success	Field	Stanley *et al.* 2016
	A		0.0024	Affected learning and short-term memory	Laboratory	Stanley *et al.* 2015
Bombus impatiens	A			Developed ataxia	Laboratory	Baines *et al.* 2017
Tetragonisca angustula	A (F)	0.28		Caused hyperactivity	Laboratory	de Oliveira Jacob *et al.* 2019
Eristalis tenax	L		0.5	Affected survival	Laboratory	Basley *et al.* 2018
Melipona quadrifasciata	A		0.18	Locomotor activity of bees was altered	Laboratory	Piovesan *et al.* 2020
Tetragonisca fiebrigi	A		2.05	Locomotor activity of bees was altered	Laboratory	Piovesan *et al.* 2020
Tetragonisca angustula	F			Induced high hyperactivity	Laboratory	de Oliveira Jacob *et al.* 2019
Megachile rotundata	A			Adverse effects	Laboratory	Baines *et al.* 2017
Drosophila melanogaster	A		1.04	Physiological, developmental, and biochemical status	Laboratory	Li *et al.* 2020
Clothianidin						
Apis mellifera	A (NE)			Developed ataxia, hyperactivity	Laboratory	Baines *et al.* 2017
	A (Q)		0.01, 0.05	Immunocompetence of queens	Laboratory	Brandt *et al.* 2017
	A		0.002	Decreased colony performance and productivity, colony growth	Field	Sandrock *et al.* 2014
	A		0.002	Impacted synergistically with mites	Laboratory	Straub *et al.* 2019
	A (Q)		0.001	Severely affected honeybee queens	Field	Williamson *et al.* 2015
	A		10, 100 nM	Affected normal function in a field setting	Laboratory	Williamson *et al.* 2014
	A		0.0003, 0.003, 0.015, 0.03	Affected foraging activity and decrease long-term memory formation	Laboratory	Christen *et al.* 2016

Continues

Table 5.3 (continued)

Pollinator	Stage	Sublethal exposure			Note	
		Dose (µg per insect)	Concentration (p.p.m.)	Sublethal effects	Field/ laboratory	Reference
	A		0.022	Reduced homing flights	Field	Matsumoto 2013b
	A		0.004	Impaired olfactory learning acquisition in honeybees	Laboratory	Piiroinen and Goulson 2016
	L	0.00067, 0.00133		Reduced hygienic and foraging activity	Field	Morfin *et al.* 2019
	L	0.000236		Reduced the bees' longevity	Laboratory	Tadei *et al.* 2019
	A	0.00074		Abandoned their hives, and were eventually dead with symptoms	Field	Chensheng *et al.* 2014
	A	0.001		AChE was downregulated	Laboratory	Li *et al.* 2017
	A		0.003	Enhanced P450 oxidase activity	Laboratory	Yao *et al.* 2018
Apis mellifera carnica	A (F)	0.0005–0.002		Reduction of foraging activity and to longer foraging flights	Field like	Schneider *et al.* 2012
	A	0.0025		Blocked the retrieval of exploratory navigation memory	Field	Fischer *et al.* 2014
	A		0.001	Reduced the mating frequency of queens	Field	Forfert *et al.* 2017
	L	0.008, 0.016, 0.032		Negatively affect survival and the cellular responses	Laboratory	López *et al.* 2017
	A	1–100 µM		Depolarisation blocks of neuronal firing and inhibit nicotinic responses.	Laboratory	Palmer *et al.* 2013
	A (NE)		0.01–0.2	Reduced haemocyte density, encapsulation response, and antimicrobial activity	Laboratory	Brandt *et al.* 2016
Apis mellifera ligustica	A			Reduced immune defense and promotes the replication of the deformed wing virus	Laboratory	Di Prisco *et al.* 2013
		0.00016, 0.0008		Altered bee survival, food consumption, and haemolymph sugar levels	Laboratory	Tosi *et al.* 2017a,b
Apis cerana	A	0.0003		AChE was downregulated and ST activities were upregulated	Laboratory	Li *et al.* 2017
Bombus terrestris	A		0.004	Slightly lower learning and faster learning in combination with clothianidin	Laboratory	Piiroinen and Goulson 2016
Bombus terrestris dalmatinus	A			Elado-dressed oilseed rape did not cause any detrimental effects on the development or reproduction	Field	Sterk *et al.* 2016
Bombus impatiens	A		0.006, 0.036	No effects on tested parameters	Field	Franklin *et al.* 2004
	A			Develop ataxia, hyperactivity	Laboratory	Baines *et al.* 2017
Megachile rotundata	A			adverse effects	Laboratory	Baines *et al.* 2017
	L		0.03, 0.3	No effects in larvae developmental time	Field/ laboratory	Abbott *et al.* 2008

Continues

Table 5.3 (continued)

Pollinator	Stage	Sublethal exposure			Note	
		Dose (µg per insect)	Concentration (p.p.m.)	Sublethal effects	Field/ laboratory	Reference
stingless bee	A		0.001	A stressor to monarch populations	Field	Pecenka and Lundgren 2015
Acetamiprid						
Apis mellifera	A (NE)	0.5, 1, 2		Affected survival and memory related behaviour	Laboratory	Shi *et al.* 2019
Apis mellifera	A	0.1		Long-term retention of olfactory learning	Laboratory	El Hassani *et al.* 2008
Apis mellifera	A (NE)	0.1		Increase water responsiveness	Laboratory	Aliouane *et al.* 2009
Apis mellifera	A (F)		250	Affected foraging activity and decrease long-term memory formation	Field	Christen *et al.* 2016
Apis mellifera	A		4.8, 48.6, 486	Affected foraging activity and decrease long-term memory formation		Christen *et al.* 2016
Apis mellifera	A (NE)	2		Reduced the lifespan, rotating day-off status, foraging flights and induced precocious foraging activity	Laboratory/ field	Shi *et al.* 2020
Apis mellifera	A (F)		189.62	Affected the speed, distance travelled, duration, and frequency of resting, and continuous mobility	Laboratory	Jacob *et al.* 2019
Bombus impatiens	A		4.52	Impacted nest growth and development, and ultimately reproduction	Field	Bovi *et al.* 2018
Scaptotrigona postica	A (F)		475.94	Affected the speed, distance travelled, duration, and frequency of resting, and continuous mobility	Laboratory	Jacob *et al.* 2019
Tetragonisca angustula	A (F)		173.3	Not impaired locomotion	Laboratory	de Oliveira Jacob *et al.* 2019

Abbreviations: A, adult; F, forager; L, larva; NE, newly emerged; Q, queen.

5.6 Risk estimation

Figure 5.6 presents the risk quotient, on the basis of the environmental exposure and toxicity (LD_{50} contact) values for insects. We have calculated the risk quotient on the basis of the available data, following the method proposed by EPPO (2010):

$$\text{Risk quotient} = \frac{\text{Application rate}}{\text{Acute } LD_{50}}$$

It was found that the data were limited for wild bee populations. The risk quotient is higher for honeybees than for bumblebees. Similar to the toxicity results, the risk quotient also reveals that imidacloprid, thiamethoxam, and clothianidin exhibit higher toxicity than acetamiprid and thiacloprid. Another index for assessing risk is the hazard quotient. A hazard quotient of less than 50 is used to define an insecticide as harmless to the pollinator, 50–2,500 as slightly to moderately toxic, and greater than 2,500 as dangerous to pollinators (EPPO 1993). On the basis of this categorisation, the hazard quotient of NNIs for honeybees is mildly to moderately hazardous.

5.7 Neonicotinoid contamination of hive products

The growing volume of evidence of the negative influence of NNIs on pollinator populations is also strengthened by the shreds of evidence of contamination of beehive products. In one study, Mitchell *et al.* (2017) demonstrated that out of 198 honey samples from across the world, 75% were found to be contaminated with at least one of the five NNIs, namely acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam, 45% of the honey samples were contaminated with two or more insecticides, and 10% of the honey samples were contaminated with four or all five insecticides. In the Asian samples, acetamiprid followed by imidacloprid were the NNIs causing contamination in honey. In a 4-year survey of pesticide residues including NNIs in pollen, nectar, bee bread, and honey in China, Xiao

Risk quotient (RQ) for pollinators

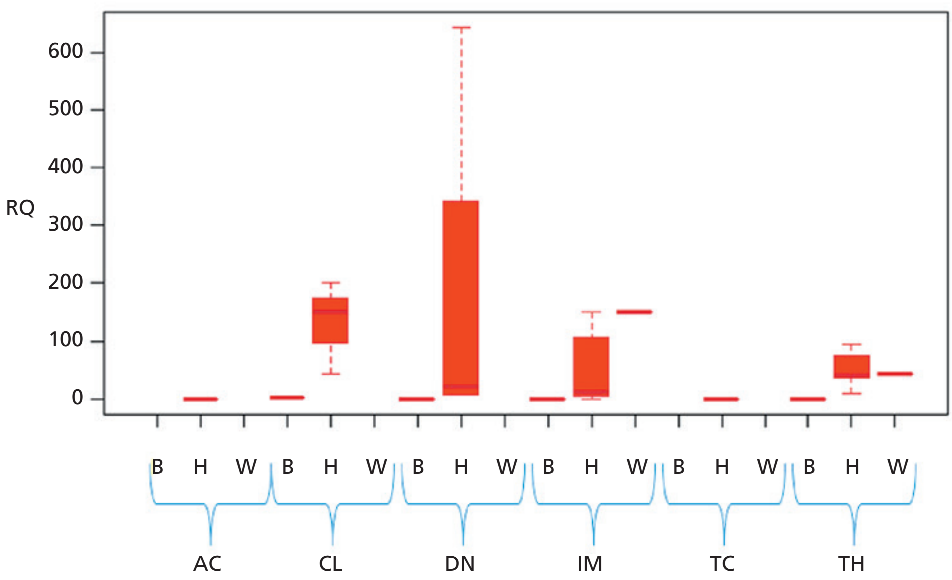


Figure 5.6 Risk quotient (RQ) of neonicotinoids to pollinator groups at 4–48 hours after treatment. B, bumblebee; H, honeybee; W, wild bee; AC, acetamiprid; CL, clothianidin; DN, dinotefuran; IM, imidacloprid; NY, nitenpyram; TC, thiacloprid; TH, thiamethoxam.

et al. (2022) found that 93.6% of pollen, 81.5% of nectar, 96.6% of bee bread, and 49.3% of honey were contaminated with at least one pesticide out of the 64 they tested. However, it is noteworthy that the NNIs were not the predominating pesticide found in the contaminated hive products (Xiao *et al.* 2022). Out of 240 bee bread, honey, pollen, and wax samples from Western Australia, 10 were contaminated with imidacloprid, and one honey sample was found to be contaminated with thiamethoxam (Manning 2018). Wen *et al.* (2021) investigated pesticides, including the NNIs acetamiprid, clothianidin, dinotefuran, and imidacloprid, residues in nectar, and pollen of oilseed rape (*Brassica napus*); they detected 48 pesticides in pollen and 34 chemicals in nectar samples. A study conducted by Jiang *et al.* (2018) investigated the concentrations of imidacloprid and thiamethoxam in pollen, nectar, and leaf samples from seed-dressed cotton crops. Imidacloprid residue was found both in pollen (1.61 to 64.58 $ng\,g^{-1}$) and in nectar samples (not detected to 1.769 $ng\,g^{-1}$), whereas thiamethoxam was found in 90% of pollen samples (not detected to 14.521 $ng\,g^{-1}$) and 60% of nectar samples (not detected to 4.285 $ng\,g^{-1}$). Pervez and Manzoor (2020) investigated pesticide residues of 100 samples each of pollen and nectar collected from different places of Khyber Pakhtunkhwa and Punjab provinces. Out of 100 pollen samples, 47% were positive; the most frequently found pesticide residue was imidacloprid, accounting for 11%, followed by thiamethoxam (7%) and carbaryl (6%). On the other hand, out of 100 nectar samples, 36% were found positive with the most abundant being imidacloprid (8%) followed by thiamethoxam (6%) and fipronil (5%). Honeybee colonies (*A. mellifera*) were used to collect pollen at monthly intervals in four provinces (Chiang Mai, Lampang, Phayao, and Phrae) of northern Thailand. One agricultural and one non-agricultural site in each of the four provinces were selected for a total of eight distinct sites. Bee-collected pollen was sorted by colour and identified to estimate plant diversity at each location; the pesticides were investigated including herbicides, fungicides, formamidines, organophosphates, pyrethroids, and NNI residues. However, the study reported that the detection of agricultural chemicals was lower relative to values obtained in other studies conducted in Europe and North America (Chaimanee *et al.* 2019). Choudhury and Sharma (2008) reported that no imidacloprid residue was found in the pollen and nectar samples collected from a mustard field in Himachal Pradesh, India; they concluded that imidacloprid seed treatment was harmless.

5.8 Conclusion

A large volume of scientific evidence indicates that NNIs have a serious negative influence on honeybees, although the extent of their influence varies and is subject to attributes such as pollinator species, their genetic makeup, geographical and environmental conditions, insecticide application patterns, etc. However, there are few toxicity studies of NNIs on solitary wild bees in Asia compared with their European or American counterparts. Regular monitoring, restricted application of NNIs, and an enforced review system are essential components to monitor NNI use to avoid the potential hazard of pollinator population loss.

6 Risk assessment of neonicotinoid pesticides on natural enemies of crop pests in agroecosystems

Chapter summary

Neonicotinoid insecticides (NNIs) are the most widely used pesticides worldwide. Because of the systemic nature of these chemicals, they are absorbed by plant tissues and travel to the growing part of plants, providing long-term preservation against phloem-feeding pests. Despite their effectiveness in pest management, the presence of these pesticides in lethal and sublethal concentrations in the environment, travelling through food webs, as well as their toxicity to non-target organisms such as pollinators and natural enemies of crop pests, has given rise to concerns about their wide use. Pesticide application may cause devastating effects on populations of natural enemies because of their lower levels of resistance to pesticides, resulting in a reduction of their populations in agroecosystems and consequently outbreaks of primary and secondary pests. Thus, it is crucial to perform a risk assessment of NNIs to natural enemies to provide a less hazardous and safer integrated pest management programme against agricultural pests. Currently, NNIs are mostly being used as a seed covering to mitigate pest damage in the early stages of crop production; however, more studies on the cost effectiveness of such applications in different crops are needed. In addition, alternative selective pest management methods are recommended to reduce the number of applications of pesticides and consequently to mitigate the adverse effect of NNIs on pests' natural enemies.

6.1 Introduction

Neonicotinoid insecticides (NNIs) are nicotinic agonists that interact with the nicotinic acetylcholine receptor (nAChR) in a very different way from nicotine, which confers selectivity to insects versus mammals (Tomizawa and Casida 2005). Binding to nAChRs, NNIs affect the central nervous system of insects. With a market share of 25%, NNIs are among the most widely used insecticides because of their efficacy against a wide range of serious agricultural pests (Bass *et al.* 2015). With approximately 23% of consumption, Asian countries are among the greatest users of NNIs in the world (Bass *et al.* 2015). Imidacloropid, clothianidin, and thiamethoxam are among the hazardous agrochemicals for non-target organisms and accounted for almost 85% of the total global use of NNIs in crop protection in 2012 (Bass *et al.* 2015).

Despite the efficacy of NNIs on many crop pests such as aphids, whiteflies, leafhoppers, and various coleopteran, lepidopteran, and dipteran insects, their adverse effects on beneficial arthropods such as pollinators and natural enemies of crop pests have received great attention (Figure 6.1) because of their value in integrated pest management and pollination (Desneux *et al.* 2007; Calvo-Agudo *et al.* 2019). Because of the negative influence of imidacloprid, thiamethoxam, and clothianidin on non-target organisms, the use of these three pesticides has recently been banned by European Commission in open agroecosystems after a risk assessment report of the European Food Safety Authority (EFSA 2018). However, they are being used in greenhouses and open agroecosystems in other continents. Given the scale of use of NNIs, their persistence in soils, their leaching into waterways, and their systemic nature within plants, there is no doubt that most organisms inhabiting arable environments will be exposed to this group of pesticides. The key question is whether typical levels of exposure are likely to lead to significant individual- or population-level impacts. Owing to the negative impact of NNIs on food quality and their effects on non-target organisms, specifically pollinators and natural enemies of agricultural pests, risk assessment of the use of NNIs is crucial.

6.2 Routes of exposure of natural enemies of crop pests to neonicotinoids

Neonicotinoids are broad-spectrum, systemic compounds which exhibit activity against piercing–sucking insects (e.g. aphids, whiteflies, leafhoppers) and several species of flies and moths. NNIs are readily absorbed by plants and act quickly at low doses compared with other insecticides (Sympathy and Rai 2006). Depending on the target pest, NNIs can be applied in a variety of methods such as foliar spraying, soil drenching, and seed dressing. Use of NNIs is not restricted to agroecosystems and they are applied as tree soil drenches or by injection to control urban and forest pests (Hladik *et al.* 2018). Depending on the pesticide application method, natural enemies of agricultural pests can be exposed to NNIs in one of two ways, direct or indirect, which are described below.

6.2.1 Direct exposure by foliage application (spraying) and pesticide drift

The main route of exposure of natural enemies of crop pests to NNIs includes direct contact with spray droplets, contaminated soil and plants, and feeding on prey items exposed to insecticides (Figure 6.2). Direct exposure

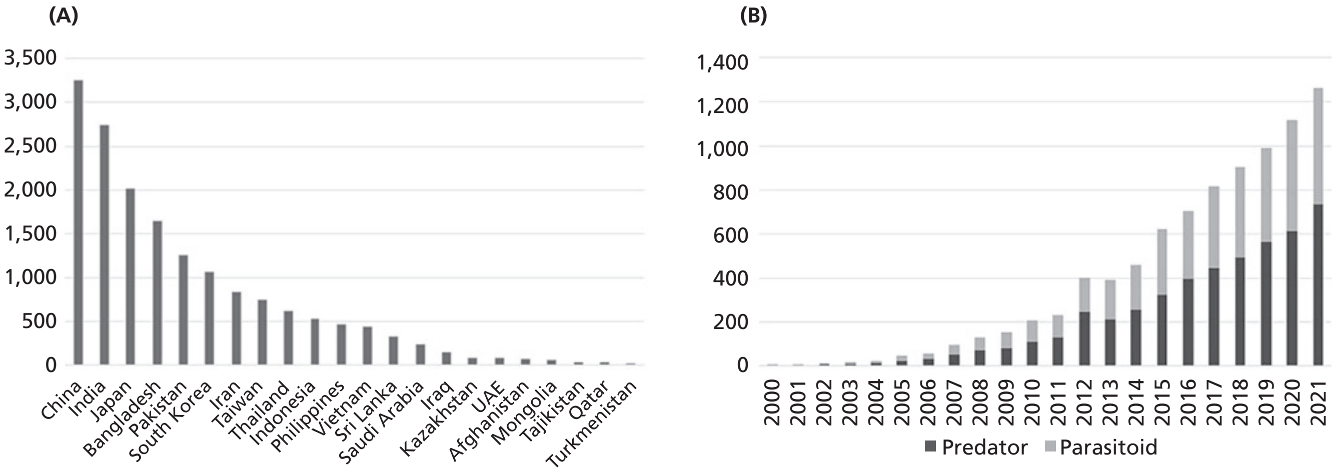


Figure 6.1 Number of studies published in scientific journals on NNIs. (A) Number of publications on NNIs and natural enemies since 2015 in different Asian countries; (B) annual number of publications on NNIs and natural enemies in Asia.

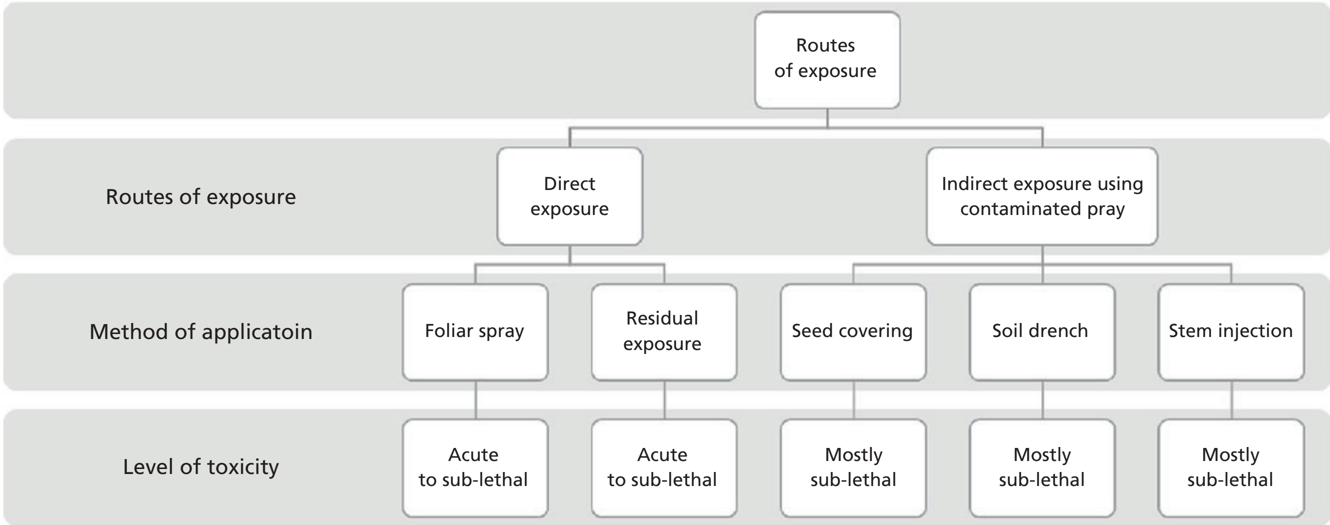


Figure 6.2 Routes of exposure of natural enemies to NNIs and their level of toxicity.

of NNIs often results in acute toxicity. Acute mortality caused by the direct impact of pesticides by topical treatment or dry residue is commonly analysed for pests and natural enemies and can be used to categorise the toxicity of pesticides for field application.

6.2.2 Indirect exposure by seed dressing and root drench application

NNIs are used primarily as plant systemic pesticides, applied as seed dressing or root drench. Because of their good water solubility, NNIs are readily absorbed and move through the vascular system to the growing parts of plants and provide long-term protection against piercing–sucking pests such as aphids, leafhoppers, and whiteflies (Iwasa *et al.* 2004). Soil and seed applications of pesticides are considered a safe method for zoophagous natural enemies of crop pests because of the lesser adverse effect of pesticides compared with foliar application. However, a negative effect has been reported in zoophytophagous species which feed on plants as well (Torres *et al.* 2010, Gontijo *et al.* 2014, Gontijo *et al.* 2015). Owing to the systemic transport and persistence of NNIs, they can be found in sublethal concentrations in plant nectar and pollen as well as honeydew secreted by the phloem-feeding insects (Calvo-Agudo *et al.* 2019). Pesticide residues in food items such as pollen, nectar, honeydew, water, and plant juice, and pesticide drift during the application of NNIs, are the major routes for indirect exposure of natural enemies (Wäcker *et al.* 2008; Calvo-Agudo *et al.* 2019), causing various levels of toxicity from acute mortality to alteration of life history and behaviour (Papachristos and Milonas 2008, Tran *et al.* 2016, Colares *et al.* 2017). For example, there was a reduction in survivorship of the parasitoid *Anagyrus pseudococci*

Table 6.1 Sublethal effects of NNIs on predators

Insecticide	Species	Classification	Sublethal effect	Reference
Imidacloprid	*Orius albidipennis*	Hemiptera: Anthocoridae	Reduce egg hatching, Nymph survival Adult fecundity	Sabahi *et al.* 2010 Moscardini *et al.* 2013
Thiamethoxam	*Platynus assimilis*	Coleoptera: Carabidae	Short-term locomotor hyperactivity Reduction in clean food consumption rate	Tooming *et al.* 2017
Imidacloprid	*Coccinella septempunctata*	Coleoptera: Coccinellidae	Reduction of longevity and daily aphid consumption, Reduction in weight Reduction in demographic parameters, such as the intrinsic rate of increase, finite rate of increase, and net reproductive rate, reduction of oviposition period	Xiao *et al.* 2016 Papachristos and Milonas 2008 Capowiez *et al.* 2005 Skouras *et al.* 2017; 2019; 2021
Imidacloprid	*Hippodamia undecimnotata*	Coleoptera: Coccinellidae	Reduction of longevity and daily aphid consumption	Xiao *et al.* 2016 Papachristos and Milonas 2008
Imidacloprid	*Serangium japonicum* *Harmonia axyridis*	Coleoptera: Coccinellidae	Affected predators' functional responses	Skouras *et al.* 2019 Taravati *et al.* 2019
Imidacloprid	*Crysoperla carnea*		Reduction in the longevity	Rogers *et al.* 2007

after feeding on the nectar of buckwheat plants treated with soil applications of imidacloprid (Krischik *et al.* 2007).

In addition, Rill *et al.* (2008) showed the safety of acetamiprid residues to immatures stages of the scale parasitoid *Aphytis melinus*. However, it was very toxic to newly emerged adults. The armoured-scale parasitoids, which develop underneath the scale coverings, may not be exposed by foliar applications of NNIs but they can be exposed to foliar residues while chewing the scale covering during emergence. Medina *et al.* (2007) reported that the ingestion of the residues of imidacloprid by larvae of *Spodoptera littoralis* caused reduction in the adult emergence of its endoparasitoid, *Hyposter didymator*, demonstrating the exposure of endoparasitoids to the NNIs during their development inside the host's body.

6.3 Toxicity of neonicotinoids to natural enemies

6.3.1 Acute toxicity pattern

Many studies have examined the toxicity of NNIs both to target and to non-target organisms, including mammals, birds, fish, insects, crustacean, molluscs, and annelids. Insects are consistently among the most sensitive taxa, whether exposed through contact or ingestion.

Typical LD_{50} values of NNIs for predators vary from 0.82 to 88 ng per insect and the contact LC_{50} values of imidacloprid vary from 0.001 to 370 p.p.b. (Table 6.1). Here, the variation between species can be attributed to the size of the insect, and the intraspecific variation is mainly due to the duration of exposure. For example, the LC_{50} for the ladybird, *Coccinella undesimpunctata*, falls from 34.2 p.p.b. at 48 hours to 28.7 p.p.b. at 72 hours (Ahmad *et al.* 2011). In addition, it has been shown that the immature stage of predators is more vulnerable than the adult stage for contact, oral, and residual exposure to NNIs (Delbeke *et al.* 1997). In contrast, Gour and Pareek (2005) reported the higher toxicity of imidacloprid to the adult stage of ladybirds compared with the larval stage. Balanza *et al.* (2019) indicated the variation in LC_{50} value in different populations of *Orius laevigatus* (Hemiptera: Anthocoridae) after being exposed to imidacloprid and thiamethoxam, which could be attributed to the race, genetic diversity, and their symbiont microbial community of different populations.

The residual LC_{50} value varies from 0.0004 to 752 p.p.m. in parasitoids intoxicated by imidacloprid (Table 6.2). It has been shown that the toxicity of NNIs varies for parasitoid species and, on the basis of the estimated LC_{50} (half lethal concentration) values, imidaclothiz possessed the least toxicity to parasitic wasps (Figure 6.3D and Table 6.2) while the rest of the NNIs were moderately harmful (Preetha *et al*. 2009; Saber 2011; Wang *et al*. 2012a; 2012b; 2013; Zhao *et al*. 2012; Khan *et al*. 2015; Ko *et al*. 2015; Cheng *et al*. 2018; Jiang *et al*. 2019). Residual toxicity tests on *Cotesia flavipes*, an important larval parasitoid for the control of the spotted stem borer, *Chilo partellus*, showed

Table 6.2 Sublethal effect of NNI pesticides on parasitoids

Insecticide	Species	Sublethal effect	Reference
NNIs	*Aphidius colemani* *Microplitis mediator* *O. insidiosus* *C. flavipes* *Trichogramma* spp.	Mitigated parasitoid performance: survival rate parasitic capacity longevity of females	Kang *et al.* 2018 D'Avila *et al.* 2018 Fontes *et al.* 2018
Imidacloprid	*T. podisi*	Reduced the emergence of the offspring parasitoids by up to 40%	Pazini *et al.* 2019
Acetamiprid, thiamethoxam	*A. gifuensis*	Wing deformities	Sun *et al.* 2014
Imidacloprid	*Encarsia inaron*	Changed the sex ratio (high number of males)	Sohrabi *et al.* 2012

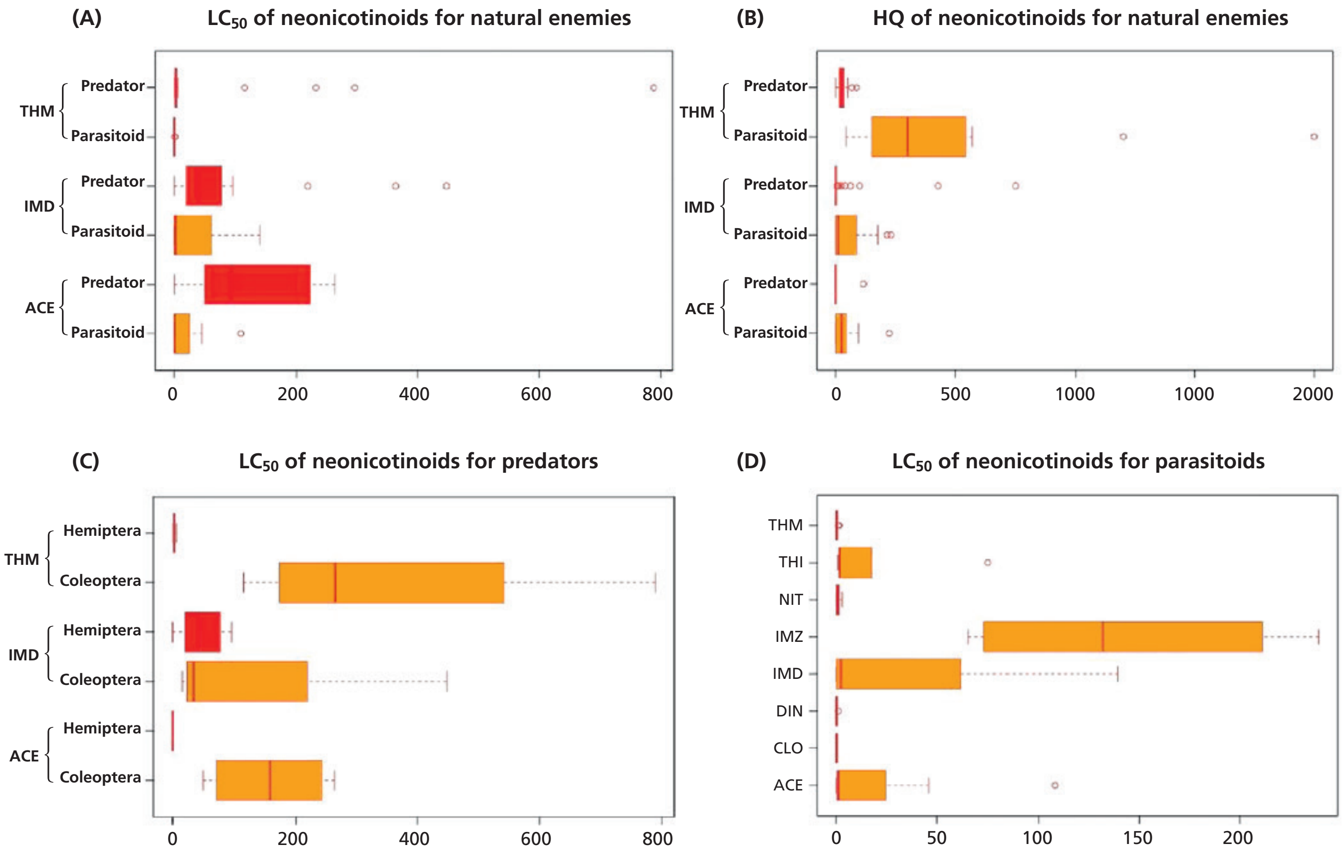


Figure 6.3 (A) Toxicity of NNIs on different natural enemies; (B) hazard quotient (HQ) of NNIs on natural enemies (hazard quotient = recommended field rate (grams of active ingredient per hectare)/LC$_{50}$ of beneficial insect (milligrams ofactive ingredient per litre)); (C) toxicity of acetamiprid, thiamethoxam, and imidacloprid on predatory insects of the orders Coleoptera and Hemiptera; (D) toxicity of NNIs on parasitoids. THM, thiamethoxam; THI, thiacloprid; NIT, nitenpyram; IMZ, imidaclothiz; IMD, imidacloprid; DIN, dinotefuran; CLO, clothianidin; ACE, acetamiprid.

that organophosphates exhibited the highest contact toxicity to *C. flavipes* adults while NNIs (acetamiprid and imidacloprid) were less toxic, showing the possibility of applying NNIs in pest management programmes against the spotted stem borer (Alhtar *et al.* 2021). Toxicology analysis on *Euschistus heros* (Hemiptera: Pentatomidae) and its parasitoid *Telenomus podisi* (Hymenoptera: Platygastridae) showed that all pesticides were classified as slightly to moderately toxic to *T. podisi* on the basis of the risk quotient (Pazini *et al.* 2019).

Comparison of three NNI pesticides indicated lower levels of toxicity and the hazard quotient of acetamiprid and imidacloprid for predators than parasitic wasps, while thiamethoxam had higher toxicity for both groups of natural enemies (Figure 6.3A,B); on the basis of the hazard quotient, thiamethoxam is considered a slightly to moderately toxic pesticide for parasitic wasps (Preetha *et al.* 2010). In addition, thiamethoxam has a higher hazard quotient for parasitoids than predators. Comparing the toxicity of NNIs for two insect orders of predators, Coleoptera and Hemiptera, demonstrated their lower toxicity on beetles which can be attributed to the presence of a hard integument, different detoxification mechanisms, and having harder forewings (elytra) generally covering the abdominal tergites and making it difficult for pesticides to penetrate inside their body. The toxicity of acetamiprid and thiamethoxam is slightly lower than imidacloprid in Coleoptera. In contrast, the toxicity of imidacloprid is lower than two other pesticides for hemipteran predators (Figure 6.3C).

NNI insecticides can affect pest and natural enemy populations differently and the study on adverse effect of pesticides on different trophic levels in agroecosystem is crucial. It has been demonstrated that the toxicity of NNIs is higher for common pistachio psylla, *Agonoscena pistaciae*, compared with its natural enemies (Amirzade *et al.* 2014). In contrast, results indicated lower toxicity of NNIs on brown planthopper, *Nilaparvata lugans*, than its mirid predator, *Cyrtorhinus lividipeNNIs* (Preetha *et al.* 2010). These contradictory results show the necessity of study for the toxic effect of pesticides on different trophic levels of each agricultural crop which will be helpful selecting better pest control programmes. Furthermore, it is crucial to notice that NNIs, which are not lethal in their field concentrations, cause many different type of sublethal effect on non-target organisms.

6.3.2 Sublethal effects of neonicotinoids on natural enemies

Most studies assess only mortality and are performed over short periods, but it is clear that important sublethal effects (such as reduced feeding, movement, and reproduction) can be elicited by much lower doses and pesticides may cause a broad variety of physiological and behavioural sublethal effects in natural enemies. Low abundances of natural enemies (parasitoids and predators) and a decreased parasitism rate have been reported after application of imidacloprid to control wheat, soybean, and blueberry pests (Roubos *et al.* 2014; Mohammed *et al.* 2018; Varehorst and O'Neal 2012). Prabhaker *et al.* (2011) also demonstrated the side effects of imidacloprid insecticide on the parasitoid species *Aphytis melinus*, *Gonatocerus ashmeadi*, *Eretmocerus eremicus* and *Encarsia formosa*, in addition to the generalist predators *Geocoris punctipes* and *Orius insidiosus* under laboratory conditions.

Recent meta-analysis demonstrated the significant negative effect of NNIs on the behaviour of predators, parasitoids, and pollinators. Among them the highest negative impact observed was in predators with an 84% reduction in the amount of prey consumption (Main *et al.* 2018) (Figure 6.4). Application of insecticides may cause sublethal effects on biological and behavioural parameters including developmental time, fecundity, longevity, sex ratio, feeding activity, predation rate, orientation, and mobility of natural enemies (Desneux *et al.* 2006; Kang *et al.* 2018) (Table 6.1). The negative effect of sublethal doses of NNIs on the longevity and

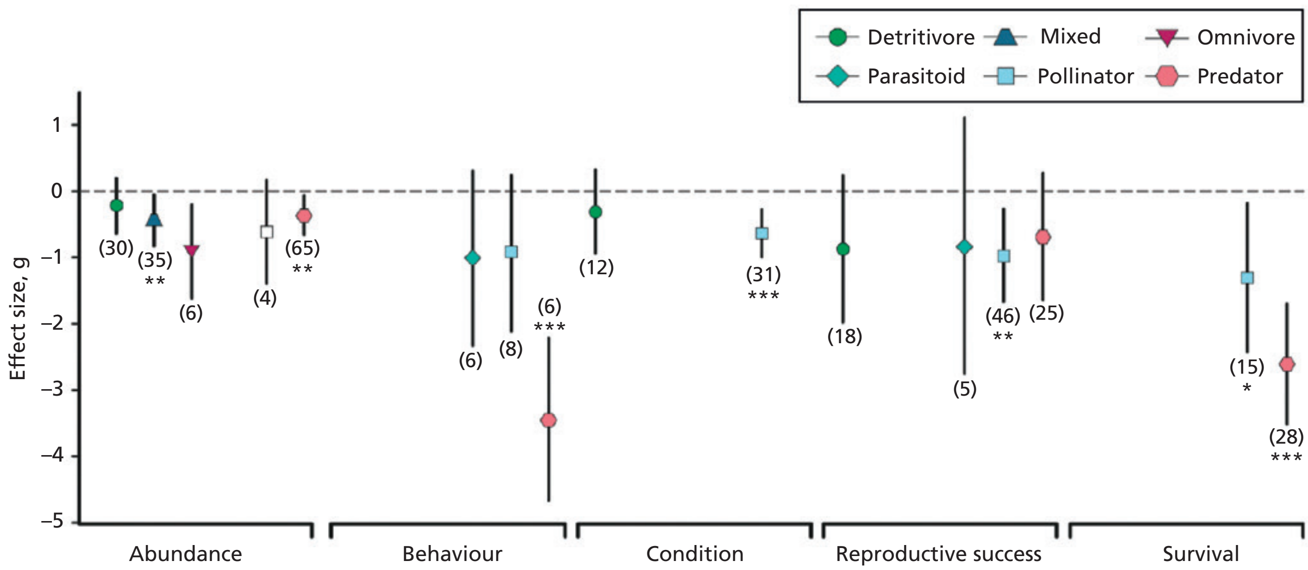


Figure 6.4 Effects (Hedges' g) of NNI treatments on performance measures of non-target arthropod functional groups. Bars around means represent 95% confidence intervals and the numbers of observations considered in each case are in parentheses. (Reprinted from Main et al. 2018).

survival and fecundity of hoverflies and ladybirds has also been documented (Calvo-Agudo *et al.* 2019; Skouras *et al.* 2019).

Exposure to pesticides also negatively affected the biocontrol efficiency of pest parasitoids (Calvo-Agudo *et al.* 2019). Reductions in the rate of parasitism, foraging efficiency, and host feeding behaviour were also documented because of exposure to the sublethal concentrations of NNIs. Several studies have proved that the oral ingestion of sublethal doses of NNIs mitigates parasitoid performance, such as survival rate, parasitic capacity, longevity of female adults, wing deformities, and change in sex ratio in parasitic wasps (Sohrabi *et al.* 2012; Sun *et al.* 2014; D'Avila *et al.* 2018; Fontes *et al.* 2018; Kang *et al.* 2018) (Table 6.2). It was shown that acetamiprid and thiacloprid possessed the least toxicity to the parasitism performance and emergence probability of *Tricogramma* species (Wang *et al.* 2012a; Jiang *et al.* 2019) while many studies have confirmed the high toxicity of dinotefuran, thiamethoxam, imidacloprid, nitenpyram, and clothianidin to *Trichogramma* species (Preetha *et al.* 2009; Saber 2011; Wang *et al.* 2012a; 2012b; 2013; Zhao *et al.* 2012; Khan *et al.* 2015; Ko *et al.* 2015; Cheng *et al.* 2018). Thus, the sublethal effect of NNIs could reduce biological control services provided by natural enemies.

NNIs may not always cause negative effects on natural enemies. For instance, the sublethal effect of thiamethoxam on the longevity of *Hippodamia variegata* was negligible (Rahmani *et al*. 2013). Moreover, the number of eggs produced by a imidacloprid-exposed predatory mite, *Amblyseius victoriensis*, was higher than in a control group (James 1997).

6.4 Food web contamination

An agroecosystem hosts hundreds or thousands of arthropod species including herbivores, predators, parasitoids, pollinators, and scavengers, forming different trophic levels of the food web. Only limited numbers of arthropods in agroecosystems are considered as pests, however, and most species can be categorised as beneficial insects since they are actively involved in pollination of agricultural crops, nutrient cycles, or reducing the population of herbivores by their predatory or parasitic behaviour. Most arthropods are exposed to lethal or sublethal doses of NNIs exclusive of their function in agroecosystems. Vulnerable pest and non-pest arthropods die after being exposed to lethal concentrations of NNIs; the remaining arthropods, including resistant individuals or the vulnerable ones that received sublethal doses of pesticides, stay alive. However, they are contaminated and expose other consumers in higher trophic levels of the food web to the insecticides (Frank and Tooker 2020). In addition, recent research has demonstrated the presence of NNIs in the honeydew of phloem-feeding insects (Hemiptera) fed on the treated host, which can adversely affect different insects that use honeydew as a food resource (Calvo-Agudo *et al.* 2019). It has been shown that NNIs did not have a lethal effect on some pests such as spider mites (Acari: Tetranichidae) and slugs (Mollusca: Gastropoda) which consumed the treated plants; however, they had lethal and sublethal effects on their hemipteran and coleopteran predators fed on contaminated prey, respectively (Szczepaniec *et al.* 2011; Douglas *et al.* 2015). In consequence, the application of NNIs has more negative effects on natural enemies than the herbivores, and results in herbivore outbreak by reducing the abundance of natural enemies.

6.5 Future considerations for neonicotinoid application

Despite having negative effects on natural enemies of crop pests, pesticide application is inevitable in the pest management of highly profitable crops with important pests (e.g. fruit-damaging pests, virus vectors, etc.). Integrated pest and pollinator management (IPPM) is a combination of control methods that can be applied against agricultural pests to mitigate pest populations to lower than economic threshold levels. It is important to create compatible, eco-friendly, and crop-specific IPPM programmes to reduce the adverse effects of pesticides on beneficial insects such as natural enemies and pollinators. Non-chemical cultural control methods such as using crop traps, changing the date of sowing, or the method of irrigation, are helpful in reducing pest populations or enhancing crop tolerance without adverse effects on beneficial insects. Intercropping and companion cropping can be helpful in increasing biocontrol by providing diversity of food for natural enemies. Using resistant plant cultivars has remained the most important and cost-effective means of control against agricultural pests. However, the effectiveness of using resistant cultivars can be increased with their pollinator attractiveness. Furthermore, some of the natural enemies such as hoverflies, parasitic wasps, lacewings, and ladybirds can provide both ecosystem services by either reducting the populations of agricultural or pollinating crops specifically in the greenhouse environment and boosting the quality and yield stability of agricultural products (Egan *et al.* 2020).

Currently, about 60% of all NNI applications are soil/seed treatments (Jeschke et al 2011). Among NNIs, clothianidin is used for seed treatment and imidacloprid and thiamethoxam are used as either seed treatment or foliar application. However, recent analysis on the yield of seed oils indicated that seed coating is often not needed and may not always contribute effectively to yield gain (Seagraves and Lundgren 2012; Goulson 2013). More studies on the cost effectiveness of using NNIs on other important crops are required. Monitoring pest populations with adequate methods and application of pesticides when the population level is above economic threshold is helpful in reducing the number of applications of NNIs (Furlan and Kreutzweiser 2015). Considering the intraspecific variation of natural enemies intoxicated by NNI pesticides (Bielza 2016; Balanza *et al.* 2019), application of resistant strains of natural enemies can reduce the negative effect of NNIs on released biocontrol agents.

Calvo-Agudo *et al.* (2019) have also shown the importance of the application method: foliar application of imidacloprid caused higher mortality in hoverfly, *Sphaerophoria rueppellii*, fed on the honeydew of *Planococcus citri* compared with soil application of same pesticide and with a control group. It is clear that pesticide exposure negatively affects the performance of natural enemies. Drench or granular applications of NNIs may have less negative impact on natural enemies and be more suitable for use in IPPM programmes (Cloyd and Bethke 2011). In addition, considering the negative effect of pesticide drift, spraying near the surface of plant and avoiding application on windy days can be helpful in reducting pesticide drift and its adverse effect on non-target organisms.

7 Risk regulation and mitigation in the Asia-Pacific region

Chapter summary

The regulations of the uses of neonicotinoid insecticides (NNIs) in the Asia-Pacific region mainly consist of registration of the pesticide products, post-registration review, and requirements of risk mitigation. The registration of the uses of NNIs in the Asia-Pacific region follows local pesticide regulatory systems, which are different from country to country. This results in very diverse data requirements and decision-making standards in the region. Continuous efforts would be needed to further harmonize the regulatory systems and promote science-based risk assessment which takes into account not only the toxic effects of the chemicals but also the local agriculture conditions and practices.

In the Asian countries with the highest number of registered NNI uses, such as Japan, China, and Australia, concerns about environmental issues are invoking risk assessment of the uses On the basis of these reviews, some NNIs might be discontinued or restricted. At the same time, owing to the importance of NNIs to agriculture in these countries, the regulatory decisions need to carefully balance the benefit and risk.

In most Asian countries, the authorities require precautionary statements on labels where potential risks are identified, and risk mitigation measures are needed. The mitigation measures are required to be acceptable to local farmers and implementable in the countries. Levelling up the review system as well as implementing risk mitigation programmes with education for farmers seems important in some Asia-Pacific countries.

7.1 Regulation of neonicotinoid uses in the Asia-Pacific region

Neonicotinoids (NNIs) are designed to kill insects; therefore their intrinsic toxicity against insects is naturally high. As such, they are some of the most heavily managed agricultural chemical groups globally. It must be remembered that such regulatory assessments are applied to all agricultural chemicals and are not specific to NNIs.

There are a range of regulatory decision-making schemes in the Asia-Pacific region. For example, there are already well-established regulatory systems in countries such as Australia, New Zealand, Japan, Korea, and China, while others have rapidly evolving registration schemes with a mixture of decision-making criteria (Thailand, Malaysia, Vietnam, Taiwan, India, and the Philippines). Some countries such as Indonesia and Cambodia have instead oriented their registration systems towards the FAO/WHO hazard standard, and a few still only have basic systems with their own criteria (e.g. Pakistan).

In general, two main types of decision-making scheme have been adopted in Asia-Pacific countries, which are based on hazard and risk respectively. Hazard assessment and classification are based on the intrinsic properties of a pesticide product itself. A hazard classification of a pesticide product may result in the assignment of a hazard symbol, signal word, or hazard statement on the product label, and in some countries the classification criteria are also used as standards for approval of uses of crop protection products, so called cut-off criteria. Risk assessment is based on both the intrinsic properties of a pesticide product and the degree of exposure of human and non-target organisms to it during its use. Obviously, the risk-based approach is more scientifically robust than the hazard-based approach because the actual harm that a pesticide could cause to humans and the environment depends not only on the properties of the pesticide but also on the methods of its use. Risk assessment will not only result in a decision as to whether to authorise the use of a pesticide product, but also provide the technical information that risk managers need in their consideration of risk mitigation measures and recommendations and instructions for safe use.

Currently, only a few countries in the Asia-Pacific region, for example China, Australia, New Zealand, Korea, and Japan, use risk-based decision-making schemes to approve the use of crop protection products. Less than a quarter have adopted a tiered approach towards assessing risk and only half of them are using FAO/WHO criteria in their assessment. Authorities in most Asia-Pacific countries are still making their decisions mainly on the basis of hazard. The technical capabilities of most Asia-Pacific regulatory authorities are still developing, which is why risk-based regulatory systems have not yet been widely adopted in the region.

With these varying regulatory systems, the specific uses of pesticides (including NNIs) are regulated differently throughout the region. This has caused concerns in some authorities, who have taken some actions to improve the situation. An example of this is the ASEAN

Pesticide Management Harmonization project, a joint project undertaken by the the Association of Southeast Asian Nations (ASEAN), US Department of Agriculture (USDA), and CropLife Asia. The project involves 10 ASEAN countries aiming to harmonise their regulatory systems and promote a science-based risk assessment approach, taking into account agriculture conditions and practices in ASEAN. Overall, in the Asia-Pacific region, a trend of increasing use of risk assessment in safety evaluation of NNIs has been observed in recent years.

Several regulatory agencies in the region have also undertaken specific reviews of NNIs in response to decisions taken towards these chemicals in the European Union:

- In China, formal re-evaluation comprising local exposure studies concluded that there is insufficient scientific evidence in the European Union decisions to trigger an immediate ban in the country and a conclusion was made to strengthen monitoring, establish risk assessments in a science-based and systematic approach, and to revise and improve labelling.
- Japan is in the process re-evaluating major NNIs registered in the country. In accordance with the revision of the Pesticide Control Act, companies that had pesticides including NNI products on the market are required to submit risk assessments for honeybees and extend the assessment to cover wild bees (mainly social bees such as bumblebees) from 2022 as part of the re-evaluation of registered items. The regulators are also preparing risk management measures for bees and examining post-monitoring methods particularly for wild bees. While the regulator is still maintaining that these products can be used safely, it is likely that labels will be modified to further optimise their protectiveness for bees as part of this process.
- The South Korean regulator has placed restrictions on new registrations of, and revised labels for, four NNIs (imidacloprid, clothianidin, thiamethoxam, and dinotefuran). The regulator has also strengthened the precautionary statements for honeybees on registered NNI products.
- In India, an All-India Pollinator project team was appointed by the government to gather data about local exposures. They submitted a report based on findings from respective higher-tier semi-field studies conducted following international guidelines. The government is in the process of considering this report and may decide to restrict uses on certain bee-attractive crops (such as applications of intrinsically bee-toxic products only during pre-blooming, cautionary statements on labels, etc.).
- In November 2019, the Australian pesticides regulator started a review of NNIs to reconsider approved active constituents, registrations of selected products containing NNIs, and all associated label approvals on the basis of risks to the environment. However, the regulator still maintains that these products are effective and safe when used according to label directions. Reports on specific products are expected to be released for consultation starting in mid-2022.

An important point to note is that in those Asia-Pacific countries with the highest number of registered NNI uses, such as Japan, China, and Australia, authorities have independently reviewed the exposure to pollinators and aquatic organisms from uses relevant in their countries, taking into account local agriculture and practices.

The regulations of the uses of NNIs have significant emphasis on environmental risks, especially on pollinators. Meanwhile, the residues of pesticides in food and drinking water are also regulated because they are considered potential dietary exposure routes of the chemicals to humans. In some Asian countries (e.g. China, Korea, India, Japan, Australia, and New Zealand), consumer risk assessment is required to ensure that the daily human intake of residues is not above the acceptable daily intake, a value derived from NOAELs (no observed adverse effect level) of chronic toxicity studies in animals and which has a safety factor (e.g. 1/100). To mitigate the risk, a pre-harvest interval) is set on the basis of the results of field residue trials. Meanwhile, maximum residue levels are also drawn from the results of field residue trials and used as important criteria to ensure that good agriculture practice is followed by farmers and to facilitate the trade of food commodities between countries.

7.2 Tier system for evaluation and assessment of risks

To fulfill the regulatory requirements for the registration of NNIs (and other agrochemicals), enormous amounts of data have been generated over recent years. Ever more regulators in the Asia-Pacific region now take a tiered approach to such product assessment to use resources effectively and appropriately, following well-established risk evaluation procedures.

In Tier I assessments, basic data sets are produced in laboratories and analysed for potential risks in the initial steps of risk assessment (USEPA 2014). Such data generation uses modelled exposure values and includes data on acute and chronic toxicity values for bee adults and larvae. Where potential risks or triggers are identified, this leads to Tier II tests being conducted, which can include measured residues in nectar or pollen and colony feeding or semi-field bee studies (in cases where the potential for pollinator risks has been identified) (USEPA

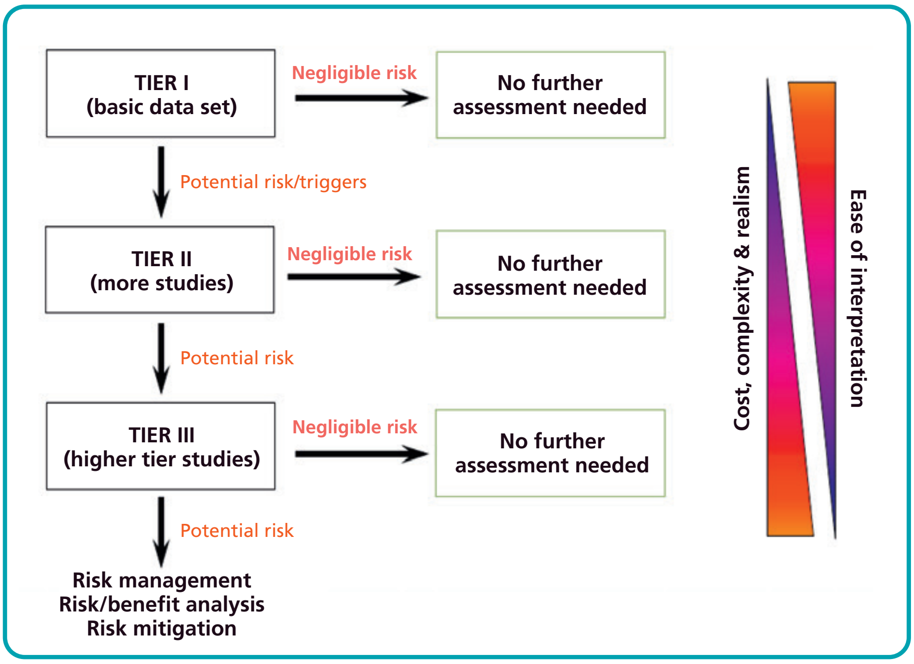


Figure 7.1 Scheme of the tiered approach in risk assessment.

2014). Where potential risks still cannot be excluded, Tier III studies are undertaken, including full-field studies involving multiple fields and multiple hives (USEPA 2014); this can be using either the target crop itself or a surrogate attractive crop (Figure 7.1).

Examples of tiered risk assessment for pollinators in Japan and Korea are presented in the Annex of this chapter.

7.3 Pollinator data requirements for the regulation of neonicotinoids

As with the overall regulatory assessment frameworks, data requirements for assessing and approving NNI uses mostly rely on identical, globally valid effect/hazard core studies. This is related to the fact that toxicity figures are an intrinsically invariable property of a substance, whereas the exposure may differ within and between cropping systems, application types, and regions.

To provide some more context, Tier 1 toxicity studies (worst-case exposure routes) are conducted in accordance with internationally established test guidelines to assess the effects on a pollinator – especially on honeybee species – using the representative formulation shown in Table 7.1. In cases where negligible exposure of pollinators to a particular product can be demonstrated, Tier 1 studies may also not be necessary.

In Australia the regulator has developed a 'Roadmap for insect pollinator risk assessment in Australia' which outlines a tiered approach to risk assessment. The first tier of assessment involves an assessment approach of calculating risk quotients. Methods for refinement at this tier are described, relying on data and approaches from both Europe and North America. At higher tiers of assessment, increasingly complex studies pertaining to exposure and effects (semi-field and full-field studies at colony level) are considered. These studies allow refinements in exposure and/or effect estimations using an increasing level of realism. Importantly, the different levels of refinement are not intended to be prescriptive. The regulator has stated that the specific set of data used in assessing potential risks of a pesticide to bees ultimately depends on multiple lines of evidence and risk management objectives, and generally accepts data packages generated for the European Union or North America without any further requests.

Table 7.1 Recommended Tier 1 test systems

Study type	Tier	Guidelines
Honeybee adult acute oral and contact	Tier 1	OECD 213 / OECD 214
Honeybee adult chronic oral	Tier 1	OECD 245
Honeybee larval single exposure	Tier 1	OECD 237
Honeybee larval repeated exposure	Tier 1	OECD GD 239
Honeybee toxicity to residues on foliage*	Tier 1	850.3030

*Only required for foliar sprays and if the honeybee acute contact endpoint is less than 11 µg a.i. (active ingredient) per bee.

The Japanese regulator has developed additional data requirements and risk assessment on bee toxicity for

NNIs but does adopt a tiered risk assessment approach in practice. The data required for bee testing include standard adult honeybee acute contact and acute oral studies, as well as adult honeybee chronic oral and larval data. Recently, the Ministry of the Environment has decided to establish a risk assessment scheme for pesticides on wild bees. A method for evaluating potential impacts on wild bees by correcting honeybee toxicity data with an uncertainty coefficient has been discussed and implemented, and an evaluation method for native honeybee (*Apis cerana japonica*) is also being proposed.

In Taiwan, bee toxicity data requirements as well as the use of tiered assessments have been established. Additional study data are not required for application methods that lead to low exposure of pollinators (e.g. indoor use, etc.). For products used in field environments, applicants need to provide study data on acute oral or acute contact toxicity to adult honeybees. If the target crops are nectariferous or polleniferous plants, or the application methods would probably cause pesticide drifting resulting in contamination to bee-attractive plants or beekeeping environments, then applicants also need to provide study data of formulated products on acute contact toxicity to adult honeybees.

South Korea currently requires acute oral and acute contact adult honeybee toxicity data to be provided and hazard quotients calculated using the field application rates of pesticides. Depending on the results of the first-tier evaluation, data on foliar residual toxicity and semi-field tests are additionally required at higher tiers of assessment. The Rural Development Administration has considered including chronic toxicity studies for adult honeybees and honeybee larvae for revised risk assessment.

In China and India, acute oral and acute contact toxicity studies on honeybees are required for both technical and formulated products. However, in China semi-field studies are a well-established optional higher-tier assessment method. Since 2017 there has been a risk assessment system. This stipulates that when a certain lower-tier trigger is reached and the targeted crop is included in a honeybee-attractive list, with applications being proposed during the flowering period, then higher-tier honeybee studies may be conducted.

Southeast Asian countries, on the other hand, have not prescribed additional data requirements for NNI-based products, although in Vietnam the US Environmental Protection Agency's (USEPA's) toxicity classification system has been used for simple cut-off criteria by the Vietnamese Government for use on any flowering crops that are attractive to honeybees (with the Vietnamese authorities not accepting for registration any products with a USEPA classification of 2 (moderate toxicity) or 1 (high toxicity) without allowing for any consideration of potential risk mitigation measures as a part of proposed use patterns, unlike in the USA).

7.4 Risk mitigation programmes in the Asia-Pacific region

Various risk factors associated with NNI insecticides have been proposed, from laboratory experiments to field demonstrations. As mitigation programmes have to be implemented not only from the policy and regulation perspectives but also into the field with farmers, consideration must be given to realistic situations and farming practices, as well as economic, social, and political factors, to balance the benefits and risks.

Mitigation of risk can then be achieved through controlling and minimising the exposure to a hazard. Regulators of agricultural chemicals do this by requiring registrants to place precautionary statements on the labels of registered products, so that the risk of the hazard is reduced to a safe and acceptable level. Again, this is true for all crop protection products.

Examples of possible approaches to mitigate specific risks to pollinators include restricting applications to occur only outside the crop flowering periods (for example, with pre-flowering intervals); the removal of flowering weeds within the crop before application (if the crop is not attractive to bees); the removal of hives during applications and for a period afterwards; use of specific application methods (such as seed treatments or granules); limiting application timing to early morning or late evening to avoid applications during bee foraging periods; prescribing the use certain agronomic practices (e.g. avoiding drift by use of low drift equipment); and reduced application rates (however, in this last case, efficacy may be reduced or fewer pests controlled, and the risk of resistance developing in targeting pests potentially increases).

Appropriate mitigation measures are, by nature, decided on a case-by-case basis and specific to the countries and farming situations being considered. However, general precautionary statements for pollinators have been recommended by the FAO on the basis of the UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS, considered one of the main indicators of progress towards sound chemical management systems). In reality though, precautionary statements are not internationally harmonised and frequently follow national requirements. Some examples of current prescribed precautionary statements on registered, intrinsically bee-toxic NNI products in the Asia-Pacific region are as follows:

- Philippines: 'Toxic to bees. Do not use where bees are actively foraging. Do not spray on flowering crops between 15 days before the start of flowering and the end of flowering.'

- India: 'Toxic to bees. Do not use where bees are actively foraging. Do not spray on flowering crops between 10 days before the flowering and the end of flowering' and 'This product is highly toxic to bees exposed to direct treatment and thus should be avoided during active foraging period of bees'.

- Thailand: 'Toxic to bees; the product should be handled with care.'

- Japan: 'This product affects bees. Do not use when drift to the beehive and surrounding beehives is possible. Do not use in orchards or other places where bees are introduced for pollination. Provide information on product use to relevant parties in order to avoid accident sto bees. For use after flowering, do not use until flowering is complete and petals have mostly fallen. For indoor use, use in a facility where bees do not gain entry.'

- Japan (seed treatment use): 'When seeding, there are no or very limited flowers at the seeded and surrounding fields due to well management of fields and the season of seeding. Therefore, no restriction and no specific safety phrases would be necessary for seed treatment.'

- Korea: 'This product is highly toxic to bees. Do not apply this product while bees are actively foraging or fields are in bloom.', 'This product has high residual toxicity. Do not apply this from (RT_{25}+ 2) days before flowering until the end of the flowering period. Do not apply this over the wide-area at once.', and 'This product has high residual toxicity. Do not apply this from the start of spring until the flowering is complete. Do not apply this over the wide-area at once.'

- Australia (seed treatment use): 'For planters that discharge dust into the air, including those using pneumatic vacuum seed metering devices, deflector equipment should be installed to reduce emission of dust and the potential for off-field deposit of dust onto flowering crops or flowering weeds. For planters that discharge dust into the air, do not perform seeding operations under very dry or windy conditions.'

7.5 Conclusion

The regulations of the uses of NNIs in the Asia-Pacific region mainly consist of registrations of pesticide products, post-registration reviews, and requirements for risk mitigation.

Registration of the uses of NNIs in the Asia-Pacific region follows local pesticide regulatory systems, which are different from country to country. This results in very diverse data requirements and decision-making standards in the region. Continuous efforts would be needed to further harmonise the regulatory systems and promote science-based risk assessment which takes into account not only the toxic effects of the chemicals but also the local agriculture conditions and practices.

In the Asian countries with the highest number of registered NNI uses, such as Japan, China, and Australia, the uses of the NNIs are under review. This is mainly driven by the environmental concerns of this class of chemical. On the basis of the results of the reviews, some uses of NNIs might be discontinued or restricted. At the same time, because of the importance of NNIs to the agriculture of these countries, the regulatory decisions need to carefully balance the benefit and risk.

In most Asian countries, the authorities require precautionary statements on labels where potential risks are identified, and risk mitigation measures are needed. The mitigation measures are required to be acceptable to local farmers and implementable in the countries. With the small-scale farming systems in the region, stewardship activities engaging industry, farmers, and other stakeholders are particularly important for effective implementation of risk mitigation measures and to promote safe use of NNI products. In some parts of the region where the education level of farmers is not high, greater stewardship efforts are needed.

7.6 Annex: detailed examination of two risk-based regulatory systems in the Asia-Pacific region

Example 1: Japan

The following is a summary of the pesticide registration legal system in Japan (https://elaws.e-gov.go.jp). The risk assessment of pesticides on honeybees in Japan is done on the basis of a tiered system of toxicological tests, including individual and colony levels. In Tier 1, individual-level tests (laboratory) are conducted. LD_{50} (in micrograms per bee) and LDD_{50} (median lethal dietary dose, in micrograms per bee per day) values are used to evaluate the toxicity of pesticides in single and repeated exposures in the Tier 1 stage, respectively (Table 7.2). The Ministry of Agriculture, Forestry, and Fisheries stipulates that Tier 1 tests are based according to the pesticide exposure routes to be performed, but it is required that adult acute contact toxicity tests should be performed.

The oral test is conducted when there is an exposure possibility of pesticides by contaminated foods. The oral test for adults is assumed as a single exposure (i.e. adult acute oral test), but a chronic test is required if the risk

Table 7.2 Tier 1 risk assessment test for pesticide registration in Japan

Stage	Toxicity test	Study name	Primary endpoint	Condition
Adult	Single contact	Adult acute contact	48-hour LD_{50}	Required
	Single oral	Adult acute oral	48-hour LD_{50}	There is the possibility of oral exposure in adults
	Repeated oral	Adult chronic oral	10-day LDD_{50}	Exceed certain toxicity in an acute oral test
Larva	Single oral	Larval acute	72-hour LD_{50}	There is the possibility of oral exposure to larvae
	Repeated oral	Larval chronic	10-day LDD_{50} or no observed effect dietary dose	

index is much more than 0.4 in the acute test. In the case of larval exposure, both acute and chronic tests are acceptable and LDD_{50} or NOEDD (no observed effect dietary dose) could substitute as a toxicity index for the acute oral test (i.e. LD_{50}). The detailed cases exempted from the oral toxicity are as follows:

- When the active ingredient of the pesticide is used in a sealed or placed without spraying (e.g. attractant, repellent, and so on).
- When the pesticide is used in the place where honeybees cannot forage (e.g. warehouses).
- When the pesticide is not used extensively in a large area at once.
- When the timing of the pesticide application does not overlap the flowering period of crops (e.g. non-flowering crop, managed non-flowering crop, and so on).
- When the pesticide is used for flowering crops which the honeybee does not visit (e.g. cereal, pine, ginkgo, and so on).

The Ministry of Agriculture, Forestry, and Fisheries considers three major exposure scenarios according to application methods of pesticides: (1) foliar application, (2) soil treatment, and (3) seed treatment. Each scenario is examined by considering exposure routes, and the expected exposure amount of pesticide on bees is calculated. The exposure route of direct contact on adult honeybees is only established in the foliar spray scenario. The expected exposure is surrogated by empirical estimation or actual quantified values. The empirical estimation methods are as follows.

Adult contact exposure amount (in micrograms per bee) = pesticide deposit per bee (nanolitres per bee)[a] × concentration of active ingredient (micrograms per nanolitre)

[a]70 nL per bee is assumed on the basis of experiments by the Ministry of Agriculture, Forestry, and Fisheries, but this can be substituted with the actual experimental value.

Adult (larva) oral exposure amount (micrograms per bee) = food consumption per bee (grams per bee per day)[b] × residue in food (micrograms per gram)[c]

[b]Adult: 9.6 mg per bee per day in pollen, and 140 mg per bee per day in nectar.
[b]Larva: 3.6 mg per bee per day in pollen, and 120 mg per bee per day in nectar.
[b]For estimation of food consumption rate, the guidance of the United States Guidance for Assessing Pesticide Risks to Bees, pages 51–52 (USEPA 2014), is also acceptable.
[c]Foliar application: 98 µg/g/kg/ha × application rate of active ingredient (kg/ha).
[c]Soil treatment: Briggs EEC (estimated environment concentration) (µg/g).
[c]Seed treatment: 1 µg/g.
[c]Residue can also be estimated from the actual experimental value.

Risk quotient is calculated as the ratio between estimated exposure and toxicity index, as suggested by USEPA guidance. The risk quotient is then compared with the level of concern, a value expected to have an impact on honeybees (Figure 7.2). If the estimated risk quotient is not exceeded by the level of concern, the pesticide can be registered. Otherwise, re-evaluation is performed by quantifying the expected exposure of pesticides and considering the applicable risk mitigation options. If the re-estimated risk quotient is below the level of concern, pesticide registration is allowed. Otherwise, Tier 2 colony-level testing is required.

In Tier 2, the impact of pesticides on honeybee colonies is evaluated in semi-field conditions. The full-field test (Tier 3 in EPPO and USEPA guidance) is not recommended by the Ministry. The test methods are established tunnel tests (OECD 75 guidance) and feeding tests (Omen method), concerning USEPA guidance. Colony indices such as strength, brood pattern, and development are compared with control groups to decide the potential impact of pesticides on honeybee colonies. If a significant impact on the colony is not identified in the Tier 2 test, pesticide registration is allowed; otherwise, the registration is withdrawn.

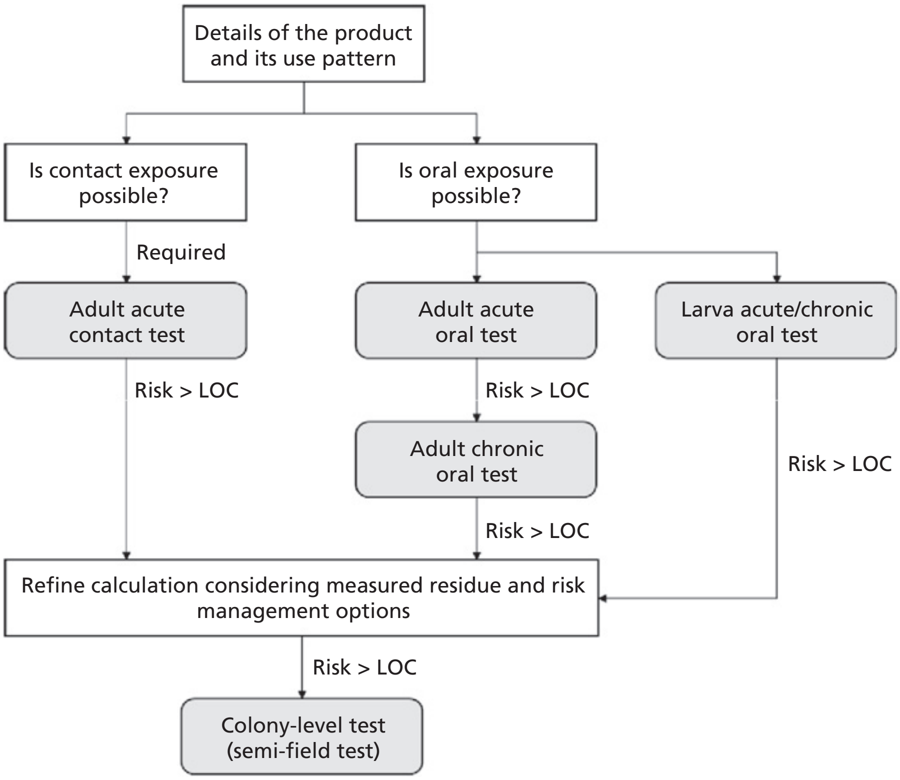


Figure 7.2 Evaluation scheme and tests for pesticide registration in Japan. LOC, level of concern.

The Ministry has stipulated labelling the information associated with uses for which efficacy and safety are confirmed in the registration process. The labels guide farmers to comply with use for minimising the unintentional impact of pesticide application on the environment. If acute contact toxicity on adult honeybees is less than 11 µg per bee, the following pictogram with a cautionary statement should be displayed on the pesticide:

'This product is highly toxic to bees. Safety measures should be fully discussed with the beekeepers before application to prevent exposure to bees.'

To mitigate the unintentional impact of pesticides on honeybees, the Ministry has stipulated (1) identifying the presence of apiaries near crop fields, (2) being careful not to drift pesticides, and (3) to share information on application plans between users and beekeepers. The Ministry has reported that bee damage has been mitigated by controlling the timing of pesticide application through a survey.

Example 2: Korea

The following is a summary of the pesticide registration legal system in South Korea (https://www.law.go.kr). The evaluation methods of honeybee toxicity were established on the basis of OECD and USEPA guidelines in Korea (Table 7.3). The risk assessment of pesticides on honeybees is done on the basis of a tiered system of toxicological tests including individual and colony levels. The Rural Development Administration requires toxicological data such as LD_{50} (in micrograms per bee), and RT_{25} (residual time to 25% mortality, in days) of pesticides on honeybees (*Apis mellifera*) in laboratory-level tests. A semi-field test is required in Tier 3 if the RT_{25} value is not less than 21 days.

For pesticide registration in Korea, both adult acute contact and oral tests are required regardless of exposure routes. LD_{50} values (in micrograms per bee) are used to evaluate the toxicity of pesticides in a single exposure to adults through contact and contaminated food. The two tests in the first step are performed following the OECD test guidelines. On the basis of the estimated LD_{50} value with a field application rate of the active ingredient (in grams per hectare), the hazard quotient is calculated to estimate the potential impact of the pesticide on honeybees when it is applied. The hazard quotient is calculated by dividing the application rate by the LD_{50} value. If the hazard quotient is not less than 50 and there is a likelihood of exposure to honeybees, a foliar residual test for honeybee adults is required for Tier 2. However, even if the hazard quotient is not less than 50, if the LD_{50} value is not less than 100, the foliar residual test is exempted.

The primary endpoint of the foliar residual test is the RT_{25}, which represents the elapsed days with less than 25% mortality of adult honeybees within a day (USEPA

Table 7.3 Honeybee risk assessment tests for pesticide registration in Korea

Tier	Stage	Study name	Primary endpoint	Test substance	
				Raw material[1]	Item
1	Adult	Adult acute contact test	LD_{50}	○	
		Adult acute oral test	LD_{50}	○	
2	Adult	Foliar residual test	RT_{25}		○
3	Colony	Semi-field test	Mortality Colony strength Brood development (Brood termination rate)		○

[1]Substance in which the active ingredients of pesticides are concentrated.

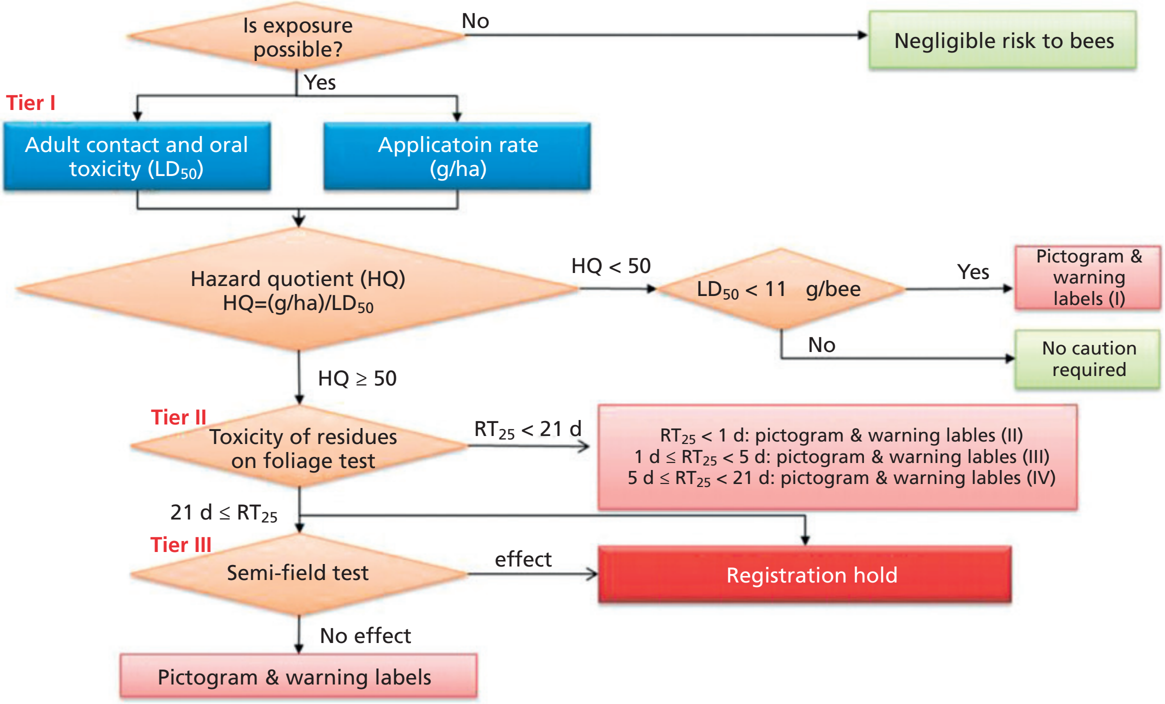


Figure 7.3 Evaluation scheme and tests for pesticide registration in Korea.

2014). If the test item shows a long residual effect (i.e. RT_{25}≥21 days), the third-step test is required. In the next step, the impacts of pesticides on honeybee colonies are evaluated in semi-field conditions providing a worst-case scenario as the bees are exposed to treated crops in tunnels covered with gauze. The test method is established following OECD 75 guidelines. Full-field testing (Tier 3 in EPPO and USEPA guidance) has not been established in Korea. Colony indices such as mortality, colony strength, and brood development are compared with control groups to decide the potential impact of pesticides on honeybee colonies. If a significant impact on the colony is not identified in the Tier 3 test, pesticide registration is allowed; otherwise, the registration is banned.

The Rural Development Administration has stipulated displaying the information associated with uses for which efficacy and safety were confirmed in the registration process. The labelling information includes the classified honeybee risk levels reflecting risk assessment data such as RT_{25} in the registration process (Figure 7.3). Table 7.4 lists the classified precautionary statements with the risk levels. If RT_{25}≥5 days, the statement 'Highly toxic to bees' is additionally seen on the label in red.

Table 7.4 Precautionary statements for honeybee risk of registered pesticides in Korea

Classification	Precautionary statement	Pictogram
I	'This product is highly toxic to bees. Be cautious when using.'	
II	'This product is highly toxic to bees. Do not apply this product while bees are actively foraging or fields are in bloom.'	
III	'This product has high residual toxicity. Do not apply this from (RT_{25} + 2) days before flowering until the end of the flowering period. Do not apply this over the wide-area at once.'	
IV	'This product has high residual toxicity. Do not apply this from the start of spring until the flowering is complete. Do not apply this over the wide-area at once.'	

8 Policy recommendations for risk management

It is of the utmost importance to conserve biodiversity from the anthropogenic activities of human civilisation to ensure sustainable development. Factors that harm sustainable ecosystem services and increase environmental pressure should be controlled. Two decades of using neonicotinoid insecticides (NNIs) have raised serious concerns about the risks associated with pollinator decline and provoked environmental risk assessments around the world. From the intensive risk evaluation of the Asia-Pacific region, we have found negative impacts on pollinator populations, on non-target organisms including natural enemies of aricultural pests, as well as contamination of the environment and perturbations to trophic levels, as demonstrated by voluminous scientific evidence in the region. However, chronic sublethal effects on terrestrial organisms are comparatively less than in North America or Europe, mainly because of the lower use of NNIs as seed coatings of rice, the major crop in Asia. However, given the environmental persistence, water solubility, and high toxicity to aquatic invertebrates and vertebrates of NNIs, certain measures should be applied to reduce or prevent environmental damage. Here, we provide our recommendations to policy-makers on the basis of the results presented in this report.

1 For policy-makers

Asian agricultural systems can be characterised by smallholder farmers, patchy landscapes, and diverse crops, although they are dominated by rice cultivation. The number of registered pesticides including NNIs is higher than in other continents or countries of other continents. The proportion of NNIs in insecticide use is expected to increase. NNIs have been applied to almost all types of crop in the Asia-Pacific region. Unlike Europe and North America, the foliar application of pesticides in Asia is more prominent than in seed coatings and soil treatments. Accordingly, we make the following recommendations.

1. Authorities should impose precautionary statements on labels to control and minimise exposure to any hazards identified with proposed uses of NNIs.

2. In particular, applications for outdoor uses should be restricted during the flowering period to protect pollinator species.

3. Initiate activities to further harmonise the use of tiered risk assessment of pesticides by regulators in the Asia-Pacific region.

4. Adopt the concept of integrated pollinator–pest management from integrated pest management. For this, ecological engineering creates habitats for pollinators, leading to agroecosystem diversification, and a selection of soft pesticides guided by pest monitoring, phenology, and targeted delivery should be used.

2 For researchers

Data show alarming signatures of the risks to aquatic and terrestrial biodiversity with trophic cascades, exemplified by the case of the fishery collapse in Lake Shinji, Japan. Also, much research has indicated the possible contamination of water bodies including rivers, lakes, and even the ocean, implying risk to aquatic biota as well as human health.

1. Consider the need for additional systemic and generic research in the region as part of a tiered risk assessment approach.

2. Intensify the monitoring systems for non-point contamination of NNIs because most drinking water comes from surface water systems in Asia-Pacific countries.

3. Surveillance and conservation of biodiversity programmes relative to environmental hazards should be prepared.

4. Expand studies on pollinator protection of honeybees such as *Apis cerana*, *Apis dorsata*, and *Apis florea*, native bumblebees such as *Bombus ignites* and *Bombus pyrosoma*, and other solitary bees which are more important in local or regional pollination services / specific crop–pollinator systems. Examples can be found in the Japanese pollinator risk assessment scheme.

5. Regular monitoring, restricted application of NNIs, and enforced review systems are essential components to monitor NNI use and avoid their potential risk of pollinator population losses.

6. Monitor pest populations with adequate methods, so that application of NNIs is done only when the population level is above the economic threshold. This will help reduce the number of applications of NNIs.

3 For academia

As Asia has the highest number of honeybee species and other pollinator species diversity, loss of pollinators due to anthropogenic activities could threaten food security in the region. However, toxicological data are heavily biased towards the management of the western honeybee, *Apis mellifera*. The risk quotient is high for honeybees compared with the European bumblebee (*Bombus terrestris*). *Bombus terrestris* has received the most attention compared with native Asian bumblebees such as *Bombus ignitus* and *Bombus pyrosoma*. Similar to the toxicity results, the risk quotient also reveals that imidacloprid, thiamethoxam, and clothianidin show more toxicity than acetamiprid and thiacloprid.

1. Educate farmers to reduce the amount of pesticides, including NNIs, that are released from paddy fields to water systems owing to the high volume of rice production.

2. Pollinator conservation initiatives from academia to the civilian sector are required, and organising a pollinator network within Asia can help preserve the region's unique, native fauna, especially in vulnerable areas such as in high mountains or plane prairies.

3. Introduce educational programmes on the importance of ecosystem services such as provisioning, cultural, supporting, and regulating ones including pollination and biological control.

4. National and international scientific bodies, and governmental and non-governmental funding agencies, should substantially strengthen research provision, training, and awareness programmes among stakeholders, and provide extension services in collaboration with agricultural and scientific research organisations.

Appendix

A1 Authors and reviewers

A1.1 Authors

Project leader: Professor Chuleui Jung, Andong National University, South Korea

Chapters	Author	Affiliation
1: Introduction	Professor Chuleui Jung	Andong National University, South Korea
2: Agrochemicals	Dr SiangHee Tan	Crop Life Asia, Singapore
	Mr Ricky Ho	Crop Life Asia, Singapore
	Dr Min-Jung Kim	National Institute of Forest Science, South Korea
3: Mechanism of Neonicotinoids to honeybee	Dr Sanghyeon Kim	Seoul National University, South Korea
	Professor Si Hyeock Lee	Seoul National University, South Korea
	Professor En-Cheng Yang	National Taiwan University, Taiwan
4: Risk of Neonicotinoids on environment	Professor Francisco Sánchez-Bayo	University of Sydney, Australia
	Professor Yongho Shin	Dong-A University, South Korea
5: Risk of Neonicotinoids on pollinators	Dr Sampat Ghosh	Andong National University, South Korea
	Dr Tekalign Begna	Andong National University, South Korea
	Professor Leknath Kafle	National Pingtung University of Science and Technology, Taiwan
	Professor Chuleui Jung	Andong National University, South Korea
6: Risk of Neonicotinoids on natural enemy	Dr Saeed Mohamadzade Namin	Islamic Azad University, Iran Andong National University, South Korea
	Professor Dharam Pal Abrol	Sher-e-Kashmir University of Agricultural Sciences and Technology, India
	Professor Chuleui Jung	Andong National University, South Korea
7: Regulation and mitigation	Dr Michael Leader	Crop Life Asia, Singapore
	Dr Min-Jung Kim	National Institute of Forest Science, South Korea
	Dr Zhaoqiang Li	Crop Asia, Singapore

A1.2 Reviewers

Name	Affiliation	Country
Professor Christian W.W. Pirk	University of Pretoria	South Africa
Professor Donghui Wu	NEIGAE, CAS	China
Dr Qingyun Diao	Honeybee research institute, CAAS	China
Professor V. Sivaram	Professor V. Sivaram	India
Dr Kouichi Goka	NIES	Japan
Dr Kiyoshi Kimura	NARO	Japan
Professor Joon-Ho Lee	Seoul National University	Korea
Professor Yonggyun Kim	Andong National University	Korea
Professor Jaesoo Kim	Chonbuk National University	Korea
Dr SooMyeong Hong	NIAS, Rural Development Administration	Korea
Dr Kyongmi Chon	NIAS, Rural Development Administration	Korea
Dr KyeChung Park	Plant and Food Research	New Zealand
Professor Irfan Kandemir	Ankara University	Turkey

A2 Workshops and symposium

A2.1 Workshops

First workshop (online)
- Date: 25 November 2021
- Host: Korean Academy of Science and Technology (KAST)
- Description: Professor Yoo Hang Kim, President of AASSA, introduced the project and explained its background, purpose, scope, and schedule. He also stressed the InterAcademy Partnership's interest in the project and cited the EASAC and NASAC reports. Then, participants discussed the major points of the project in groups.

Second workshop (online)
Date: 22 December 2021
Host: Korean Academy of Science and Technology (KAST)
Description: to specify the objectives, scopes, team up, and to check working progress of the project. Every participant introduced their area of research work and their potential contribution. Professor Mooha Lee and Professor Yoohang Kim gave feedback and emphasised the importance of cooperation and data collection.

Third workshop (online)
Date: 26 January 2022
Host: Korean Academy of Science and Technology (KAST)
Description: reports on the three following chapters were shared for an update on the progress: (1) trends in NNI use; (2) environment risks of NNIs; (3) regulatory status of NNIs in the Asia-Pacific region. The leader or co-leader of each working group presented progress and suggestions.

Fourth workshop (online)
Date: 23 February 2022
Host: Korean Academy of Science and Technology (KAST)
Description: the outlines for, and working progress of, the following chapters of the report were discussed: (1) agrochemicals and crops; (2) the environment; (3) pollinators; (4) natural enemies; and (5) regulation and mitigation.

Fifth workshop (online)
Date: 30 March 2022
Host: Korean Academy of Science and Technology (KAST)
Description: in line with the fourth workshop, drafts of the reports and manuscripts of chapters were presented. Key points of discussion/comments were discussed in the following session.

Sixth workshop (hybrid)
Date: 19 May 2022
Venue: The Plaza, Seoul, Korea
Description: several presentations on some scientific matters were made by experts. They were followed by extensive discussions by the participants. Then, Professor Yoo Hang Kim, Past President of AASSA, explained the remaining procedures to successfully complete the project.

Seventh workshop (online)
Date: 1 August 2022
Host: Korean Academy of Science and Technology (KAST)

A2.2 Symposium

Title: Ecological Risk Assessment of Neonicotinoids in Asia
Date: 29 April 2022
Venue: SONO Bell Byeongsan, Korea
Host: Korean Society of Applied Entomology

A2.3 Participants

Name	Affiliation	Country
Professor Hassan Taj	Hajee Mohammad Danesh Science and Technology University	Bangladesh
Professor Chen Lihong	Chinese Academy of Agricultural Science	China
Professor Wu Donghui	Northeast Normal University	China
Professor Meirong Zhao	Zhejiang University of Technology	China
Dr Gen Shubao	Xinyang Agriculture and Forestry University	China
Professor Parthib Basu	University of Calcutta	India
Professor Damayanti Buchori	Bogor Agricultural University	Indonesia
Professor Shimano Satoshi	Heisei University	Japan
Dr Hong-Hyun Park	Rural Development Administration	Korea
Dr Kyongmi Chon	Rural Development Administration	Korea
Dr Soo-Myung Hong	Rural Development Administration	Korea
Mr Seong-pil Cho	Korea Crop Protection Association	Korea
Mr Jaehak Lee	Korea Crop Protection Association	Korea
Mr Hakyong Kim	Korea Crop Protection Association	Korea
Professor Kong Luen Heong	Zhejiang University	Malaysia

Name	Affiliation	Country
Professor Badamdorj Bayartogtokh	National University of Mongolia	Mongolia
Professor Hlaing Minoo	Maejo University	Myanmar
Dr Sunil Aryal	Nepal Agricultural Research Council	Nepal
Dr Kye Chung Park	Horticulture Research	New Zealand
Dr Waqas Wakil	University of Agriculture Fiaslabad	Pakistan
Professor Siriwat Woonsiri	Chulalongkorn University	Thailand
Professor Panuwan Chantawannakul	Chiang Mai University	Thailand
Professor Irfan Kandemir	Ankara University	Turkey
Professor Baymat Kaxramanov	Tashkent State Agrarian University	Uzbekistan
Professor Pham HongThai	Vietnam National University	Vietnam

A2.4 Administrative and secretarial support

AASSA secretariat:

Professor Yoo Hang Kim, Former President
Professor Mooha Lee, Member of Executive Board
Professor Seungbok Choi, Director
Mr Jaehyoung Lee, Head of International Cooperation Division
Ms Lyunhae Kim, Senior Programme Officer
Mr Dong Hyun Kim, Programme Officer

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Chapter 5 Risk assessment of neonicotinoids on pollinator populations

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Chapter 6 Risk assessment of neonicotinoid pesticides on natural enemies of crop pests in agroecosystems

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Chapter 7 Risk regulation and mitigation in the Asia-Pacific region

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