Exposure Assessment and Toxicological Evaluations of Neonicotinoid Insecticides

(殺虫剤ネオニコチノイドへのばく露と毒性学的評価)

Dissertation Summary

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CHAPTER 1: General Background

Neonicotinoid insecticides (NNIs) are a relatively new class of insecticides used for various pest control regimens across the globe. As neurotoxicants, NNIs are highly effective against different species of biting, chewing and sucking insects; with minimal toxicological profiles in mammalian species (Simon-Delso *et al.,* 2015; Wang Y. *et al.,* 2018). Due to their systematic properties, NNIs are effective against insecticide resistant pests; and their field applications are highly characterized with low environmental resurgence. These unique insecticidal properties have made NNIs the most preferred replacements for organophosphates, carbamates and pyrethroids. Currently, NNIs are the most popular pest extermination agents across the globe (Wang Y. *et al.,* 2018); in 2014, NNIs alone accounted for more than 25% of the global insecticide market **(**Kathage *et al*., 2018).

However, the momentous dependence on NNIs have generated several concerns about their residual infiltrations into various segments of the ecosystem. A plethora of studies have consistently reported high concentrations of NNI residues in food and other environmental matrices. However, (i) data on the human exposure levels of NNIs are highly limited in many developing countries, (ii) the potential toxicological ramifications of long-term low-dose exposures of NNIs in mammalian species are not well clarified, (iii) epidemiological data needed for validating mechanistic findings on NNIs are highly scarce, (iv) countries with records of high NNI exposure frequencies, do not have validated countermeasures for ameliorating their exposure rates within affected human populations.

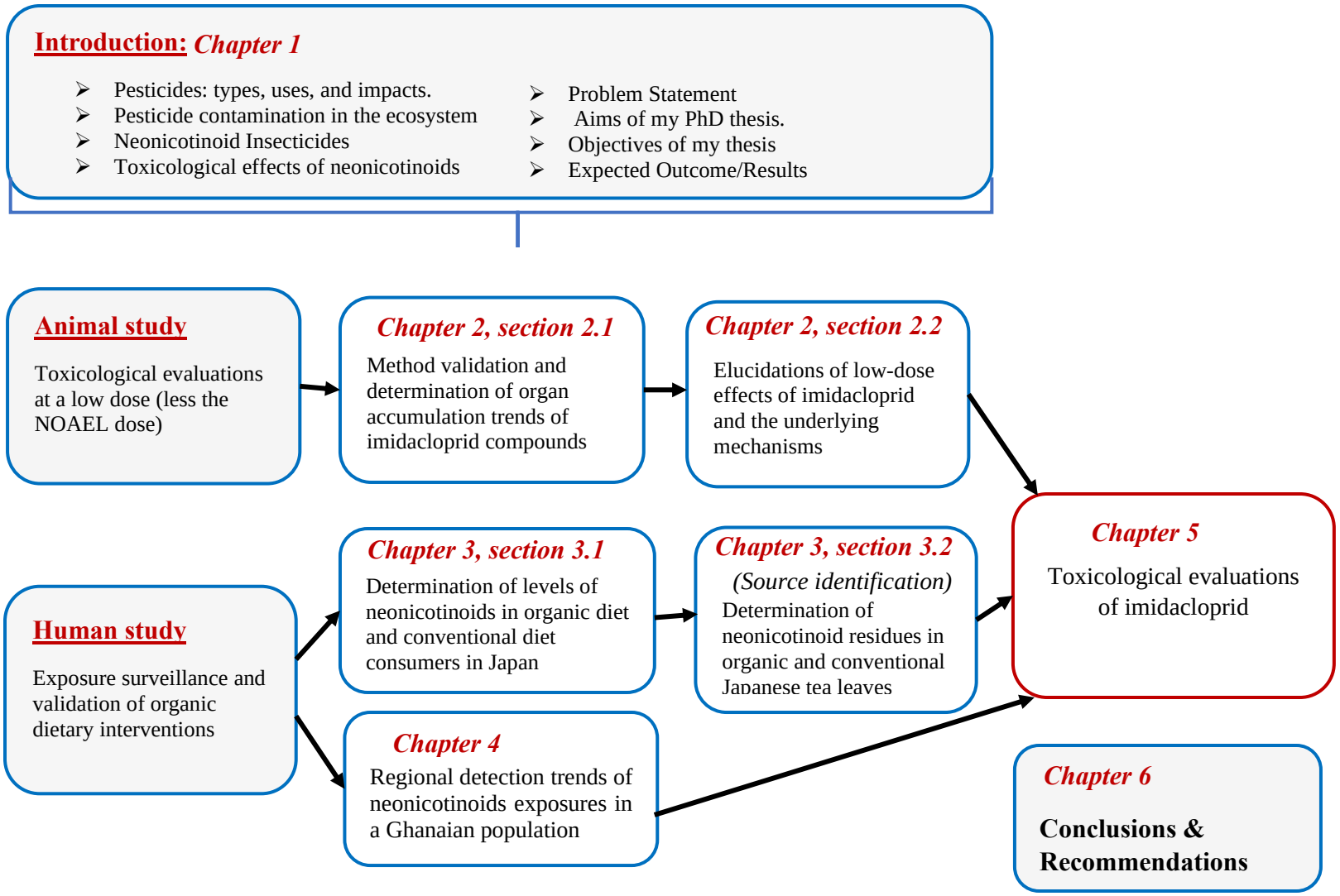
Aims of my PhD thesis

My PhD Research aimed to elucidate the potential toxicological effects of chronic low-dose exposures to NNIs, evaluate the exposure dynamics of NNIs in a Ghanaian population; and to validate organic dietary intervention as a countermeasure for ameliorating NNI exposure rates in a Japanese population (Fig. 1.2). I sought to achieve these set of goals by using *in vivo* techniques (using rodent models) and biomonitoring strategies (exposure surveillance in human populations. In my PhD. research, I used

imidacloprid (IMI) as a representative compound for the toxicological assessments of NNIs because (i) IMI is the most commonly used NNI compound across the globe and (ii) biomonitoring data on IMI is extensively reported.

Objectives of my thesis

I. To determine the long-term exposure-associated biodistribution and bioaccumulation trends of IMI and its metabolites in mammalian system, using a C57BL/6J male mice.
II. To elucidate the dietary interactive effects of low doses of IMI in mammalian system, upon chronic co-exposure of $1/10^{th}$ the NOAEL of IMI and high fat diet to C57BL/6J male mice.
III. To determine NNI exposure trends in a Japanese population; and to validate organic dietary intervention as a countermeasure for ameliorating NNI exposure levels in Japan.
IV. To determine the exposure levels and potential health risks of NNIs in the consumer population of Kumasi, Ghana.


Introduction: Chapter 1
Pesticides: types, uses, and impacts.
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Aims of my PhD thesis.
Objectives of my thesis
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Chapter 2, section 2.2
Elucidations of low-dose effects of imidacloprid and the underlying mechanisms
Human study
Exposure surveillance and validation of organic dietary interventions
Chapter 3, section 3.1
Determination of levels of neonicotinoids in organic diet and conventional diet consumers in Japan
Chapter 3, section 3.2
(Source identification)
Determination of neonicotinoid residues in organic and conventional Japanese tea leaves
Chapter 4
Regional detection trends of neonicotinoids exposures in a Ghanaian population
Chapter 5
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Chapter 6
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CHAPTER 2: ***Method validations, biological fates, and toxicological evaluations of chronic low-dose exposures to imidacloprid and its associated metabolites in C57BL/6J male mice.***

In Chapter 2, the biological fates and toxicological effects of chronic low-dose exposures to IMI and its associated metabolites were elucidated in C57BL/6J male mice; and this was studied under two thematic areas.

Chapter 2, section 2.1 of my thesis aimed to (i) develop a sensitive method for simultaneous detection and quantification of the most popular IMI and seven of its metabolites in tissue specimens, and to (ii) determine the biodistribution of the IMI compounds in tissues of C57BL/6J male mice; after exposure to 0.6 mg/kg bw/day of IMI (10% of non-observable adverse effect level [NOAEL] of IMI) through a powdered diet for 24 weeks. I successfully developed a method which was accurate (recoveries were ≥ 70% for most compounds), sensitive (limit of detections (LODs) ≤ 0.47 ng/mL and limit of quantifications (LOQs) ≤ 1.43 ng/mL were recorded for all detected compounds, $R^2 \geq 0.99$) and precise (RSDs ≤ 20%) for routine analysis of IMI and seven of its metabolites in blood and various tissue matrices. After bio-distributional analysis, IMI and five of its metabolites were detected in mice. Brain, testis, lung, kidney, inguinal white adipose tissue, and gonadal white adipose tissue mainly accumulated IMI, blood and mesenteric white adipose tissue mainly accumulated IMI-olefin; liver mainly accumulated desnitro-IMI; pancreas predominately accumulated 4-hydroxy-IMI. The desnitro-dehydro-IMI and the desnitro-IMI metabolites recorded tissue-blood concentration ratios ≥ 1.0 for testis, brain, lung and kidney. The cumulative levels of the six detected IMI compounds (Σ6 IMI compounds) were found in the decreasing order: blood > testis > brain > kidney > lung > inguinal white adipose tissue > gonadal white adipose tissue > mesenteric white adipose tissue > liver > pancreas. Altogether, this study provided essential data needed for effective mechanistic elucidation of compound-specific adverse outcomes associated with chronic exposures to IMI in mammalian species.

Chapter 2, section 2.2 aimed to elucidate the mechanistic role of IMI in the prevalence of high fat diet (HFD)-induced liver steatosis, using a C57BL/6J mice model. Mice (3 weeks

old) were fed with HFD and treated with 0.6 mg/kg body weight/day (1/10th of the NOAEL) of IMI through water or diet, for 24 weeks. In a controlled group, mice were fed with only HFD. At the end of the study, IMI treatment significantly potentiated HFD-induced body weight gain in mice. Also, IMI increased the liver weights of mice, with complimentary reductions in mesenteric and gonadal white adipose tissue weights. Histopathological analysis of liver revealed a drastic steatosis in IMI treated mice. Following a real-time qPCR analysis, IMI upregulated transcriptions of hepatic fatty acid biosynthesis-related transcription factors and genes. Imidacloprid also induced hepatic expression of the gene encoding pregnane X receptor; but had no significant effect on hepatic expressions of liver X receptor and aryl hydrocarbon receptor. The IMI treatment further enhanced serum alanine aminotransferase levels but downregulated hepatic antioxidant mRNA expressions. Ultimately, this study suggested an IMI-potentiation effects on prevalence of HFD-induced liver steatosis via transcriptional modulations of the hepatic fatty acid biosynthesis pathway.

CHAPTER 3: ***Neonicotinoid exposure levels in Japanese population and assessments of organic dietary interventions as a countermeasure for neonicotinoid exposures***.

In Chapter 3, I determined NNI exposure levels and validated the efficiency of organic dietary interventions in a Japanese population. This was studied under two themes.

In Chapter 3, Section 3.1, I sought to assess the impacts of organic dietary interventions on NNI exposures in a Japanese population. A total of 103 volunteers were recruited into the study by convenience. The subjects were fed with organic diets for 5 or 30 days; and conventional diets for 30 days. A total of 947 repeated urine samples were collected from the participants; and then subjected to LC-MS/MS analysis to determine NNI concentrations. Eight NNIs were detected; with a decreasing detection frequency (%Dfs) pattern; dm-acetamiprid (65.3%) > dinotefuran (52.2%), imidacloprid (40.1%) > clothianidin (33.6%) > thiamethoxam (28.5%) > acetamiprid (12.3%) > nitenpyram (5.6%) > thiacloprid (2.7%). Dinotefuran, dm-acetamiprid and clothianidin recorded the highest concentrations in the subjects. The %Df of NNIs in the 5-days or 30-days organic diet consumers were lower than those of the conventional diet consumers. The organic diet

consumers showed lower multiple NNI exposure trends, compared to the conventional diet consumers. The mean and median cumulative levels of NNIs (median M_{eq}) were significantly lower in the organic diet consumers than the conventional diet consumers ($P<0.0001$). The estimated daily intakes (EDIs) of NNIs were higher in adults compared to children, but less than 1% of NNI cRfDs, except for clothianidin which recorded %cRfD of 1.32 in children. The 30-day organic diet intervention showed drastic reductions in EDIs, compared to the conventional diet group. The current findings indicated that an adoption of a long-term organic dietary intervention may offer optimum ameliorative outcomes on NNI exposure rates in human populations.

In Chapter 3, Section 3.2, I sought to assess the residual levels NNIs in organic and conventional green tea leaves produced in Japan. A total of 103 tea leaves (thus, 42 organic and 61 conventional), were sampled from grocery stores in Japan. Concentrations of NNIs in the tea leaves were quantified using LC-MS/MS; and the data was used to estimate maximum daily intakes of NNIs within the Japanese population. Eight NNI compounds were detected in both organic and conventional tea leaves. Detection frequencies (%Dfs) of NNIs in the tea samples (n=103) were found in the decreasing order; thiacloprid (84.47%) > dinotefuran (74.76%) > imidacloprid (69.90%) ≈ clothianidin (69.90%) > dm-acetamiprid (63.11%) > thiamethoxam (58.25%) > acetamiprid (4.85%) > nitenpyram (1.94%). About 94.20% of the tea leaves contained two or more NNI compounds simultaneously. The %Dfs of NNIs were relatively lower in organic tea leaves, compared to the conventional tea leaves. Various percentile concentrations of NNIs were far lower in organic tea leaves, compared to the conventional tea leaves. The maximum daily intakes of NNIs through consumption of tea (MDIgt) were also lower for organic tea leaves, compared to the conventional tea samples.

CHAPTER 4: *Region exposure trends of neonicotinoids in Ghana*

In chapter 4 of my thesis, I evaluated NNI exposures in the consumer population of Kumasi, a cosmopolitan city in Ghana. A total of 75 human urine samples were collected from healthy volunteers (nonfarmers, aged 13–80 yr) and analysed with a liquid chromatography electrospray ionization tandem mass spectrometry system. Seven NNIs

and 3 NNI metabolites were detected in the following pattern (frequency, median concentration, maximum concentration): *N*-dm-acetamiprid (94.7%, 0.41 µg/L, 8.79 µg/L) > imidacloprid (70.7%, 0.15 µg/L, 211.62 µg/L) > *N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl-*N′*-methylformamidine (62.2%, 0.43 µg/L, 53.85 µg/L) > 2-[*N*-(6-chloro-3-pyridylmethyl)-*N*ethylamino]-2-(methylimino)acetic acid (56.8%, 0.10 µg/L, 3.53 µg/L) > clothianidin (40%, >limit of quantification [LOQ], 0.45 µg/L) > nitenpyram (18.7%, >LOQ, 0.14 µg/L) ≈ thiamethoxam (18.7%, >LOQ, 0.21 µg/L) > dinotefuran (12.0%, >LOQ, 1.01 µg/L) > acetamiprid (2.7%, >LOQ, 0.08 µg/L) ≈ thiacloprid (2.7%, >LOQ, 0.14 µg/L). Approximately 92% of the subjects were found to be exposed to multiple neonicotinoids simultaneously. The mean, median, and maximum imidacloprid equivalent of the relative potency factor of NNIs were found to be 1.6, 0.5, and 22.52, respectively. The median estimated daily intakes of acetamiprid, imidacloprid, and nitenpyram were 0.47, 1.27, and 0.02 µg/kg/d for females and 0.91, 0.66, and 0.08 µg/kg/d for males, respectively. The maximum daily intakes of all the NNIs were <1% of their chronic reference doses (cRfDs), except for imidacloprid and thiacloprid which recorded maximum daily intakes corresponding to 17.97 and 8.28% of cRfDs, respectively.

CHAPTER 5: *Toxicological evaluations of low-dose exposures to imidacloprid in human populations*

In the human studies presented in Chapter 3, section 3.1 and Chapter 4 of the current thesis, the maximum daily intakes of IMI recorded in Japanese and Ghanaian populations were found to be equivalent to 0.21% and 18% of the IMI RfD, respectively. These findings seemed to suggest that the daily exposure levels of IMI recorded in both populations are of low toxicological essence. However, these findings are counterintuitive in that, findings from Chapter 2, section 2.2 of this thesis and a previous finding from Sun *et al. (*2016) found various adverse outcomes in relation to IMI exposures at doses far below IMI RfDs (thus, less than the NOAEL doses of IMI).

In Chapter 2, section 2.2 of this thesis, an exposure to 1/10th the NOAEL of IMI (0.57 mg/kg) was found to induce liver weight gains and hepatic steatosis in a C57BL6/J mice model. This indicated that, an exposure to a dose below the RfD of IMI may alter

hepatic lipid homeostasis in mammalian systems. In a precious study (Sun *et al.*, 2016) a daily exposure to 1/100th the NOAEL of IMI (0.057 mg/kg) was found to promote high-fat diet–induced adiposity and insulin resistance in C57BL/6 J male mice. This suggested that, even at 1% of the NOAEL (5.7 mg/kg), IMI may interrupt energy metabolism via the AMP-activated protein kinase-α pathway in mammals.

Hence, by setting the NOAEL of IMI at 0.6 mg/kg (based on findings from Chapter 2, section 2.2 of this thesis) or 0.06 mg/kg (based on findings from Sun *et al.*, 2016); and by using 100 as the uncertainty factor, the reference dose of IMI was estimated (RfDest) from my study and that of Sun *et al.*, (2016) as 0.006 mg/kg /bw and 0.0006 mg/kg /bw, respectively. By considering the average body weight of adults as 62 kg and that of children as 15 kg, the potential toxicological implications of human exposures to less than the NOAELs of IMI were evaluated as ratios of IMI EDIs (in humans) to the estimated IMI RfDs (RfDest).

The 95th percentile EDI:RfD$_{est}$ ratios and 100th percentile EDI:RfD$_{est}$ ratios of IMI estimated within the Japanese population were found below 1. This tendency suggests that the Japanese population may be less likely to suffer from the toxicological implications of low-dose exposures to IMI. However, the highest 100th percentile EDI:RfD$_{est}$ ratio of IMI obtained within the Japanese population (0.7) was found to be associated with children, suggesting that children may be more susceptible to the low-dose exposure effects of IMI, compared to adults. The minimal toxicological tendencies of IMI observed in the Japanese population might be due to the low application rates of IMI containing formulations in Japan. According to Taira *et al.*, (2014), the domestic consumption rate of dinotefuran is highest, compared to all the NNIs used in Japan.

In the Ghanaian population, the 95th percentile EDI:RfD$_{est}$ ratio estimated with regards to Sun *et al.*`s study, was found to be higher than 1. Moreover, the 100th percentile EDI:RfD$_{est}$ ratios of IMI estimated from my study and that of Sun *et al.*, (2016) were far higher than 1 (1.8 and 18, respectively). These findings suggest that a section of individuals who are exposed to high levels of IMI within the Ghanaian population may be more liable to the low-dose toxicological implications of IMI.

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